LABORATORY ANALYSES IN TREATMENT PLANT OPERATIONS

TRAINING MANUAL

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF WATER PROGRAMS

LABORATORY ANALYSES IN TREATMENT PLANT OPERATIONS

This course is offered for operators and other personnel responsible for laboratory control and management in wastewater treatment.

ENVIRONMENTAL PROTECTION AGENCY
Water Programs Operations
TRAINING PROGRAM

December 1972

FOREWORD

These manuals are prepared for reference use of students enrolled in scheduled training courses of the Office of Water Programs, Environmental Protection Agency.

Due to the limited availability of the manuals, it is not appropriate to cite them as technical references in bibliographies or other forms of publication.

References to products and manufacturers are for illustration only, such references do not imply product endorsement by the Office of Water Programs, Environmental Protection Agency.

The reference outlines in this manual have been selected and developed with a goal of providing the student with a fund of the best available current information pertinent to the subject matter of the course. Individual instructors may provide additional material to cover special aspects of their own presentations

This manual will be useful to anyone who has need for information on the subjects covered However, it should be understood that the manual will have its greatest value as an adjunct to classroom presentations. The inherent advantages of classroom presentation is in the give-and-take discussions and exchange of information between and among students and the instructional staff.

Constructive suggestions for improvement in the coverage, content, and format of the manual are solicited and will be given full consideration.

Joseph Bahnick

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TRAINING PROGRAM

Through the Office of Water Program, Environmental Protection Agency conducts programs of research, technical assistance, enforcement, and technical training for water pollution control.

Training is available at five installations of the Agency. These are· the National Training Center located at the Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio, the Robert S. Kerr Water Research Center, Ada, Oklahoma, the Southeast Water Laboratory, Athens, Georgia, the Pacific Northwest Water Laboratory, Corvallis, Oregon, and the Hudson-Delaware Basins Office, Edison, New Jersey.

The objectives of the Training Program are to provide specialized training in the field of water pollution control which will lead to rapid application of new research findings through updating of skills of technical and professional personnel, and to train new employees recruited from other professional or technical areas in the special skills required. Increasing attention is being given to development of special courses providing an overview of the nature, causes, prevention, and control of water pollution.

Scientists, engineers, and recognized authorities from other Agency programs, from other government agencies, universities, and industry supplement the training staff by serving as guest lecturers. Most training is conducted in the form of short-term courses of one or two weeks' duration. Subject matter includes selected practical features of plant operation and design, and water quality evaluation in field and laboratory. Specialized aspects and recent developments of sanitary engineering, chemistry, aquatic biology, microbiology, and field and laboratory techniques not generally available elsewhere, are included.

The primary role and the responsibility of the States in the training of wastewater treatment plant operators are recognized. Technical support of operator-training programs of the States is available through technical consultations in the planning and development of operator-training courses. Guest appearances of instructors from the Environmental Protection Agency, and the loan of instructional materials such as lesson plans and visual training aids, may be available through special arrangement. These training aids, including reference training manuals, may be reproduced freely by the states for their own training programs. Special categories of training for personnel engaged in treatment plant operations may be developed and made available to the States for their own further production and presentation.

An annual Bulletin of Courses is prepared and distributed by the Office of Water Programs. The Bulletin includes descriptions of courses, schedules, application blanks, and other appropriate information. Organizations and interested individuals not on the mailing list should request a copy from one of the training centers mentioned above.

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I INTRODUCTION

Wastewater Treatment Plant Operation involves the combined efforts of process and equipment knowledge, diverse performance skills related to acquiring information, and using this information effectively within the framework of situation, time, place and human events to enhance the reuse potential of our water resources. An operator is many things, conversely, it requires many individuals contributing to the dynamics of effective operations.

Contents of Section I

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The Operator's Responsibilities and the Analyst's Part in Them	1

THE OPERATOR'S RESPONSIBILITIES AND THE ANALYST'S PART IN THEM

I INTRODUCTION

A Considered as a whole, most wastewater treatment plants serve rather small populations. Most operating staffs consist of less than five operators, often only two.

B Analysts usually have some responsibilities as operators. Not infrequently the plant superintendent and shift operator share the laboratory work and record keeping.

C In all plants, regardless of size and complexities, meaningful analyses and records are essential and vital to effective operational control, for planning and for communication with others both within the plant and outside interests.

D Todays higher goals for water resource management require refinements and intensification of process control. Increasingly complex and sophisticated analytical procedures will be required in an ever increasing percentage of plants.

II Working and living with others requires assumption of broad and varied aspects of responsibility - often automatically.

A Teamwork Requires

1 Coordination and direction

2 Cooperation of each member

3 Subordination of individual to total objectives

4 Sharing in rewards of success

5 Sharing in burdens of disappointment or failure.

B Responsibility

1 Implies understanding between

a One performing a task or duty and

b One assigning it

2 Proper relationship requires understanding of

a Nature of the obligation

b Conditions governing its execution

3 Must be accepted willingly - not grudgingly

III RESPONSIBILITIES OF THE OPERATOR

At any given plant the responsibilities and obligations of the operating staff change markedly as facilities age, undergo modification, and as performance requirements are elevated.

A New Plant Breakin

1 Become familiar with

a Each element of the plant

b Treatment objectives

c Characteristics of receiving waters

2 Identify deficiencies

3 Recommend changes and modifications to

a Correct deficiencies

b Add needed improvements

4 Acquaint designer with reasons for recommendations

5 Determine waste characteristics

6 Establish sampling and testing program

7 Develop operating schedules

8 Plan training program as personnel are assigned to plant

9 Prepare initial budget

10 Confer with industries on

a Waste problems

b Restrictions and limitations

c Pretreatment

11 Suggest values for sewer use ordinance

B Routine Operation

The operator and analyst must look constantly for ways to strengthen, refine and modify methods to improve plant performance.

1 Establish effective process control by

a Application of test results and other observations

b Adjustments in operational and maintenance practices

2 Identify adverse effects of industrial wastes on

a Plant processes

b Receiving stream

3 Correct adverse effects of industrial wastes

4 Keep useful meaningful records

a Physical

b Chemical

c Bacteriological - biological

5 Identify deficiencies in facilities

6 Provide officials with information for

a Budgeting and accounting

b Overall management

c Planning

7 Provide subordinates with

a Good working conditions

b Training opportunities

c Incentives

8 Prepare for plant improvements

a Enlargements

b Replacements

c Higher degree of treatment

9 Establish and maintain good public relations

10 Communicate effectively with

a Other members of staff

b Responsible local officials

c Local industries

d Selected groups (schools, service clubs)

e General public

f Regulatory officials

g Other operators

IV KEEPING INFORMED

The operator and the analyst are professionals or should be. To be successful they must keep abreast of effective methods and practices of others in their field and of new developments as they are reported.

A Selective Reading of Technical Literature

1 Journals

2 Manufacturer's bulletins

3 Texts

B Attend Technical Meetings

1 Water Pollution Control Associations

2 Professional societies

C Participate in Training Courses

1 In plant

2 Water Pollution Control Association

3 Regulatory agencies

D Consult with Regulatory Agency Personnel

E Visit with Other Operators and Analysts

1 At meetings

2 At their plants and yours

V DIVIDENDS OF PROFESSIONALISM

Many dividends accrue from top level performance, some tangible, others not easily defined.

A Tangible

1 Top performance of facilities

2 Complete, accurate, useful records

3 Salary benefits

4 Advancement

B Less Tangible

1 Respect and appreciation of

a Associates

b The boss

c The public

2 Leadership opportunities

3 Helping others

4 Passing Operator Certification Exam

5 Self esteem

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II BASIC LABORATORY OPERATIONS

The team effort among individuals requires an effective chain of events before, during, and after the action for guidance on what to do, how to do it, and informing others with respect to the action taken. This section considers certain elements essential for information procurement, validity and availability.

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PRINCIPLES OF RECORD KEEPING

I Records may not be used extensively by those who prepare them, hence, they may be regarded as an unessential evil. They need to be reviewed for functional use to limit pyramiding but its never quite certain whether a given item will be needed. The only common feature is the frustration associated with the lack of an item that is being sought.

A All functional records have one common objective - communication among those who have and those who need information.

1 Records may convey information from those who do the work to those who direct it.

2 Budget considerations are based upon past records and future plans.

3 Stock records are a means to determine what must be on hand to give a reasonable probability of having a given item when and where it is needed.

4 Performance records indicate:

a Operation efficiency

b Compliance or non-compliance with objectives

c Help to identify and enumerate problem areas.

5 Records may be used to provide a key for optimization or selection of processes, methods, individuals.

6 The record is an indication of good and bad decisions and a source of satisfaction for a job well done.

7 It may be used to create awareness and recognition among the people served.

B Reports are of interest to a variety of personnel of different backgrounds.

1 Supervisors are interested in what is being done in terms of the performance of operations, individuals, machines, finance, etc., for planning and controlling performance.

2 The public is interested in what they are getting for their investment.

3 Plant employees are interested in their particular contribution to the whole operation in performance or improvement.

a The report may be a summary for comparison of one plant in relation to that of other plants of a similar nature.

4 Governmental planning agencies or administrators are interested in comparisons among the what is and what ought to be done.

5 Apprentices or students seek reports for guidance in their development.

6 Engineering problems involving selection, consultation, design, are generally based upon reports of full-scale or development scale operations.

C Records and reports may be classified roughly according to the source or type of information noted, purpose, and intended contact.

1 Sensory impressions of color, odor, appearance, and feel, convey real and understandable concepts to the public and to interested parties. These may not be scientifically precise but the impact may be tremendous.

2 Reports may contain strictly physical measurements of length, width, flow, volume, rate, time in relation to events.

3 The laboratory record may be expected to contain sample identifications, numeration of direct results and techniques applied whether for biology, chemistry or microbiological disciplines. These are integrated into derived information including that necessary to recheck calculations, observations on possible interference and related effects or conclusions.

4 The engineering report may include conclusions and recommendations based upon reports of the physical features, economics, history, scientific data, etc., for planning, design, etc.

5 The supervisors annual report may contain performance items including total, maximum, minimum, budget review, major difficulties, special developments during the year and future plans.

6 The report is likely to represent a single concept item giving pertinent observations and integration along one subject or cover multiple subject areas in relation to some pertinent characteristic.

7 Any report is intended for some contact or audience - it should be written and structured to communicate accordingly.

a The log book for personal reference or guidance should contain a detailed list of work done, observed items, techniques applied, dates, calculations and conclusions at the time.

b A report to the supervisor should delete minor details but convey what was done, briefly describe methods and present results and recommendations.

c Reports to the administrator should stress the subject matter according to broad aspects, giving pertinent information and conclusions in brief, concise, language with a minimum of technical jargon.

d Public information releases should contain brief single concept items indicating some phase of the work that is important to them, what was done about it, or needs to be done to contribute to their welfare. Technical language is acceptable if it can be explained briefly in terms meaningful to the public. Candor, briefness, and enthusiasm are essential to create and hold public trust.

II The annual operating report is a recurrent obligation that describes your efforts to meet a public trust and should reflect your capabilities in management, scheduling, technical and public information.

A Information should be assembled promptly month by month to be ready for compilation into an annual shortly after the closing month.

B Review the annual report general plan with your administrative board and solicit their enthusiasm and backing for an informative and interesting production. Obtain a commitment on budgeting, possible use of outside services, and personnel. Present an outline of the annual.

C Assemble information and accessories for production in accordance with previous commitments. Work up layout and production facilities and roll the presses. An annual report is ancient history if it is released a year later.

D It should include in an attractive format:

1 A Title Page including identification of the plant, the city, Administrative Board or Commission with names of principal officials and that it is a report of operations for the stated interval. This preferably should be associated with a letter of transmittal to the mayor or other principal designated official.

2 Provide a brief resume of major problems or developments within the period covered. Volume or pounds removed per day or per year in comparison to previous load is a good start. Mention progress of new construction or planning with principal difficulties mentioned in terms of personnel, equipment, loading industrial wastes, total sewer extension. This should be in narrative style of 1/2 to 1 1/2 page length. This is a thumbnail sketch keep it that way, but it may be the only part read.

3 Present a diagram of the plant showing operations and flow pattern. Pictures increase interest. Review purpose, plant and receiving water.

4 Present an organization chart with principal officials and backup support if possible outlining responsibilities of each major slot.

5 Present a resume of plant history and changes in facilities; objectives, community, or receiving water.

6 Present a resume of future plans or progress of construction.

7 Review area or sewer extensions.

8 Review special services, delegated or not, that may be of interest to plant-community relations.

9 Present a summary chart of flow, strength, solids received/removed by month with plant efficiencies indicated.

10 Review receiving water conditions, use, trouble spots during the period covered. Stress improvements or plans for improvement. If objectionable conditions occurred what happened? What can or is being done about it? Do not hide complaints, use them.

11 Review financial and administrative developments.

12 Summarize recommendations for future or immediate attention.

13 Present charted details on:

a Flow received, strength, treatment efficiency by stage or process (overall operation).

b Cost of operation by category and general terms for operation, bonded debt, or special funding.

c Present population - load data comparisons for at least 1 or 2 years in addition to the current period.

d Data for unit operations, processes or functions.

E Completeness, clearness and conciseness characterize a good report. Remember that few will read all of it; so index it for ready reference to information sought. It should be possible to get the picture by referring to 1 to 3 pages of special interest. If these do not attract attention the message is lost. Completeness in backup information is essential for those who want it but do not engulf the reader in it.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, Water Programs Operations, Environmental Protection Agency, Cincinnati, OH 45268.

SAMPLING IN WASTEWATER TREATMENT OPERATIONS

I INTRODUCTION

A A sample is defined as a representative part, aliquot or single item from a larger group or whole to be presented for inspection or testing for quality, also, a portion of a statistical population whose characteristics are studied to gain information about the whole.

B A sample representing materials at a given time, place and flow may be called a "catch" or "grab" sample. A series of grab samples obtained over a specified period of time from the sample flow becomes a "composite" sample when all individual samples are combined into one. If the individual grab sample volumes are adjusted in proportion to flow at the time they were obtained and before combining them the resulting mixture is a "proportional composite."

C Sampling requires careful observance of procedures to be meaningful.

1 The scale up factor from the sample to the whole commonly is very large. Behavior of a 1 ml sample of the incoming flow in a BOD dilution may be used to estimate loading, a 0.001 ml sample may be used for estimation of membrane filter coliform group organisms for many million gallons of flow.

2 The sampler is obligated to insure that his sample does represent the material sampled and is protected from inadvertent changes. Proper attention to detail and identification is imperative.

3 Grab samples of flow into and out of process are likely to give misleading information if process detention time has been ignored. If both are obtained at about the same time a mid morning influent sample may represent peak loading while the effluent sample represents what entered the plant at minimum load period.

D Changes are the principal reason for sampling and testing for evaluation of treatment performance.

1 Loading may be expected to change by the hour, day, season and population activity both in characteristics and concentration. The long term trend is upward.

2 Water use and requirements change.

3 Physical facilities tend to change with time and use.

4 Operating personnel, capabilities, numbers and motivation change with budgets, organization and public interest.

E Sampling and testing are used to·

1 Estimate work done or remaining to be done.

2 Identify problems.

3 Evaluate problems and response for planning, process techniques or equipment selection, and operational competence purposes.

4 To evaluate compliance with requirements.

F Sampling is the initial step in a complex series such as inspection testing interpretation and active response to information gathered. Many factors affect situation dynamics such as objectives, probabilities, situations, time, money, items to be sampled and particular needs. Manpower capabilities, skills, background, motivation and bias have a large effect upon usefulness of information gathered.

1 There are no fixed rules of sampling applicable to every type of material, item sought situation or purpose.

2 Sampling must be coordinated among those who have or can secure and those who need information. Each must be aware of and respond consistently to site selections, scheduling, techniques and communication to be effective.

3 Integrity and reliability of all personnel concerned are essential. Any lapse in consistency results in loss of control time money with concurrent suspicions with respect to validity of subsequent actions.

G This outline reviews selected factors pertinent to sampling. It is not intended to be complete because situations and needs differ. References (1, 2, 3) cite specifics for each type of item to be analyzed. Each regulatory agency selects certain items from standard compendia that are important to their interest and to those in their jurisdiction. This outline stresses considerations or judgment factors. Authorities specify procedures.

II SAMPLING OBJECTIVES:

Samples are obtained because someone needs precise information about some material, situation or action. Constraints and requirements for sampling and testing depends upon who needs information, what kind, and why. Cost commonly modifies the action.

A Routine Record Sampling Reports:

The major wastewater treatment plant sampling effort commonly is associated with periodic reports to supervisors or regulatory agencies. Sites, frequency, techniques and handling usually are prescribed specifically for the situation by management and/or consultants according to requirements of the regulatory agency. Requirements vary depending upon:

1 Discharge or receiving water quality requirements.

2 Size of the facility.

3 Discharge location in relation to critical reuse of the receiving water.

4 Manpower budget and complexity of treatment.

5 Load characteristics, variation, and their relationships to design loading.

6 Public interest and activity

7 Enforcement

B Process Control Sampling:

Control sampling is intended to estimate what is likely to happen in plant performance rather than what has happened as in routine operating reports. Both record and control sampling and testing are used for performance control. The emphasis for control sampling and testing is to recover information in time for corrective action before little problems become big problems.

1 Control sampling usually is used to characterize in-process variables with respect to stage of completion, environmental condition or rate of change.

2 Control sampling and testing are used to provide rapid estimates of process variables that are relateable to treatment performance and to suggest corrective action when atypical values are evident. Useability is more important than high precision and accuracy.

3 Control information includes sensory impressions, meter readings, biological, chemical or physical criteria. The sampler is expected to note any unusual conditions that may be useful for interpretation of results.

4 Control applications, nature, extent of sampling and testing vary with the treatment and requirements and with staff personnel customs, capabilities, background, motivation and working budget.

C Sampling for Enforcement Purposes:

Much more attention to detail is required for legally acceptable sampling for courtroom procedures than for most other sampling operations.

1 Professionally and legally accepted methods by recognized capable trained personnel are mandatory.

2 Documentation is essential for the history of the sampling operation and subsequent handling. A signed record of time, place, conditions and sample identification number must be logged. The sample requires sealing and tagging for identification and record of who did what and when. Storage and transfer history by responsible individuals is essential.

3 Precise identification procedures, and protection from tampering are main sampling considerations. Any fact of sample history is subject to recall by the court.

4 Careful review of procedures and legal guidance with respect to specifics of the given jurisdiction are important to enhance possibilities for information acceptance by the court.

D Complaint Follow-up·

This usually means a special purpose inspection and possible sampling to evaluate the complainant's claim(s) and to guide subsequent action. The complaint may refer to routine or nonroutine characteristics in the collection system treatment plant or receiving water. Prompt and knowledgeable handling in an honest and diplomatic manner including feedback of communication to the complainant through channels are good public relations.

E Treatment Plant Design Data

Much of the information essential for design purposes is available from previous records. Sampling and testing to check routine data and to evaluate unusual or difficulty treatable wastewater mixtures are essential to provide a versatile and reliable facility capable of meeting foreseeable treatment requirements.

1 Loading amount and characteristics including anticipated new contributions and their effects are pertinent.

2 Selection of new processes or new equipment and their performance under conditions similar to that anticipated commonly require sampling and testing.

3 Sampling and testing to meet upgraded performance requirements with supplementary or alternate treatment are indicated.

F Research and Development·

Investigation of new schemes, recombinations of established operations, upgrading treatment with respect to conventional removals or the removal new or different contaminants require a more comprehensive sampling and testing effort than any subsequent application of the same nature. Establishment of guidelines for methodology and interpretation are essential for the different situation.

1 Sampling techniques and frequency for that situation may be different depending on feed or process variability.

2 New interferences may be encountered that require different handling techniques in sampling or testing.

3 Sampling and testing are intended to minimize surprises that may reduce cost effectiveness during subsequent applications.

III SAMPLING PRECAUTIONS BASED UPON ITEMS TO BE SAMPLED

Biological, chemical or physical criteria have their own characteristics and tendencies to change. The sampler should apply suitable techniques to obtain a representative portion of the intended material and to minimize changes before the assay.

A Rapidly reacting chemicals may hydrolyze, oxidize, form addition substitution or complexed products different from those

existing at the time of sampling. High rates of component reaction in the sample always complicates sampling precautions and may require the sampler to become an analyst.

1 Storage at low temeratures is common practice for decreasing sample reaction rates. Freezing may be advisable.

2 Biological changes may be reduced by adjusting the sample pH to unfavorably high or low values, or by addition of toxic chemicals.

3 Sample component hydrolysis such as formation of free fatty acids and ammonia from more complex materials is difficult to control. Low temperatures reduce the rate but added preservatives may increase hydrolysis. Prompt determination of unmodified fresh sample material is the best choice in these situations.

4 Chemical addition to convert the item sought into a less reactive derivative may be used effectively such as using zinc salts to enhance possibilities for recovery of sample hydrogen sulfide.

5 Any modification of sample components by addition or other treatment must not interfere with subsequent determinations or alter the quantities of the items sought. The sampler is obligated to use accepted sample modifications only and to clearly identify treatment provided for guidance in testing and interpretation.

B Gas - liquid sampling presents troublesome situations because gas solubility varies with pressure. If the gas is highly reactive such as chlorine, HCN, CO_2, H_2S, O_2, or NH_3 the problems are magnified.

1 Sampling profiles from top to bottom in tanks means that each 2 feet of depth is associated with a pressure increase of about 1 p.s.i. Gases such as carbon dioxide are much more soluble with increasing depth, CO_2 also tends to be produced in active solids suspensions or deposits. The pH in treatment plant systems changes rapidly with CO_2, HCO_3^- and $CO_3^=$ dynamics. Any pressure reduction or turbulence at reduced pressure enhances loss of CO_2 and concurrent rise in pH of the displaced sample. Electronic sensors and in place measurement of pH permits rapid readout without pressure change effects upon results.

2 Dissolved oxygen solubility varies with temperature and pressure. It also tends to be rapidly used in active systems and may be depleted in deposit vicinities. An oxygen deficient sample rapidly increases in oxygen content upon contact with air. In-place measurement by electronic sensors avoids possible changes due to pressure and reaction.

3 Sampling controls including pressure, chemical fixation, protection from exposure etc., are possible. Chemical fixation such as acid treatment to retain ammonia as ammonium hydrogen sulfate, alkali treatment to retain HCN as sodium cyanide and others have a long history of common use. Elaborate apparatus are available for DO sampling to flush the sample bottle and to minimize subsequent exposure to the air. The apparatus tends to become too elaborate for common use if pressure control is built in.

4 The sampler has become accustomed to measurement of temperature in place and now because he knows that it will change. The treatment plant sampler will perform more effectively if he learns to use additional remote sensors where possible particularly where dissolved gases are concerned.

C Sampling Suspended Materials:

The sampler faces real problems in sampling water containing a conglomerate of operationally defined suspended solids. He is expected to manage representative sampling of a non-homogenous mixture containing items such as organisms, detritus, fibers, grease and chunks of inorganic or organic residues with a wide range of sizes, densities, shapes, and behavior.

1 To be considered as suspended (particulate) material, the mixture must be dispersed in water and tend to move with it. Dissolved materials and rocks are excluded but the suspended material invariably includes dissolved materials, greases, oils, organisms etc. that are mechanically entrapped, adsurbed or otherwise associated with the solids. Suspended solids usually have an additional arbitrary limitation based upon size or ease of separation from water. For example, it is called "grit" if it separates from water suspension within about one minute at a flow velocity of about 1 ft/sec. Chunks, chips and sticks are intended to be removed from the sample.

2 Mixing of the main body of material or flow is essential to disperse components adequately for representative sampling. This requires particular care for raw wastewaters because many contained items tend to slide along the bottom or float at usual flow velocities. Mixing may be performed by a pump, drop box, baffling or other mixing device and the sample site located accordingly.

3 Clearing and cleanup of manual or automatic sample lines and devices are essential. Flushing the lines to obtain free flow before sampling is particularly important for sludge lines because of grease, fibers, sticks etc. in the flow that may partially plug lines.

4 Frequency of sampling depends upon variability if the item tested, requirements of the needed information, money and means to do it. Sludge characteristics may be so variable that it is advisable to sample at 5 minute intervals and composite the series to effectively estimate material pumped.

5 Clarified or stabilized wastewaters are easier to sample because the particulates remaining tend to be more uniformly dispersed. The stabilized wastewaters are easier to sample because the particulates remaining tend to be more uniformly dispersed. The stabilized sample has been equalized and treated in process to decrease the rate of change.

6 Solids have a tendency to change. Dissolved solids may be converted to suspended solids by growth, complexation, sorption or precipitation. Fine particulates tend to become larger particulates by coagulation and agglomeration. Conversely large particulates may be dissolved or dispersed by mixing hydrolysis etc. Cold storage is the usual means of decreasing change rate on samples for solids classification and quantitation. Settling rates should be tested promptly on fresh samples.

D Sampling for Pathogen Determinations

Health hazards related to the likely presence of disease producing organisms in wastewater are a major concern. The sampler is concerned with specific routines characteristic of the particular test organism group, water mass to be sampled and use to be made of the acquired information.

1 Clean sampling containers are required in all sampling routines, in this case, they must also be sterile. Special precautions are required to protect the sample from introduction of extraneous organisms introduced during the sampling operation or subsequent handling.

2 Samples may be stored or transported to the test site under low temperature conditions. No other treatment affecting growth or death rate of the sample organisms is permitted.

3 Time lapse between sampling and determination is critical. Best results are obtained for holding times of less than six hours.

E Other Aquatic Organism Sampling:

Relationships of families of organisms and habitats lead to inferences about conditions depending upon the number and variety of significant organisms

in the system. The sampler may be concerned with identification, relative numbers, varieties, predominant groups, selectivity, nutritional status, net growth, environmental conditions, or activities of a biological, chemical or physical nature. Usually treatment plant objectives limit concern to environmental conditions, growth rates, selectivity or activities that are pertinent to treatment plant conditions and can be observed or measured effectively.

1 Biomonitoring, oxygen use rates, growth rates and dynamics of organism or substrate selection necessarily require fresh samples under favorable environmental conditions.

2 Net changes, accumulated cell mass and residue accumulation may be preserved and tested later.

3 Frequent and varied sampling and testing of environmental conditions are essential to permit interpretations of behavior. Toxicity information on a test material lacks validity if later information suggests that oxygen starvation may have been a factor.

4 Direct observation on fresh process samples of selected indicator organisms commonly found under favorable performance conditions are useful. Sudden disappearance or inactivity of the usual critters suggest drastic changes in conditions or process.

IV SUMMARY:

A The sampler is obligated to follow prescribed sampling sites, methodology, scheduling and routing of samples agreed upon for his situation.

B Sampling integrity is essential. The sample should be clearly identified and represent the material to be sampled at the time and place designated. Any unusual conditions on site that may affect results should be noted in the sample record. The sample should be protected from tampering. Preservatives, storage conditions, and routine or special handling should be as specified and part of the record.

C Sampling information in this outline is deliberately selective and incomplete for guidance considerations. References cited include much more detail concerning sampling and sample handling depending on sampled material, determinations to be made, and use of the information. It must be noted that any "Standard" methods compendium includes professionally recognized and recommended procedures including certain alternates and options. These do not become legally recognized methods until accepted by statute or by the designated statutory authorities, rules and regulations for their jurisdiction. It is necessary to determine which of the possible options are acceptable within a given jurisdiction.

REFERENCES:

1 Standard Methods. Water and Wastewater 13th Edition 1971 APHA, AWWA, WPCF.

2 1971 Annual Book of ASTM Standards, Part 23 Water: Atmospheric Analysis. American Society for Testing and Materials.

3 Methods for Chemical Analysis of Water and Wastes 1971. USEPA, Office of Water Quality, Analytical Quality Control Laboratory.

4 Rules and Regulations of the Water Quality Enforcement Agency(s) including your area of operations.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, Ohio 45268

LABORATORY HOUSEKEEPING

I Housekeeping in the general sense refers to the management of a house and home and the provision of equipment and services for general welfare.

A Laboratory housekeeping places a much greater emphasis upon organization of facilities, equipment, and utensils into an effective service operation.

1 Glassware must be effectively cleaned as required and stored for use in an orderly and accessible manner.

2 Equipment should be maintained in effective working order, calibrated regularly, and operated with suitable respect for specific capabilities and limitations.

3 Facilities should be organized in line with conventional work loads.

4 Solutions and reagents should be precisely identified, stored in an orderly fashion considering stability or contamination, and replaced as necessary.

5 Schedule the operational routine with requirements so that first things are first.

6 Locate unit or allied operations so that they can be performed with a minimum of travel, lost time, or relocation of needed items.

7 Schedule cleanup regularly to be ready for analytical operations when samples arrive.

8 Organize record operations for prompt and easy accessibility when required.

II Facilities and equipment arrangement may represent the difference between orderly and hectic performance.

A Sinks, incubators, cold storage, benches, wash-up, hoods, etc., are likely to be fixed.

1 The problem is to arrange movable items around these to best advantage.

2 Equipment for the BOD test should be centered near a sink and suitable drainboard with items used frequently stored in the vicinity. Operations such as filtration or microbial tests should be suitably arranged in separate locations.

3 A laboratory cart saves time by providing a moveable platform for samples and a place for temporary storage of glassware to or from the job.

4 Electronic instruments such as the pH meter, balance, spectrophotometers should be located away from major wet operations with line voltage control if necessary.

5 Some bench space should be reserved for non-routine operations.

6 Storage of glassware should be provided near the main line of traffic with most commonly used items in the favored spots for access. Supplies should be maintained adequate to avoid washup stops during determination sessions.

7 Chemicals preferably should be stored near the balance table in closed cabinets except for special precaution items such as volatile of flammable items.

8 Prepared reagents or solutions used in determinations should be stored near the point of use.

9 Each item should have its place and be returned to that location after use.

10 An inventory control of some sort is essential to maintain chemicals, solutions and glassware ready for use when it is needed. The analyst will know or can determine requirements for routine use. Be sure that it is there beforehand.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, Water Programs Operations, Environmental Protection Agency, Cincinnati, OH 45268.

VARIATIONS IN TEST RESULTS

I INTRODUCTION

In any type of analytical work, the analyst should ask the question "how well am I doing?" or "am I getting the right results?" It is not enough that these questions should be continually asked, but they should also be continually answered. The use of an organized approach to answering these questions is known as a quality control procedure. It will be the purpose of this discussion to point out some of these procedures which can be applied in the chemistry laboratory.

II FUNDAMENTAL CONCEPTS

A Accuracy

For results to be accurate, the analysis used must give values close to the true value (see Figure 1). Errors which introduce bias into results cause them to be inaccurate. Such errors can be given an "assignable cause" and are known as determinate errors. There are three types of determinate errors.

1 Equipment errors

An error of this type could be the use of a buret which is incorrectly calibrated.

2 Method error

The most common type of error is that associated with the presence of interferences in the sample. A low or high yield factor for a desired constituent may need to be developed.

3 Personal errors

These errors are attributable to individual mistakes which are consistently made by an analyst (e.g., not rinsing a buret with a standard solution prior to titration).

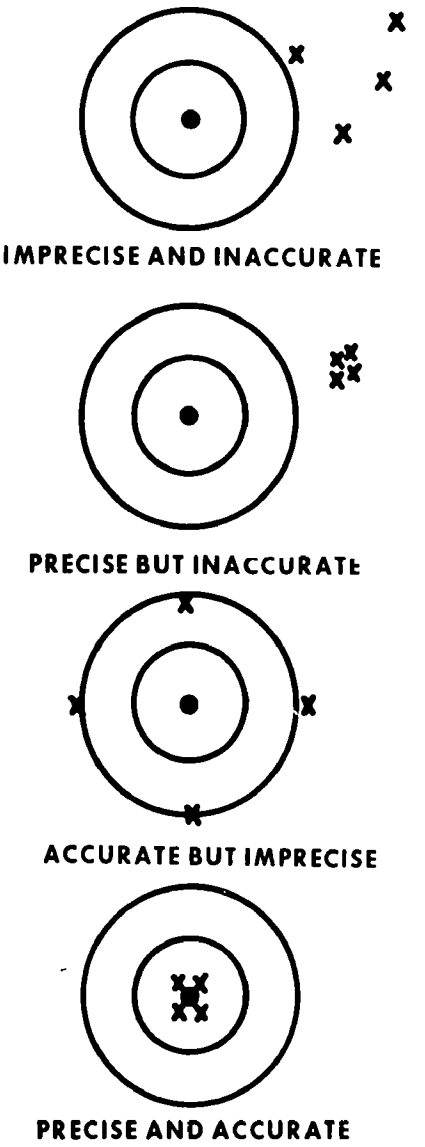

Figure 1. PRECISION AND ACCURACY

B Precision

Precision is the degree of agreement among results obtained by repeated measurements on a single sample under a given set of conditions. In other words, it is a measure of the degree to which results "check." Even when all determinate errors are eliminated, every replicate analysis will not give the same value. Errors which introduce such variation into results are due to "chance causes" (e.g., variation in reagent addition, instrument response, inadvertent contamination of sample or glassware, etc.). These errors are known as indeterminate or random errors.

III EVALUATION OF ACCURACY

A Spiked Samples

1 Samples which can be determined before and after the addition of a known constituent (in the concentration range of interest) provide a way to detect determinate errors. Spiked samples should be representative and resemble actual conditions as closely as possible. The quantitation of bias can then be obtained with the following measures.

a Mean error - the difference between the mean of the data and the true result.

b Relative error - the mean error of a set of data expressed as a percentage of the true result.

EXAMPLE: An analyst determines the nitrate content of the effluent from his sewage treatment plant to be 0.5 mg/l. He then adds 1 mg/l of standard nitrate solution to the sample. Table 1 shows the replicate results obtained on the spiked sample.

Table 1

Sample: Effluent
Determination: Nitrate (Modified Brucine)

X_1
1.55 mg/l
1.58
1.60
1.47
1.35

1 Calculation of mean error

$$\bar{X} = \frac{7.55}{5} = 1.51 \text{ mg/l}$$

2 Calculation of relative error

$$\text{Relative Error} = \frac{0.01 \times 100}{1.50} = 0.7\%$$

2 In some cases, it is impossible to spike a sample so that it resembles actual conditions (e.g., BOD and pesticide samples). Youden[5] provides excellent techniques for detecting bias in this situation.

B Independent Method

Analysis of a sample for a desired constituent by two or more methods that are entirely different in principle (gravimetric and volumetric) may aid in the estimation of determinate error.

IV EVALUATION OF PRECISION

A measure of the degree of agreement among results can be obtained by analyzing a single sample repeatedly under a given set of conditions.

A Range

The range of the replicate results (difference between the lowest and the highest value) provides a measure of indeterminate variations.

B Standard Deviation

1 Since these indeterminate variations conform to the laws of statistical distributions the standard deviation of the replicates also can be used as a measure.

$$s = \sqrt{\frac{\Sigma (X_1 - \bar{X})^2}{n - 1}} \qquad (1)$$

X_1 = value of single result

$\bar{X}$ = average of results on same sample

n = number of results

Example: Table 2 contains a set of 5-day BOD results obtained on a synthetic sample containing 150 mg/l of glucose and 150 mg/l glutamic acid. (Note: A 1% dilution was used in the actual test). The results are those submitted by laboratories participating in a collaborative study. Calculate the standard deviation of these results

$$\bar{X} = 192 \text{ mg/l}$$

$$n = 36.0$$

$$\Sigma (X_1 - \bar{X})^2 = 58,200$$

$$s = \sqrt{\frac{58,200}{35}}$$

$$s = 41 \text{ mg/l}$$

2 For a small number of replicates ($n < 10$), the range can be used to estimate the standard deviation (see Table 3).

Table 2*

SAMPLE 150 mg/l glucose + 150 mg/l glutamic acid (1% dilution)
DETERMINATION 5-day Biochemical Oxygen Demand

X_1	$X_1 - \bar{X}$	$(X_1 - \bar{X})^2$	X_1	$X_1 - \bar{X}$	$(X_1 - \bar{X})^2$
100 mg/l	-92 mg/l	8464 $(mg/l)^2$	198 mg/l	6 mg/l	36 $(mg/l)^2$
117	-75	5625	199	7	49
125	-67	4489	200	8	64
132	-60	3600	200	8	64
142	-50	2500	204	12	144
147	-45	2025	210	18	326
153	-39	1521	211	19	361
160	-32	1024	212	20	400
165	-27	729	215	23	529
165	-27	729	223	31	961
167	-25	625	224	32	1024
173	-19	361	227	35	1225
178	-14	196	229	37	1369
189	- 3	9	238	46	2116
190	- 2	4	247	55	3025
196	4	16	250	58	3344
196	4	16	259	67	4489
197	5	25	274	82	6724

*Data taken from Water, Oxygen Demand Report (July, 1960), Analytical Reference Service, Training Program, R.A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

$$\frac{R}{d_N} = s \qquad (2)$$

Example: Table 4 contains a set of replicate nitrate results. Calculate the standard deviation of these results.

a Use of formal (1)

$X = 0.72$ mg/l

$n = 5$

$\Sigma (X_i - \bar{X})^2 = .0134$

$$s = \sqrt{\frac{.0134}{4}}$$

$s = .058$ mg/l

b Use of formula (2)

$R = .14$ mg/l

$d_N = 2.33$

$$s = \frac{.14 \text{ mg/l}}{2.33}$$

$s = .060$ mg/l

Table 3*

Factors Used to Estimate the Standard Deviation from Range

Size of sample (n)	d_N	$\frac{1}{d_N}$
2	1.13	.887
3	1.69	.391
4	2.06	.486
5	2.33	.430
6	2.53	.395
7	2.70	.370
8	2.85	.351
9	2.97	.337
10	3.08	.325

*Natrella, Experimental Statistics, pp. 2-6.

Table 4

Sample: Ohio River Water
Determination: Nitrate (Modified Brucine)

X_i	$X_i - \bar{X}$	$(X_i - \bar{X})^2$
0.65 mg/l N	-.07	.0049
0.68	-.04	.0016
0.70	-.02	.0004
0.76	+.04	.0016
0.79	+.07	.0049

C Relative Standard Deviation

The standard deviation expressed as a percentage of the mean is known as the relative standard deviation.

Example. Table 2

$s = 41$ mg/l

$\bar{X} = 192$ mg/l

$$\text{Relative Standard Deviation} = \frac{41 \times 100}{192}$$

$= 21\%$

V CONCLUSION

The concepts of precision and accuracy and their evaluation have been presented.

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ANALYTICAL REACTIONS - STANDARD SOLUTIONS

I INTRODUCTION

A A standard solution is one whose composition and concentration are known to a high degree of accuracy. In chemical analyses, it is used to determine the concentration of a particular component in a sample.

B These chemical analyses very frequently involve an acid-base reaction or an oxidation-reduction reaction.

C Three important terms connected with these two types of chemical reactions are· mole, equivalent weight, and normality. These terms will be defined in following sections.

II ANALYTICAL CHEMICAL REACTIONS

A As stated above, in a chemical analysis a volume of standard solution is brought into contact with a volume of sample in order to determine the concentration of some particular component of the sample.

B For a given volume of sample, it is necessary to use a definite amount of the standard solution--too much or too little would give erroneous results.

C For example, one cannot simply mix together random volumes of sodium hydroxide and hydrochloric acid solutions and expect the only two substances produced to be sodium chloride and water.

D Unless the concentrations of the two reagents are known and the amounts measured accurately, excess sodium hydroxide or hydrochloric acid will also remain at the end of the reaction.

E The reason for these limitations is that when molecules react with one another, they do so in definite ratio. Unless the number of molecules of each reactant is known, there will always be an excess of one of the reactants remaining at the end of the chemical reaction. As mentioned before, this leads to erroneous analytical results.

F Because of their size, it is not possible to count out numbers of molecules. However, the number of molecules in a quantity of a chemical may be found by determining its weight and consulting a table which lists the weights of the atoms making up the chemical.

G For example, sodium hydroxide has the formula NaOH. It can also be stated that a molecule of sodium hydroxide consists of one sodium atom, one hydrogen atom and one oxygen atom.

One sodium atom weighs 23 atomic mass units (amu). An oxygen atom weighs 16 amu; and a hydrogen atom weighs 1 amu.

A molecule of sodium hydroxide, therefore, weighs 40 amu; all amu values have been rounded off. Forty amu is the molecular weight of sodium hydroxide.

H A mole of any chemical is a number of grams numerically equal to the molecular weight of that chemical. One mole of sodium hydroxide, therefore, contains 40 grams (40 g).

I Similarly, the atomic weight of a chlorine atom is 35 amu; that of a hydrogen atom is 1 amu, and the molecular weight of the hydrogen chloride molecule is 36 amu. One mole of hydrogen chloride weighs 36 g.

J Synonyms for mole are: mol, gram mol, gram mole, and gram molecular weight.

K Tables listing atomic weights of the elements can be found in virtually all

of the texts used for high school and first year college chemistry courses.

L The remaining two terms mentioned in I C (equivalent weight and normality) will be considered as they are related to acid-base and oxidation-reduction reactions.

III ACID-BASE REACTIONS

A Recall that an acid is a substance which donates a hydrogen ion, or proton, (H^+) in a chemical reaction and a base is a substance which donates a hydroxide ion (OH^-) in a chemical reaction. Reactions involving these two ions are termed acid-base reactions.

B In the case of sodium hydroxide, the molecular weight is 40 amu and one mole of sodium hydroxide weighs 40 g. One hydroxide ion is contained in the sodium hydroxide molecule.

1 For a base, the number of grams in a mole divided by the number of hydroxide ions equals a quantity called the equivalent weight. Examples are given below. All amu values have been rounded off.

a Base - potassium hydroxide KOH

Atoms	Number	Wt/atom	Total
K	1	39 amu	39 amu
O	1	16	16
H	1	1	1
			56 amu

One mole of KOH = 56 g
Number of hydroxide ions = 1
Equivalent weight of KOH = 56 g

b Base - magnesium hydroxide $Mg(OH)_2$

Atoms	Number	Wt/atom	Total
Mg	1	24 amu	24 amu
O	2	16	32
H	2	1	2
			58 amu

One mole of $Mg(OH)_2$ = 58 g
Number of hydroxide ions = 2
Equivalent weight of $Mg(OH)_2$ = 29 g

2 For an acid, the number of grams in a mole divided by the number of hydrogen ions is the equivalent weight. Examples are given below. All amu values have been rounded off.

a Acid - nitric acid HNO_3

Atoms	Number	Wt/atom	Total
H	1	1 amu	1 amu
N	1	14	14
O	3	16	48
			63 amu

One mole of HNO_3 = 63 g
Number of hydrogen ions = 1
Equivalent weight of HNO_3 = 63 g

b Acid - sulfuric acid H_2SO_4

Atoms	Number	Wt/atom	Total
H	2	1 amu	2 amu
S	1	32	32
O	4	16	64
			98 amu

One mole of H_2SO_4 = 98 g
Number of hydrogen ions = 2
Equivalent weight of H_2SO_4 = 49 g

C Normality is a method of expressing solution concentrations. If one equivalent weight of a chemical is dissolved in a solvent and the volume brought to one liter (l), the concentration of the solution is one normal (N).

1 The equivalent weight of KOH was calculated to be 56 g. This amount of the solid dissolved in water and diluted to a liter would give a 1 N solution.

2 The equivalent weight of HNO_3 was found to be 63 g. This quantity of acid diluted to a liter would give a 1 N solution.

IV OXIDATION-REDUCTION REACTIONS

A The concepts of mole, equivalent weight and normality, as described in previous sections, apply also to oxidation-reduction reactions.

B One definition of an oxidation is that it involves an increase in the oxidation state (charge) of an atom.

For example: $FeCl_2 \rightarrow FeCl_3$. In this conversion the Fe has been oxidized from +2 to +3.

C A reduction is a decrease in the oxidation state (charge) of an atom.

For example: $KMnO_4 \rightarrow MnO_2$. In this conversion the Mn has been reduced from +7 to +4.

D The equivalent weight of an oxidizing or reducing agent is calculated by dividing the number of grams in a mole of the reagent by the change in charge involved.

For example: $FeCl_2$ (used as a reducing agent).

Atoms	Number	Wt/atom	Total
Fe	1	56 amu	56 amu
Cl	2	70	70
			126 amu

One mole of $FeCl_2$ = 126 g
Change in charge = 1
Equivalent weight of $FeCl_2$ = 126 g

The concentration of a liter of solution which contains 126 g of $FeCl_2$ is 1 N.

V PRIMARY STANDARDS

A A reagent of known purity is called a primary standard. Primary standard grade chemicals are available from chemical supply houses and the National Bureau of Standards. An accurately measured quantity of a primary standard is used for the preparation of standard solutions.

B Other requirements of a primary standard are:

1 It must be stable at 105° C (the temperature used for drying).

2 It should not be reactive with components of the air, such as O_2 and CO_2.

3 It should have a high equivalent weight so as to minimize any errors in the analysis.

4 It should be readily available at a reasonable cost.

VI STORAGE OF STANDARD SOLUTIONS

A Standard solutions should be prepared using high quality distilled water.

B Care should be taken to insure the cleanliness of the glass or plastic bottle used for storage.

C Some solutions may decompose on exposure to light and should be stored in dark bottles.

D The stopper or cap should fit tightly.

VII CALCULATIONS

A The basic formula used in volumetric analysis is

$$(l \times N) \text{ of standard solution} = (l \times N) \text{ of sample}$$

In a typical analysis, three of the four quantities are known, or found, and

$$N \text{ of sample} = \frac{(l \times N) \text{ of standard}}{l \text{ of sample}}$$

B This formula can be rearranged to give

$$g = l \times N \times \text{equivalent weight}$$

where g and equivalent weight (ew) refer to the component being analyzed and l and N to the standard solution.

C For example: How many g of NaOH are present in a sample if 100.0 ml of 0.2 N HCl are required for its titration?

$$g = l \times N \times \text{equivalent weight} = \frac{100.0 \text{ ml}}{1000.0 \text{ ml/l}} \times 0.2 \times \frac{40.0}{1} = 0.8$$

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III THE PHYSICAL NATURE OF WATER

Water has many properties which are unusual for liquids, upon which depend most of the familiar aspects of the world about us.

Contents of Section III

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The Aquatic Environment Part 1:	7
The Physical Nature of Water and Waters	

THE AQUATIC ENVIRONMENT

Part 1· The Nature and Behavior of Water

I INTRODUCTION

The earth is physically divisible into the lithosphere or land masses, and the hydrosphere which includes the oceans, lakes, streams, and subterranean waters.

A Upon the hydrosphere are based a number of sciences which represent different approaches. Hydrology is the general science of water itself with its various special fields such as hydrography, hydraulics, etc. These in turn merge into physical chemistry and chemistry.

B Limnology and oceanography combine aspects of all of these, and deal not only with the physical liquid water and its various naturally occurring solutions and forms, but also with living organisms and the infinite interactions that occur between them and their environment.

C Water quality management, including pollution control, thus looks to all branches of aquatic science in efforts to coordinate and improve man's relationship with his aquatic environment.

II SOME FACTS ABOUT WATER

A Water is the only abundant liquid on our planet. It has many properties most unusual for liquids, upon which depend most of the familiar aspects of the world about us as we know it.

TABLE 1

UNIQUE PROPERTIES OF WATER

Property	Significance
Highest heat capacity (specific heat) of any solid or liquid (except NH_3)	Stabilizes temperatures of organisms and geographical regions
Highest latent heat of fusion (except NH_3)	Thermostatic effect at freezing point
Highest heat of evaporation of any substance	Important in heat and water transfer of atmosphere
The only substance that has its maximum density as a liquid (4°C)	Fresh and brackish waters have maximum density above freezing point. This is important in vertical circulation pattern in lakes.
Highest surface tension of any liquid	Controls surface and drop phenomena, important in cellular physiology
Dissolves more substances in greater quantity than any other liquid	Makes complex biological system possible. Important for transportation of materials in solution.
Pure water has the highest di-electric constant of any liquid	Leads to high dissociation of inorganic substances in solution
Very little electrolytic dissociation	Neutral, yet contains both H^+ and OH^- ions
Relatively transparent	Absorbs much energy in infra red and ultra violet ranges, but little in visible range. Hence "colorless"

B Physical Factors of Significance

1 Water substance

Water is not simply "H_2O" but in reality is a mixture of some 33 different substances involving three isotopes each of hydrogen and oxygen (ordinary hydrogen H^1, deuterium H^2, and tritium H^3, ordinary oxygen O^{16}, oxygen 17, and oxygen 18) plus 15 known types of ions. The molecules of a water mass tend to associate themselves as polymers rather than to remain as discrete units. (See Figure 1)

2 Density

a Temperature and density Ice. Water is the only known substance in which the solid state will float on the liquid state. (See Table 2)

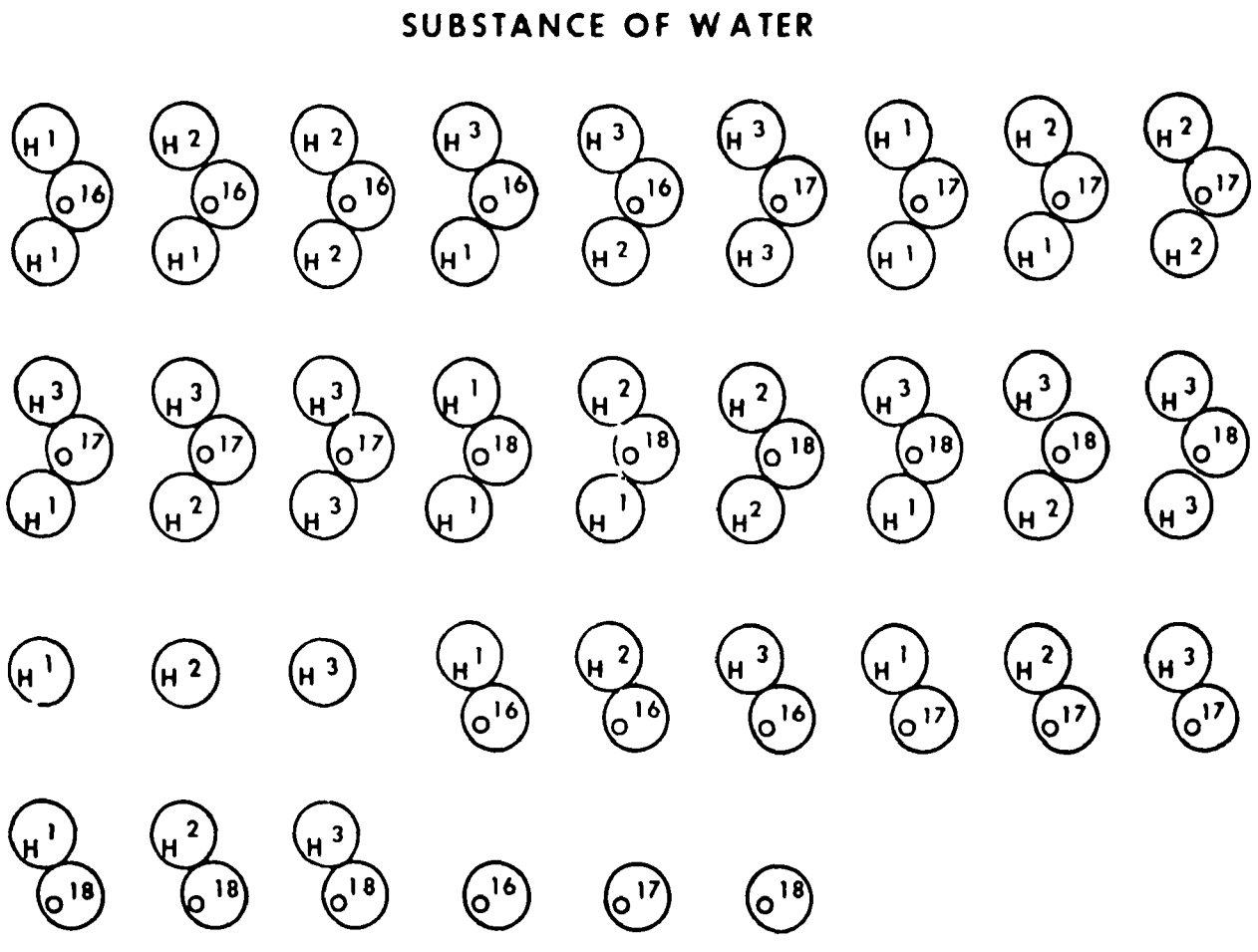

Figure 1

TABLE 2

EFFECTS OF TEMPERATURE ON DENSITY OF PURE WATER AND ICE*

Temperature (°C)	Density	
	Water	Ice **
-10	.99815	.9397
- 8	.99869	.9360
- 6	.99912	.9020
- 4	.99945	.9277
- 2	.99970	.9229
0	.99987----	.9168
2	.99997	
4	1.00000	
6	.00997	
8	.00988	
10	.00973	

* Tabular values for density, etc., represent statistical estimates by various workers rather than absolute values, due to the variability of water.

** Regular ice is known as "ice I". Four or more other "forms" of ice are known to exist (ice II, ice III, etc.), having densities at 1 atm. pressure ranging from 1.1595 to 1.67. These are of extremely restricted occurrence and may be ignored in most routine operations.

This ensures that ice usually forms on top of a body of water and tends to insulate the remaining water mass from further loss of heat. Did ice sink, there could be little or no carryover of aquatic life from season to season in the higher latitudes. Frazil or needle ice forms colloidally at a few thousandths of a degree below 0° C. It is adhesive and may build up on submerged objects as "anchor ice", but it is still typical ice.

1) Seasonal increase in solar radiation annually warms surface waters in summer while other factors result in winter cooling. The density differences resulting establish two classic layers. the epilimnion or surface layer, and the hypolimnion or lower layer, and in between is the thermocline or shear-plane.

2) While for certain theoretical purposes a thermocline is defined as a zone in which the temperature changes one degree centigrade for each meter of depth, in practice, any transitional layer between two relatively stable masses of water of different temperatures (and probably other qualities too) may be regarded as a thermocline.

3) Obviously the greater the temperature differences between epilimnion and hypolimnion and the sharper the gradient in the thermocline, the more stable will the situation be.

4) From information given above, it should be evident that while the temperature of the hypolimnion rarely drops much below 4° C, the epilimnion may range from 0° C upward.

5) It should also be emphasized that when epilimnion and hypolimnion achieve the same temperature, stratification no longer exists, and the entire body of water behaves hydrologically as a unit, and tends to assume uniform chemical and physical characteristics. Such periods are called overturns and

usually result in considerable water quality changes of physical, chemical, and biological significance.

6) When stratification is present, however, each layer behaves relatively independently, and considerable quality differences may develop.

7) Thermal stratification as described above has no reference to the size of the water mass; it is found in oceans and puddles.

8) The relative densities of the various isotopes of water also influence its molecular composition. For example, the lighter O_{16} tends to go off first in the process of evaporation, leading to the relative enrichment of air by O_{16} and the enrichment of water by O_{17} and O_{18}. This can lead to a measurably higher O_{18} content in warmer climates Also, the temperature of water in past geologic ages can be closely estimated from the ratio of O_{18} in the carbonate of mollusc shells.

b Dissolved and/or suspended solids may also affect the density of natural waters.

TABLE 3
EFFECTS OF DISSOLVED SOLIDS ON DENSITY

Dissolved Solids (Grams per liter)	Density (at 4° C)
0	1.00000
1	1.00085
2	1.00169
3	1.00251
10	1.00818
35 (mean for sea water)	1.02822

c Density caused stratification

1) Density differences produce stratification which may be permanent, transient, or seasonal.

2) Permanent stratification exists for example where there is a heavy mass of brine in the deeper areas of a basin which does not respond to seasonal or other changing conditions.

3) Transient stratification may occur with the recurrent influx of tidal water in an estuary for example, or the occasional influx of cold muddy water into a deep lake or reservoir.

4) Seasonal stratification involves the annual establishment of the epilimnion, hypolimnion, and thermocline as described above. The spring and fall overturns of such waters materially affect biological productivity.

5) Density stratification is not limited to two-layered systems; three, four, or even more layers may be encountered in larger bodies of water.

d A "plunge line" may develop at the mouth of a stream. Heavier water flowing into a lake or reservoir plunges below the lighter water mass of the epiliminium to flow along at a lower level. Such a line is usually marked by an accumulation of floating debris.

2 The viscosity of water is greater at lower temperatures (see Table 4). This is important not only in situations involving the control of flowing water as in a sand filter, but also since overcoming resistance to flow generates heat, it is significant in the heating of water by internal friction from wave and current action. Living organisms more easily support themselves in the more viscous (and also denser) cold waters of the arctic than in the less viscous warm tropical waters.

TABLE 4

VISCOSITY OF WATER (In millipoises at 1 atm)

	Dissolved solids in g/L			
Temp. °C	0	5	10	30
-10	26.0	----	----	----
- 5	21.4	----	----	----
0	17.94	18.1	18.24	18.7
5	15.19	15.3	15.5	16.0
10	13.10	13.2	13.4	13.8
30	8.00	8.1	8.2	8.6
100	2.84	----	----	----

3 Surface tension has biological as well as physical significance. Organisms whose body surfaces cannot be wet by water can either ride on the surface film or in some instances may be "trapped" on the surface film and be unable to re-enter the water.

4 Incident solar radiation is the prime source of energy for virtually all organic and most inorganic processes on earth. For the earth as a whole, the total amount (of energy) received annually must exactly balance that lost by reflection and radiation into space if climatic and related conditions are to remain relatively constant over geologic time.

a For a given body of water, immediate sources of energy include in addition to solar irradiation: terrestrial heat, transformation of kinetic energy (wave and current action) to heat, chemical and biochemical reactions, convection from the atmosphere, and condensation of water vapor.

b The proportion of light reflected depends on the angle of incidence, the temperature, color, and other qualities of the water. In general, as the depth increases arithmetically, the light tends to decrease geometrically. Blues, greens, and yellows tend to penetrate most deeply while ultra violet, violets, and orange-reds are most quickly absorbed. On the order of 90% of the total illumination which penetrates the surface film is absorbed in the first 10 meters of even the clearest water, thus tending to warm the upper layers.

5 Water movements

a Waves or rhythmic movement

The best known are traveling waves caused by wind. These are effective only against objects near the surface. They have little effect on the movement of large masses of water.

Standing waves or seiches occur in lakes, estuaries, and other enclosed bodies of water, but are seldom large enough to be observed. An "internal wave or seich" is an oscillation in a submersed mass of water such as a hypolimnion, accompanied by compensating oscillation in the overlying water such that no significant change in surface level is detected. Shifts in submerged water masses of this type can have severe effects on the biota and also on human water uses where withdrawals are confined to a given depth. Descriptions and analyses of many other types and sub-types of waves and wave-like movements may be found in the literature.

b Tides

Tides are the longest waves known in the ocean, and are evident along the coast by the rhythmic rise and fall of the water. While part and parcel of the same phenomenon, it is often convenient to refer to the rise and fall of the water level as "tide", and to the accompanying currents as "tidal currents".

Tides are basically caused by the attraction of the sun and moon on water masses, large and small; however, it is only in the oceans and certain of the larger lakes that true tidal action has been demonstrated. The patterns of tidal action are enormously complicated by local topography, interaction with seiches, and other factors. The literature on tides is very large.

c Currents (except tidal currents) are steady a rhythmic water movements which have had major study only in oceanography although they are best known from rivers and streams. They primarily are concerned with the translocation of water masses. They may be generated internally by virtue of density changes, or externally by wind or terrestrial topography. Turbulence phenomena or eddy currents are largely responsible for lateral mixing in a current. These are of far more importance in the economy of a body of water than mere laminar flow.

d Coriolis force is a result of interaction between the rotation of the earth, and the movement of masses or bodies on the earth. The net result is a slight tendency for moving objects to veer to the right in the northern hemisphere, and to the left in the southern hemisphere. While the result in fresh waters is usually negligible, it may be considerable in marine waters. For example, other factors permitting, there is a tendency in estuaries for fresh waters to move toward the ocean faster along the right bank, while salt tidal waters tend to intrude farther inland along the left bank. Effects are even more dramatic in the open oceans.

e Langmuir circulation (or L. spirals) is the interlocking rotation of somewhat cylindrical masses of surface water under the influence of wind action. The axes of the cylinders are parallel to the direction of the wind.

To somewhat oversimplify the concept, a series of adjoining cells might be thought of as chains of interlocking gears in which at every other contact the teeth are rising while at the alternate contacts, they are sinking (Figure 2).

The result is elongated masses of waste rising or sinking together. This produces the familiar "wind rows" of foam, flotsam and jetsam, or plankton often seen streaking

windblown lakes or oceans. Certain zoo-plankton struggling to maintain a position near the surface often collect in the down current between two Langmuir cells, causing such an area to be called the "red dance", while the clear upwelling water between is the "blue dance".

This phenomenon may be important in water or plankton sampling on a windy day.

6 The pH of pure water has been determined between 5.7 and 7.01 by various workers. The latter value is most widely accepted at the present time. Natural waters of course vary widely according to circumstances.

C The elements of hydrology mentioned above represent a selection of some of the more conspicuous physical factors involved in working with water quality. Other items not specifically mentioned include: molecular structure of waters, interaction of water and radiation, internal pressure, acoustical characteristics, pressure-volume-temperature relationships, refractivity, luminescence, color, dielectrical characteristics and phenomena, solubility, action and interactions of gases, liquids and solids, water vapor, ices, phenomena of hydrostatics and hydrodynamics in general.

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IV MICROBIOLOGICAL ASPECTS OF WASTEWATER TREATMENT

Organisms in water may be implicated in purification of it, existence in it, or in making it hazardous for man or animal. This section considers the background and techniques for evaluating water with respect ot pathogen carriage.

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COLLECTION AND HANDLING OF SAMPLES FOR BACTERIOLOGICAL EXAMINATION

I INTRODUCTION

The first step in the examination of a water supply for bacteriological examination is careful collection and handling of samples. Information from bacteriological tests is useful in evaluating water purification, bacteriological potability, waste disposal, and industrial supply. Topics covered include: representative site selection, frequency, number, size of samples, satisfactory sample bottles, techniques of sampling, labeling, and transport.

II SELECTION OF SAMPLING LOCATIONS

The basis for locating sampling points is collection of representative samples.

A Take samples for potability testing from the distribution system through taps. Choose representative points covering the entire system. The tap itself should be clean and connected directly into the system. Avoid leaky faucets because of the danger of washing in extraneous bacteria. Wells with pumps may be considered similar to distribution systems

B Grab samples from streams are frequently collected for control data or application of regulatory requirements. A grab sample can be taken in the stream near the surface.

C For intensive stream studies on source and extent of pollution, representative samples are taken by considering site, method and time of sampling. The sampling sites may be a compromise between physical limitations of the laboratory, detection of pollution peaks, and frequency of sample collection in certain types of surveys. First, decide how many samples are needed to be processed in a day. Second, decide whether to measure cycles of immediate pollution or more average pollution.

Sites for measuring cyclic pollution are immediately below the pollution source. Sampling is frequent, for example, every three hours.

A site designed to measure more average conditions is far enough downstream for a complete mixing of pollution and water.

Keep in mind that averaging does not remove all variation but only minimizes sharp fluctuations. Downstream sites sampling may not need to be so frequent.

Samples may be collected 1/4, 1/2 and 3/4 of the stream width at each site or other distances, depending on survey objectives. Often only one sample in the channel of the stream is collected. Samples are usually taken near the surface

D Samples from lakes or reservoirs are frequently collected at the drawoff and usually about the same depth and may be collected over this entire surface.

E Collect samples of bathing beach water at locations and times where the most bathers swim.

III NUMBER, FREQUENCY AND SIZE OF SAMPLES

A For determining sampling frequency for drinking water, consult the USPHS Standards.

1 The total number, frequency, and site are established by agreement with either state of PHS authorities.

2 The minimum number depends upon the number of users. Figure 1 indicates that the smaller populations call for relatively more samples than larger ones. The numbers on the left of the graph refer to actual users and not the population shown by census

3 In the event that coliform limits of the standard are exceeded, daily samples must be taken at the same site. Examinations should continue until two consecutive samples show coliform level is satisfactory. Such samples are to be considered as special samples and shall not be included in the total number of samples examined.

4 Sampling programs described above represent a minimum number which may be increased by reviewing authority.

B For stream investigations the type of study governs frequency of sampling.

C Collect swimming pool samples when use is heavy. The high chlorine level rapidly reduces the count when the pool is not in use Residual chlorine tests are necessary to check neutralization of chlorine in the sample.

D Lake beaches may be sampled as required depending on the water uses.

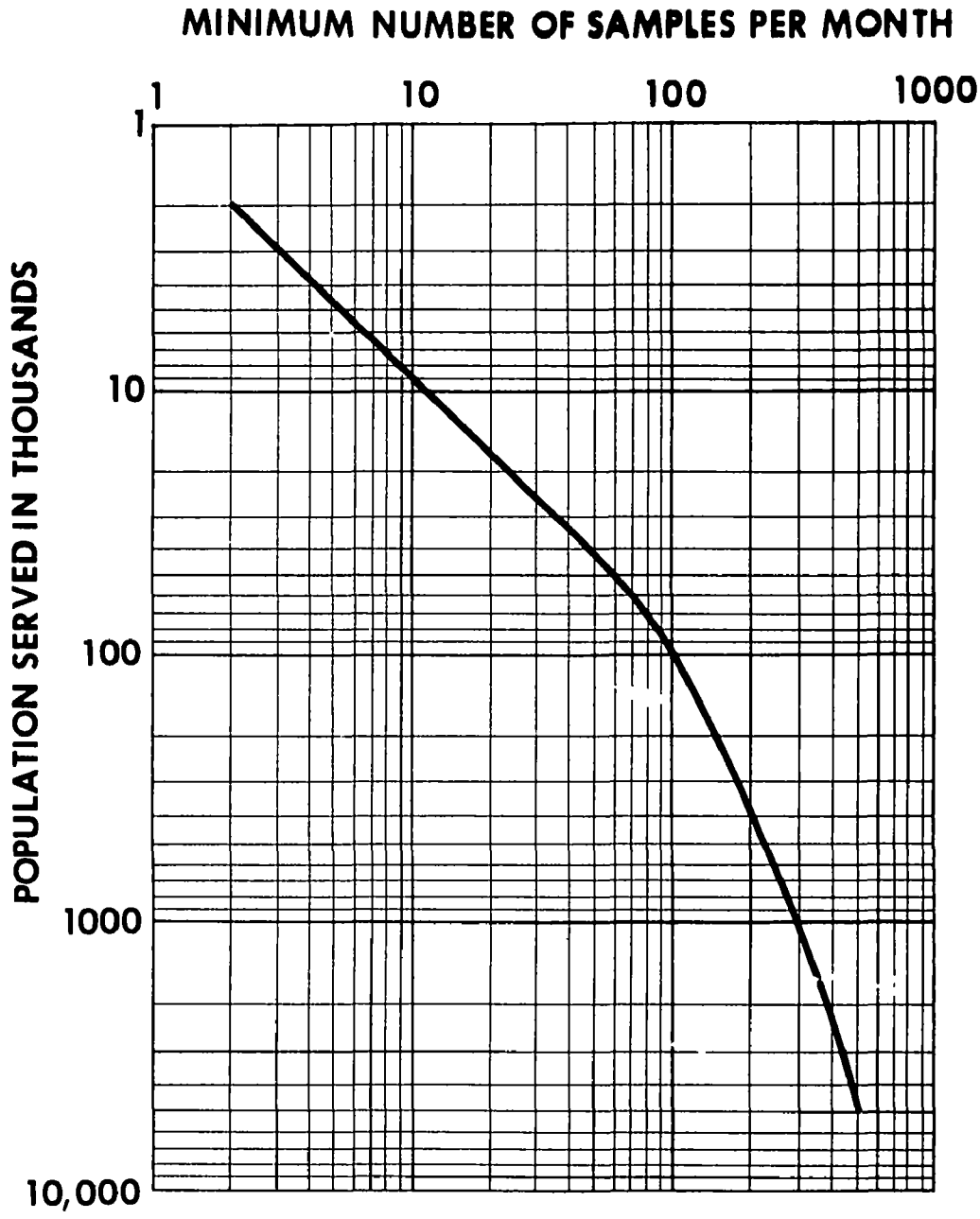

FIGURE 1

E Salt water or estuarine beaches are sampled as needed with frequency depending on use.

F Size of samples depends upon examination anticipated. Generally 100 ml is the minimum size.

IV BOTTLES FOR WATER SAMPLES

A The sample bottles should have capacity for at least 100 ml of sample, plus an air space. The bottle and cap must be of bacteriological inert materials. Resistant glass or heat resistant plastic are acceptable. At the National Training Center, wide mouth ground-glass stoppered bottles (Figure 2) are used.

All bottles must be properly washed and sterilized. Protect the top of the bottles and cap from contamination by paper or metal foil hoods. Both glass and heat resistant plastic bottles may be sterilized in an autoclave. Hold plastic at 121°C for at least 10 minutes. Hot air sterilization, 1 hour at 170°C, may be used for glass bottles.

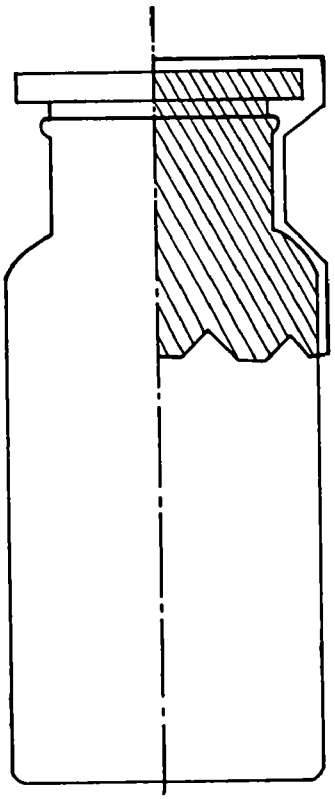

FIGURE 2

B Add sodium thiosulfate to bottles intended for halogenated water samples. A quantity of 0 1 ml of a 10% solution provides 100 mg per liter concentration in a 100 ml sample. This level shows no effect upon viability or growth

C Supply catalogs list wide mouth ground glass stoppered bottles of borosilicate resistance glass, specially for water samples.

V TECHNIQUE OF SAMPLE COLLECTION

Follow aseptic technique as nearly as possible. Nothing but sample water must touch the inside of the bottle or cap. To avoid loss of sodium thiosulfate, fill the bottle directly and do not rinse. Always remember to leave an air space.

A In sampling from a distribution system, first run the faucet wide open until the service line is cleared. A time of 3-5 minutes generally is sufficient. Reduce the flow and fill the sample bottle without splashing. Some authorities stress flaming the tap before collection. A chlorine determination is often made on the site.

B The bottle may be dipped into some waters by hand. Avoid introduction of bacteria from the human hand and from surface debris. Some suggestions follow:
Hold the bottle near the base with one hand and with the other remove the hood and cap. Push the bottle rapidly into the water mouth down and tilt up towards the current to fill. A depth of about 6 inches is satisfactory. When there is no current move the bottle through the water horizontally and away from the hand. Lift the bottle from the water, spill a small amount of sample to provide an air space, and return the uncontaminated cap

C Samples may be dipped from swimming pools. Determine residual chlorine on the pool water at the site. Test the sample at the laboratory to check chlorine neutralization by the thiosulfate.

D Sample bathing beach water by wading out to the two foot depth and dipping the sample up from about 6 inches below the surface. Use the procedure described in V. B.

E Wells with pumps are similar to distribution systems. With a hand pumped well, waste water for about five minutes before taking the sample. Sample a well without a pump by lowering a sterile bottle attached to a weight. A device which opens the bottle underneath the water will avoid contamination by surface debris.

F Various types of sampling devices are available where the sample point is inaccessible or depth samples are desired. The general problem is to put a sample bottle in place, open it, close it, and return it to the surface. No bacteria but those in the sample must enter the bottle.

1 The J - Z sampler described by Zobell in 1941, was designed for deep sea sampling but is useful elsewhere (Figure) 3). It has a metal frame, breaking device for a glass tube, and sample bottle. The heavy metal messenger strikes the lever arm which breaks the glass tubing at a file mark. A bent rubber tube straightens and the water is drawn in several inches from the apparatus. Either glass or collapsible rubber bottles are sample containers.

Commercial adaptations are available.

2 Note the vane and lever mechanism on the New York State Conservation Department's sampler in Figure 4. When the apparatus is at proper depth the suspending line is given a sharp pull. Water inertia against the vane raises the stopper and water pours into the bottle. Sufficient sample is collected prior to the detachment of the stopper from the vane arm allowing a closure of the sample bottle.

The New York State Conservation Department's sampler is useful for shallow depths and requires nothing besides glass stoppered sample bottles.

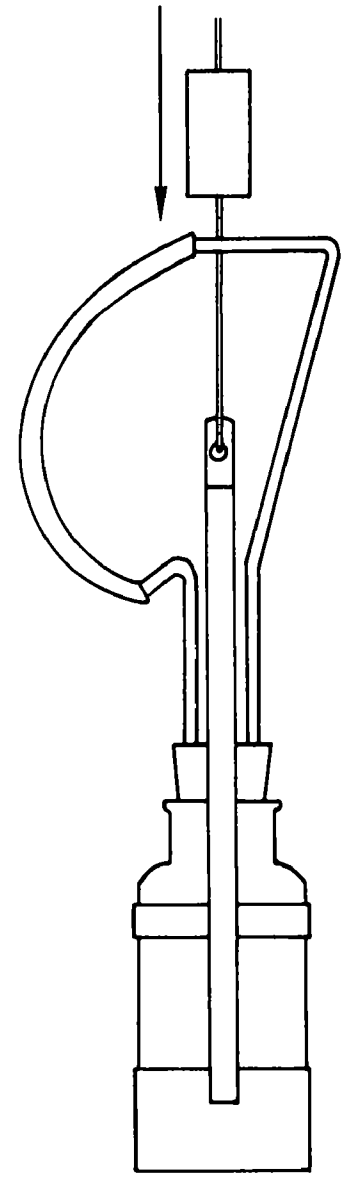

FIGURE 3

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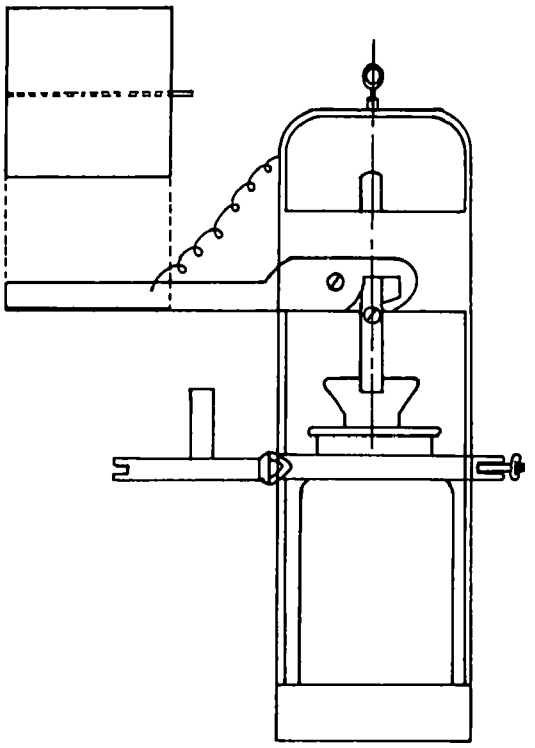

FIGURE 4

3 A commercial sampler is available which is an evacuated sealed tube with a capillary tip. When a lever on the support rack breaks the tip, the tube fills. Other samplers exist with a lever for pulling the stopper, while another uses an electromagnet.

VI DATA RECORDING

A Information generally includes: date, time of collection, temperature of water, location of sampling point, and name of the sample collector. Codes are often used. The location description must be exact enough to guide another person to the site. Reference to bridges, roads, distance to the nearest town may help. Use of the surveyors' description and maps are recommended. Mark identification on the bottles or on securely fastened tags. Gummed tags may soak off and are inadvisable.

B While a sanitary survey is an indispensable part of the evaluation of a water supply, its discussion is not within the scope of this lecture. The sample collector could supply much information if desired

VII SHIPPING CONDITIONS

A The examination should commence as soon as possible, preferably within one hour. A maximum elapsed time between collection and examination is 30 hours for potable water samples and 8 hours for other water samples (collection 6 hours and laboratory procedures 2 hours). Standard Methods (13th Edition) recommends icing of samples between collection and testing.

VIII PHOTOGRAPHS

A photograph is a sample in that it is evidence representing water quality. Sample collectors and field engineers may carry cameras to record what they see. Pictures help the general public and legal courts to better understand laboratory data.

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BACTERIOLOGICAL INDICATORS OF WATER POLLUTION

Part 1. General Concepts

I INTRODUCTION

A Bacterial Indication of Pollution

1 In the broadest sense, a bacterial indicator of pollution is any organism which, by its presence, would demonstrate that pollution has occurred, and often suggest the source of the pollution.

2 In a more restrictive sense, bacterial indicators of pollution are associated primarily with demonstration of contamination of water, originating from excreta of warm-blooded animals (including man, domestic and wild animals, and birds).

B Implications of Pollution of Intestinal Origin

1 Intestinal wastes from warm-blooded animals regularly include a wide variety of genera and species of bacteria. Among these the coliform group may be listed, and species of the genera Streptococcus, Lactobacillus, Staphylococcus, Proteus, Pseudomonas, certain spore-forming bacteria, and others.

2 In addition, many kinds of pathogenic bacteria and other microorganisms may be released in wastes on an intermittent basis, varying with the geographic area, state of community health, nature and degree of waste treatment, and other factors. These may include the following·

a Bacteria· Species of Salmonella, Shigella, Leptospira, Brucella, Mycobacterium, and Vibrio comma.

b Viruses· A wide variety, including that of infectious hepatitis, Polioviruses, Coxsackie virus, ECHO viruses (enteric cytopathogenic human orphan -- "viruses in search of a disease"), and unspecified viruses postulated to account for outbreaks of diarrheal and upper respiratory diseases of unknown etiology, apparently infective by the water-borne route.

c Protozoa: Endamoeba histolytica

3 As routinely practiced, bacterial evidence of water pollution is a test for the presence and numbers of bacteria in wastes which, by their presence, indicate that intestinal pollution has occurred. In this context, indicator groups discussed in subsequent parts of this outline are as follows.

a Coliform group and certain subgroupings

b Fecal streptococci and certain subgroupings

c Miscellaneous indicators of pollution

4 Evidence of water contamination by intestinal wastes of warm-blooded animals is regarded as evidence of health hazard in the water being tested.

II PROPERTIES OF AN IDEAL INDICATOR OF POLLUTION

A An "ideal" bacterial indicator of pollution should:

1 Be applicable in all types of water

2 Always be present in water when pathogenic bacterial constituents of fecal contamination are present. Ramifications of this include --

a Its density should have some direct relationship to the degree of fecal pollution.

b It should have greater survival time in water than enteric pathogens.

c It should disappear rapidly from water following the disappearance of pathogens, either through natural or man-made processes.

d It always should be absent in a bacteriologically safe water.

3 Lend itself to routine quantitative testing procedures without interference or confusion of results due to extraneous bacteria

4 Be harmless to man and other animals

B In all probability, an "ideal" bacterial indicator does not exist. The discussion of bacterial indicators of pollution in the following parts of this outline include consideration of the merits and limitations of each group, with their applications in evaluating bacterial quality of water.

III APPLICATIONS OF TESTS FOR POLLUTION INDICATORS

A Tests for Compliance with Bacterial Water Quality Standards

1 Potability tests on drinking water to meet Interstate Quarantine or other standards of regulatory agencies.

2 Determination of bacterial quality of environmental water for which quality standards may exist, such as shellfish waters, recreational waters, water resources for municipal or other supplies.

3 Tests for compliance with established standards in cases involving the protection or prosecution of municipalities, industries, etc.

B Treatment Plant Process Control

1 Water treatment plants

2 Wastewater treatment plants

C Water Quality Surveys

1 Determination of intestinal pollution in surface water to determine type and extent of treatment required for compliance with standards

2 Tracing sources of pollution

3 Determination of effects on bacterial flora, due to addition of organic or other wastes

D Special Studies, such as

1 Tracing sources of intestinal pathogens in epidemiological investigations

2 Investigations of problems due to the Sphaerotilus group

3 Investigations of bacterial interference to certain industrial processes, with respect to such organisms as Pseudomonas, Achromobacter, or others

IV SANITARY SURVEY

The laboratory bacteriologist is not alone in evaluation of indication of water pollution of intestinal origin. On-site study (Sanitary Survey) of the aquatic environment and adjacent areas, by a qualified person, is a necessary collateral study with the laboratory work and frequently will reveal information regarding potential bacteriological hazard which may or may not be demonstrated through laboratory findings from a single sample or short series of samples.

Part 2. The Coliform Group and Its Constituents

I ORIGINS AND DEFINITION

A Background

1 In 1885, Escherich, a pioneer bacteriologist, recovered certain bacteria from human feces, which he found in such numbers and consistency as to lead him to term these organisms "the characteristic organism of human feces."

He named these organisms Bacterium coli-commune and B. lactis aerogenes. In 1895, another bacteriologist, Migula, renamed B. coli commune as Escherichia coli, which today is the official name for the type species.

2 Later work has substantiated much of the original concept of Escherich, but has shown that the above species are in fact a heterogeneous complex of bacterial species and species variants.

a This heterogeneous group occurs not only in human feces but representatives also are to be found in many environmental media, including sewage, surface freshwaters of all categories, in and on soils, vegetation, etc.

b The group may be subdivided into various categories on the basis of numerous biochemical and other differential tests that may be applied.

B Composition of the Coliform Group

1 Current definition

As defined in "Standard Methods for the Examination of Water and Wastewater" (12th ed): "The coliform group includes all of the aerobic and facultative anaerobic, Gram-negative, nonspore-forming rod-shaped bacteria which ferment lactose with gas formation within 48 hours at 35° C."

2 The term "coliforms" or "coliform group" is an inclusive one, including the following bacteria which may meet the definition above

a Escherichia coli, E. aurescens, E. freundii, E. intermedia

b Aerobacter aerogenes, A. cloacae

c Biochemical intermediates between the genera Escherichia and Aerobacter

3 There is no provision in the definition of coliform bacteria for "atypical" or "aberrant" coliform strains.

a An individual strain of any of the above species may fail to meet one of the criteria of the coliform group.

b Such an organism, by definition, is not a member of the coliform group, even though a taxonomic bacteriologist may be perfectly correct in classifying the strain in one of the above species.

II SUBDIVISION OF COLIFORMS INTO "FECAL" AND "NONFECAL" CATEGORIES

A Need

Single-test differentiations between coliforms of "fecal" origin and those of "nonfecal" origin are based on the assumption that typical E. coli and closely related strains are of fecal origin while A. aerogenes and its close relatives are not of direct fecal origin. (The latter assumption is not fully borne out by investigations at this Center. See Table 1, IMViC Type --++). A number of single differential tests have been proposed to differentiate between "fecal" and "nonfecal" coliforms.

Without discussion of their relative merits, several may be cited here:

B Types of Single-Test Differentiation

1 Determination of gas ratio

Fermentation of glucose by E. coli results in gas production, with hydrogen and carbon dioxide being produced in equal amounts. Fermentation of glucose by A. aerogenes results in generation of twice as much carbon dioxide as hydrogen.

Further studies suggested absolute correlation between H_2/CO_2 ratios and the terminal pH resulting from glucose fermentation. This led to the substitution of the methyl red test.

2 Methyl red test

Glucose fermentation by E. coli typically results in a culture medium having terminal pH in the range 4.2 - 4.6 (red color a positive test with the addition of methyl red indicator). A. aerogenes typically results in a culture medium having pH 5.6 or greater (yellow color, a negative test).

3 Indole

When tryptophane, an amino acid, is incorporated in a nutrient broth, typical E. coli strains are capable of producing indole (positive test) among the end products, whereas A. aerogenes does not (negative test).

In reviewing technical literature, the worker should be alert to the method used to detect indole formation, as the results may be greatly influenced by the analytical procedure.

4 Voges-Proskauer test (acetylmethyl carbinol test)

The test is for detection of acetylmethyl carbinol, a derivative of 2, 3, butylene-glycol, as a result of glucose fermentation in the presence of peptone. A. aerogenes produces this end product (positive test) whereas E. coli gives a negative test.

a Experience with coliform cultures giving a positive test has shown a loss of this ability with storage on laboratory media for 6 months to $2\frac{1}{2}$ years, in 20 - 25% of cultures (105 out of 458 cultures).

b Some workers consider that all coliform bacteria produce acetylmethyl carbinol in glucose metabolism. These workers regard acetylmethyl carbinol-negative cultures as those which have enzyme systems capable of further degradation of acetylmethyl carbinol to other end products which do not give a positive test with the analytical procedure. Cultures giving a positive test for acetylmethyl carbinol lack this enzyme system.

c This reasoning leads to a hypothesis (not experimentally proven) that the change of reaction noted in certain cultures in 4.a above is due to the activation of a latent enzyme system.

5 Citrate utilization

Cultures of E. coli are unable to use the carbon of citrates (negative test) in their metabolism, whereas cultures of A. aerogenes are capable of using the carbon of citrates in their metabolism (positive test).

Some workers (using Simmons Citrate Agar) incorporate a pH indicator (brom thymol blue) in the culture medium in order to demonstrate the typical alkaline reaction (pH 8.4 - 9.0) resulting with citrate utilization.

6 Elevated temperature (Eijkman) test

a The test is based on evidence that E. coli and other coliforms of fecal

origin are capable of growing and fermenting carbohydrates (glucose or lactose) at temperatures significantly higher than the body temperature of warm-blooded animals. Organisms not associated with direct fecal origin would give a negative test result, through their inability to grow at the elevated temperature.

b While many media and techniques have been proposed, EC Broth, a medium developed by Perry and Hajna, used as a confirmatory medium for 24 hours at 44.5 ± 0.2°C are the current recommended medium and method of choice. While the "EC" terminology of the medium suggests "E. coli" the worker should not regard this as a specific procedure for isolation of E. coli.

c A similar medium, Boric Acid Lactose Broth, has been developed by Levine and his associates. This medium gives results virtually identical with those obtained from EC Broth, but requires 48 hours of incubation.

d Elevation temperature tests require incubation in a water bath. Standard Methods 13th Ed. requires this temperature to be 44.5 ± 0.2°C. Various workers have urged use of temperatures ranging between 43.0°C and 46.0°C. Most of these recommendations have provided a tolerance of ± 0.5°C from the recommended levels. However, some workers, notably in the Shellfish Program of the Public Health Service, stipulate a temperature of 44.5 ± 0.2°C. This requires use of a water bath with forced circulation to maintain this close tolerance. This tolerance range has been instituted in the 13th Edition of Standard Methods and the laboratory worker should conform to these new limits.

e The reliability of elevated temperature tests is influenced by the time required for the newly-inoculated cultures to reach the designated incubation temperature. Critical workers insist on placement of the cultures in the water bath within 30 minutes, at most, after inoculation.

7 Other tests

Numerous other tests for differentiation between coliforms of fecal vs. nonfecal origin have been proposed. Current studies suggest little promise for the following tests in this application: uric acid test, cellobiose fermentation, gelatin liquefaction, production of hydrogen sulfide, sucrose fermentation, and others.

C IMViC Classification

1 In 1938, Parr reported on a review of a literature survey on biochemical tests used to differentiate between coliforms of fecal vs. nonfecal origin. A summary follows:

Test	No. of times used for differentiation
Voges-Proskauer reaction	22
Methyl red test	20
Citrate utilization	20
Indole test	15
Uric acid test	6
Cellobiose fermentation	4
Gelatin liquefaction	3
Eijkman test	2
Hydrogen sulfide production	1
Sucrose fermentation	1
a-Methyl-d-glucoside fermentation	1

2 Based on this summary and on his own studies, Parr recommended utilization of a combination of tests, the indole, methyl red, Voges-Proskauer, and the citrate utilization tests for this differentiation. This series of reactions is designated by the mnemonic "IMViC". Using this scheme, any coliform culture can be described by an "IMViC Code" according to the reactions for each culture. Thus, a typical culture of E. coli would have a code ++--, and a typical A. aerogenes culture would have a code --++.

3 Groupings of coliforms into fecal, non-fecal, and intermediate groups, as shown in "Standard Methods for the Examination of Water and Wastewater" are shown at the bottom of this page.

D Need for Study of Multiple Cultures

All the systems used for differentiation between coliforms of fecal vs. those of nonfecal origin require isolation and study of numerous pure cultures. Many workers prefer to study at least 100 cultures from any environmental source before attempting to categorize the probable source of the coliforms.

III NATURAL DISTRIBUTION OF COLIFORM BACTERIA

A Sources of Background Information

Details of the voluminous background of technical information on coliform bacteria recovered from one or more environmental media are beyond the scope of this discussion. References 2, 3, and 4 of this outline are suggested routes of entry for workers seeking to explore this topic.

B Recent Studies on Coliform Distribution

1 Since 1960, workers at this Center have engaged in a continuing study of the natural distribution of coliform bacteria and an evaluation of procedures for differentiation between coliforms of fecal vs. probable nonfecal origin. Results of this work have special significance because:

a Rigid uniformity of laboratory methods have been applied throughout the series of studies

b Studies are based on massive numbers of cultures, far beyond any similar studies heretofore reported

Groupings of Coliforms into Fecal, Nonfecal and Intermediate Groups

Organism	Indole	Methyl red	Voges-Proskauer	Citrate
E. coli, Variety I	+	+	-	-
Variety II	-	+	-	-
E. freundii (Intermediates)				
Variety I	-	+	-	±
Variety II	+	+	-	+
A. aerogenes				
Variety I	-	-	+	±
Variety II	±	-	+	+

c A wider variety of environmental and biological sources is being studied than in any previous series of reports.

d All studies are based on freshly recovered pure culture isolates from the designated sources.

e All studies are based on cultures recovered from the widest feasible geographic range, collected at all seasons of the year. It is believed that no more representative series of studies has been made or is in progress.

2 Distribution of coliform types

Table 1 shows the consolidated results of coliform distributions from various biological and environmental sources.

a The results of these studies show a high order of correlation between known or probable fecal origin and the typical E. coli IMViC code (++--). On the other hand, human feces also includes numbers of A. aerogenes and other IMViC types, which some regard as "nonfecal" segments of the coliform group. (Figure 1)

b The majority of coliforms attributable to excretal origin tend to be limited to a relatively small number of the possible IMViC codes, on the other hand, coliform bacteria recovered from undisturbed soil, vegetation, and insect life represent a wider range of IMViC codes than fecal sources, without clear dominance of any one type. (Figure 2)

c The most prominant IMViC code from nonfecal sources is the intermediate type, -+-+, which accounts for almost half the coliform cultures recovered from soils, and a high percentage of those recovered from vegetation and from insects. It would appear that if any coliform segment could be termed a "soil type" it would be IMViC code -+-+.

d It should not be surprising that cultures of typical E. coli are recovered in relatively smaller numbers from sources judged, on the basis of sanitary survey, to be unpolluted. There is no known way to exclude the influence of limited fecal pollution from small animals and birds in such environments

e The distribution of coliform types from human sources should be regarded as a representative value for large numbers of sources. Investigations have shown that there can be large differences in the distribution of IMViC types from person to person, or even from an individual.

3 Differentiation between coliforms of fecal vs. nonfecal origin

Table 2 is a summary of findings of SEC workers, based on a number of different criteria for differentiating between coliforms of fecal origin and those from other sources.

a IMViC type ++-- is a measurement of E. coli, Vairety I, and appears to give reasonably good correlation between known or highly probable fecal origin and doubtful fecal origin.

b The combination of IMViC types, ++--, +---, and -+--, gives improved identification of probable fecal origin, and appears also to exclude most of the coliforms not found in excreta of warm-blooded animals in large numbers.

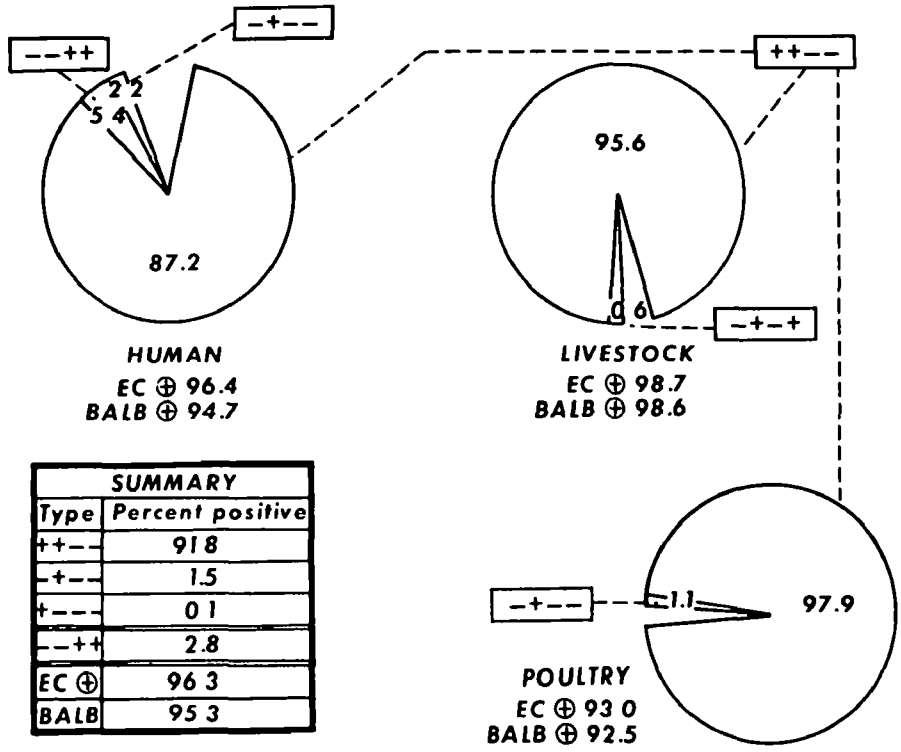
c While the indole, methyl red, Voges Proskauer, and citrate utilization tests, each used alone, appear to give useful answers when applied only to samples of known pollution from fecal sources, the interpretation is not as clear when applied to coliforms from sources believed to be remote from direct fecal pollution.

Table 1. COLIFORM DISTRIBUTION BY IMVIC TYPES AND ELEVATED TEMPERATURE TEST FROM ENVIRONMENTAL AND BIOLOGICAL SOURCES

IMVIC type	Vegetation		Insects		Soil				Fecal sources				Poultry	
					Undisturbed		Polluted		Human		Livestock			
	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total	No. strains	% of total
++--	128	10.6	134	12.4	131	5.6	536	80.6	3932	87.2	2237	95.6	1857	97.9
--++	237	19.7	113	10.4	443	18.8	13	2.0	245	5.4	0	<0.1	1	0.1
-+--	23	1.9	0	<0.1	78	3.3	1	0.2	99	2.2	14	0.6	20	1.1
+++-	2	0 2	0	<0.1	7	0.3	0	<0.1	106	2.4	59	2.5	0	<0.1
-+-+	168	14.0	332	30.6	1131	48.1	87	13.0	50	1.1	1	<0.1	5	0.3
++-+	116	9.6	118	10.9	87	3.7	22	3.3	35	0.8	27	1.2	11	0.6
-+++	32	2.7	28	2.6	181	7.7	5	0.7	21	0.5	0	<0.1	0	<0.1
++++	291	24.2	254	23.4	159	6.8	0	<0.1	6	0.1	0	<0.1	0	<0.1
+-++	88	7.3	46	4.2	67	2.9	0	<0.1	14	0.2	0	<0.1	0	<0.1
---+	87	7.2	42	3.9	4	0.2	1	0.2	2	<0.1	0	<0.1	0	<0.1
-++-	5	0.4	0	<0.1	1	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
--+-	19	1.6	0	<0.1	53	2.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+-+-	2	0.2	0	<0.1	6	0.3	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+--+	5	0.4	8	0.7	0	<0.1	0	<0.1	0	<0.1	0	<0.1	0	<0.1
+---	0	<0.1	9	0.8	0	<0.1	0	<0.1	2	<0.1	0	<0.1	2	<0.1
Total	1203		1084		2348		665		4512		2339		1896	
No. EC +	169*		162*		216		551		4349		2309		1765	
% EC +	14.1*		14.9*		9.2		82.9		96.4		98.7		93.0	

*120 of these were ++--, 15 --++, 11 -+--

*129 of these were ++--, 27 -+-+, 5 ++-+



SUMMARY	
Type	Percent positive
++−−	91.8
−+−−	1.5
+−−−	0.1
−−++	2.8
EC ⊕	96.3
BALB	95.3

FIGURE 1

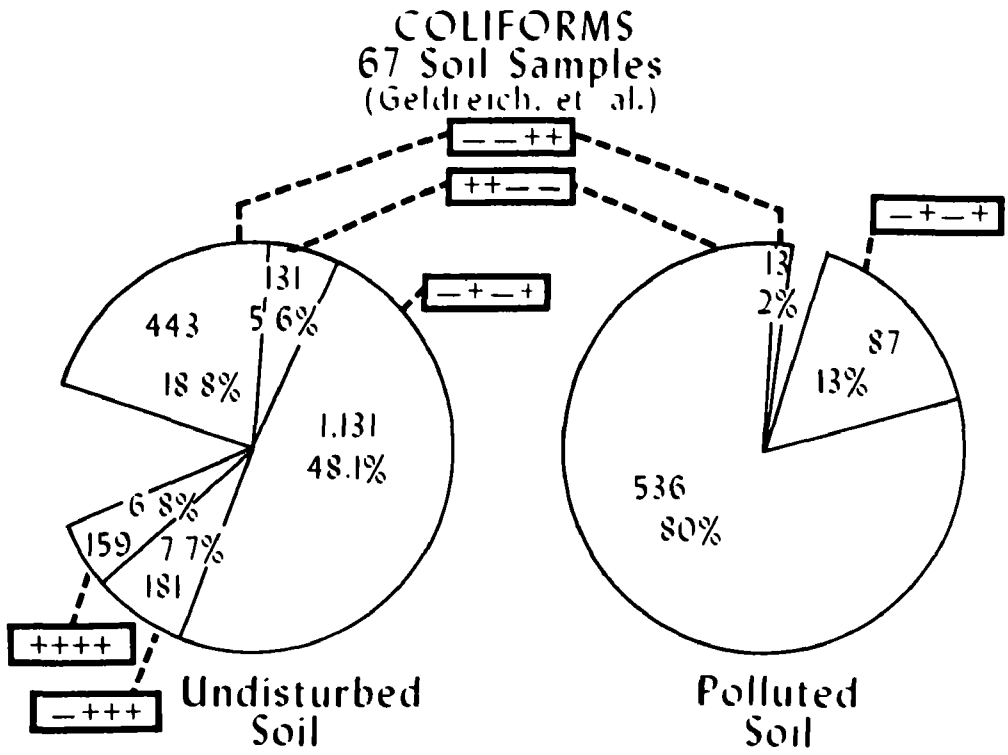


FIGURE 2

Table 2. COMPARISON OF COLIFORM STRAINS ISOLATED FROM WARM-BLOODED ANIMAL FECES, FROM UNPOLLUTED SOILS AND POLLUTED SOILS WITH USE OF THE IMViC REACTIONS AND THE ELEVATED TEMPERATURE TEST IN EC MEDIUM AT 44.5° C (±0.5°) (12th ed. 1965, Standard Methods for the Examination of Water and Wastewater)

Test	Warm-blooded animal feces	Soil, Unpolluted	Soil Polluted	Vegetation	Insects
+ + - -	91.8%	5.6%	80.6%	10.6%	12.4%
+ + - -, + - - - and - + - -	93.3%	8.9%	80.7%	12.5%	13.2%
Indole positive	94.0%	19.4%	82.7%	52.5%	52.4%
Methyl red positive	96.9%	75.6%	97.9%	63.6%	79.9%
Voges-Proskauer positive	5 1%	40.7%	97.3%	56.3%	40.6%
Citrate utilizers	3.6%	88.2%	19.2%	85 1%	86.7%
Elevated temperature (EC) positive	96.4%	9.2%	82.9%	14.1%	14.9%
Number of cultures studied	8,747	2,348	665	1,203	1,084

Total Pure Cultures Studied 14,047

d The elevated temperature test gives excellent correlation with samples of known or highly probable fecal origin. The presence of smaller, but demonstrable, percentages of such organisms in environmental sources not interpreted as being polluted could be attributed largely to the warm-blooded wildlife in the area, including birds, rodents, and other small mammals.

e The elevated temperature test yields results equal to those obtained from the total IMViC code. It has marked advantages in speed, ease and simplicity of performance, and yields quantitative results for each water sample. Therefore, it is regarded as the method of choice for differentiation between coliforms of probable direct fecal origin and those which may have become established in the bacterial flora of the aquatic or terrestrial habitat.

IV EVALUATION OF COLIFORMS AS POLLUTION INDICATORS

A The Coliform Group as a Whole

1 Merits

a The absence of coliform bacteria is evidence of a bacteriologically safe water.

b The density of coliforms is roughly proportional to the amount of excretal pollution present.

c If pathogenic bacteria of intestinal origin are present, coliform bacteria also are present, in much greater numbers.

d Coliforms are always present in the intestines of humans and other warm-blooded animals, and are eliminated in large numbers in fecal wastes.

e Coliforms are more persistent in the aquatic environment than are pathogenic bacteria of intestinal origin.

f Coliforms are generally harmless to humans and can be determined quantitatively by routine laboratory procedures.

2 Limitations

a Some of the constituents of the coliform group have a wide environmental distribution in addition to their occurrence in the intestines of warm-blooded animals.

b Some strains of the coliform group may multiply in certain polluted waters ("aftergrowth"), of high nutritive values thereby adding to the difficulty of evaluating a pollution situation in the aquatic environment. Members of the A. aerogenes section of the coliform are commonly involved in this kind of problem.

c Because of occasional aftergrowth problems, the age of the pollution may be difficult to evaluate under some circumstances.

d Tests for coliforms are subject to interferences due to other kinds of bacteria. False negative results sometimes occur when species of Pseudomonas are present. False positive results sometimes occur when two or more kinds of non-coliforms produce gas from lactose, when neither can do so alone (synergism).

B The Fecal Coliform Component of the Coliform Group (as determined by elevated temperature test)

1 Merits

a The majority (over 95% of the coliform bacteria from intestines of warm-blooded animals grow at the elevated temperature.

b These organisms are of relatively infrequent occurrence except in association with fecal pollution.

c Survival of the fecal coliform group is shorter in environmental waters than for the coliform group as whole. It follows, then, that high densities of fecal coliforms is indicative of relatively recent pollution.

d Fecal coliforms generally do not multiply outside the intestines of warm-blooded animals. In certain high-carbohydrate wastes, such as from the sugar beet refineries, exceptions have been noted.

2 Limitations

a Feces from warm-blooded animals include some (though proportionately low) numbers of coliforms which do not yield a positive fecal coliform test when the elevated temperature test is used as the criterion of differentiation. These organisms are E. coli varieties by present taxonomic classification.

b There is at present no established and consistent correlation between ratios of total coliforms/fecal coliforms in interpreting sanitary quality of environmental waters.

In domestic sewage, the fecal coliform density commonly is greater than 90% of the total coliform density. In environmental waters relatively free from recent pollution, the fecal coliform density may range from 10-30% of the total coliforms. There are, however, too many variables relating to water-borne wastes and surface water runoff to permit sweeping generalization on the numerical relationships between fecal- and total coliforms.

c At this time, evaluations are underway regarding the survival

of fecal coliforms in polluted waters compared with that of enteric pathogenic bacteria. In recent pollution studies, species of Salmonella have been found in the presence of 220 fecal coliforms per 100 ml (Spino), and 110 fecal coliforms per 100 ml (Brezenski, Raritan Bay Project).

V APPLICATIONS OF COLIFORM TESTS

A Current Status in Official Tests

1 The coliform group is designated, in "Standard Methods for the Examination of Water and Wastewater" (13th ed., 1971), through the Completed Test MPN procedure as the official test for bacteriological potability of water. The Confirmed Test MPN procedure is accepted where it has been demonstrated, through comparative tests, to yield results equivalent to the Completed Test. The membrane filter method also is accepted for examination of waters subject to interstate regulation.

2 The 12th edition of Standard Methods has introduced a standard test for fecal coliform bacteria. It is emphasized that this is to be used in pollution studies, and does not apply to the evaluation of water for potability. This procedure has been carried to the 13th Edition.

B Applications

1 Tests for the coliform group as a whole are used in official tests to comply with interstate drinking water standards, state standards for shellfish waters, and in most, if not all, cases where bacterial standards of water quality have been established for such use as in recreational or bathing waters, water supplies, or industrial supplies. Laboratory personnel should be aware of possible implementation of the fecal coliform group as the official test for recreational and bathing waters.

2 The fecal coliform test has application in water quality surveys, as an adjunct to determination of total coliform density. The fecal coliform test is being used increasingly in all water quality surveys.

3 It is emphasized that no responsible worker advocates substitution of a fecal coliform test for total coliforms in evaluating drinking water quality.

Part 3. The Fecal Streptococci

I DEFINITIONS

A A fecal streptococcus as used in this discussion refers to any streptococcus commonly found in significant numbers in the feces of human or other warm-blooded animals. Other terms used are "enterococci" or "Group D streptococci."

B Enterococci are characterized by specific taxonomic biochemistry. Serological procedures differentiate the Group D streptococci from the various groups. Although they overlap, the three groups, fecal streptococcus, enterococcus, and Group D streptococcus, are not synonymous. Because our emphasis is on indicators of unsanitary origin, fecal streptococcus is the more appropriate term and will include the enterococcus as well as other groups.

C A rigid definition of the fecal streptococcus group is not possible with our present knowledge. The British Ministry of Health (1956) defines the organisms as "Gram-positive" cocci, generally occurring in pairs or short chains, growing in the presence of bile salt, usually capable of development at 45° C, producing acid but not gas in mannitol and lactose, failing to attack raffinose, failing to reduce nitrate to nitrite, producing acid in litmus milk and precipitating the casein in the form of a loose, but solid curd, and exhibiting a greater resistance to heat, to alkaline conditions and to high concentrations of salt than most vegetative bacteria." However, it is pointed out that "streptococci departing in one or more particulars from the type species cannot be disregarded in water."

D Some workers consider that growth at 45° C and multiplication in 40% bile are the most significant indications of fecal origin of streptococci.

E 1 The first group, the Pyogenic Group, not growing at 10° C or 45° C, pathogenic for man and animals, causes total lysing of red blood cells, giving them the common name of beta hemolytic streptococci. These organisms are not considered as belonging to the fecal streptococci.

2 A second group, or Viridans group, not growing at 10° C, growth at 45° C, is an intermediate group, some members resembling group 1 and others.

3 The third group, the Enterococcus Group, growing at 10° C and 45° C, and typically exhibit vigorous biochemical reactions. Members of these groups (2 and 3) are considered to be fecal streptococci.

4 The fourth, the Lactic Group, growing at 10° C and not at 45° C, is a common harmless inhabitant of plants and dairy products and bear no relationship to the definition of a fecal streptococcus.

Examples of specific strains or species representative of each group are·

a Streptococcus pyogenes, cause of scarlet fever

b Streptococcus bovis, from cow manure

c Streptococcus fecalis, from human feces

d Streptococcus lactis, from cheese

F For special purposes, strains of individual fecal streptocci are identified by streptocci are identified by biochemical tests. The techniques are discussed elsewhere. Strains or species likely to be fecal streptococci are· Streptococcus fecalis, S. fecalis variety liquefaciens, S. fecalis variety zymogenes, S. durans, S. bovis, S. equinus, S. salivarius, and S. fecalis biotypes usually growing at 10° C and 45° C.

II HISTORY

From their discovery to the present time the fecal streptococci appear characteristic of fecal pollution, being consistently present in both the feces of all warm-blooded animals and in the environment associated with animal discharges. As early as 1910 fecal streptococci were proposed as indicators to the Metropolitan Water Board of London. However, little progress resulted in the United States until improved methods of detection and enumeration appeared after World War II.

Renewed interest in the group as indicators began with the introduction of azide dextrose broth in 1950, (Mallmann & Seligmann, 1950). The method which is in the current edition of Standard Methods appeared soon after. (Litsky, et al. 1955).

With the advent of improved methods for detection and enumeration of fecal streptococci, a significant body of technical literature has appeared.

III SIGNIFICANCE OF FECAL STREPTOCOCCAL INDICATORS

A The presence of fecal streptococci indicates the presence of warm-blooded animal pollution. Many types of wild life may harbor fecal streptococci. One study showed 71% of various mammalian species yielding enterococci. Similarly, reptiles showed 85% incidence. However, the common rodents yielded few and sporadic isolations. Wild birds also showed few individuals with enterococci. (Mundt 1963). Insects may be only accidental mechanical carriers of fecal streptococci. However, Geldreich, et al (1964) found high numbers in some insects.

B Evidence indicates that fecal streptococci do not occur in pure water or virgin soil.

In one study, Medrek and Litsky (1960) looked specifically for occurrence of enterococci in undisturbed or virgin soil. Modern methods showed 8 out of 369 samples with enterococci. With only one exception, the presence of enterococci in a given soil sample was associated with the presence of coliforms. The presence of indicators in a given soil sample is considered indicative of chance pollution of the sampling area by animal or bird droppings or drainage.

C Fecal streptococci do not multiply in water. A recent report is illustrative (Morris and Weaver, 1954). Duplicate samples were taken of 52 polluted Kentucky wells and examined before and after storage for 24 hours. While the coliform numbers increased, decreased or changed little, the streptococcal numbers never increased.

Other experiments on a variety of Louisiana surface water gave same results for streptococcal numbers. Fecal streptococci enter a water, survive for a period of time and die without multiplying.

D On the basis of laboratory data, the fecal streptococci could be expected to be more resistant to electrolytes like salt. If true, the group would have special value for salt water investigation. Further research is needed to determine this point.

E The fecal streptococci are not considered pathogenic. However, such strains as S. fecalis have been known to occur with endocarditis and other human infections. S. fecalis var zymogenes, a beta hemolytic streptococcus normally inhabiting the human intestine, is also nonpathogenic.

The fecal streptococci are alleged causes of food-borne infections. However, the circumstantial evidence has not been supported by intensive studies.

IV LIMITATIONS

A Knowledge of relative survival times of fecal streptococci and pathogens is needed. Comparable results with many types of pathogens under many environmental conditions are needed. Insufficient time

has elapsed since adequate media have been developed for evidence to accumulate. The coliforms have over 60 years of accumulated validation for their use. The fecal streptococci have had only about 15 years of serious consideration. A few comparisons exist, however.

When sewage is added to the soil, the coliforms persist for long periods. Fecal streptococci disappear rapidly. Typhoid bacilli, added experimentally with sewage, persist less time than the fecal streptococci. However, mineral-organic content of the receiving soil has much influence on persistence so that only relative rates may be determined. (Mallman and Litsky, 1950).

B Although the enterococci are considered as having their origin, in the intestines of warm-blooded animals, other sources may exist. The resistance and tolerance of these streptococci, together with their low minimum and high maximum limits of growth may not only enable them to survive but also to grow under diverse conditions of nature. Reports have appeared that growth occurs in food products, fresh plants and silage. For example, some investigations (Mundt, 1962) show that fecal streptococci of the S. fecalis and the S. liquefaciens types occur commonly on plants. However, Geldreich et al, 1963 concluded after an exhaustive study of vegetation that indicator contribution was negligible.

V FECAL COLIFORM-FECAL STREPTOCOCCUS RELATIONSHIPS

Because coliforms may derive from non-fecal sources, the fecal coliforms are a more reliable standard of comparison with streptococcal densities. Even so, variation in known ratios is marked.

A Variation may arise from different methods, different species, different individuals, and different geographic locations.

Table 3. ESTIMATED PER CAPITA CONTRIBUTION OF INDICATOR MICROORGANISMS FROM SOME ANIMALS*

		Average indicator density per gram of feces		Average contribution per capita per 24 hr		
Animals	Avg wt of Feces/24 hr, wet wt, g	Fecal coliform, million	Fecal streptococci, million	Fecal coliform, million	Fecal streptococci, million	Ratio FC/FS
Man	150	13.0	3.0	2,000	450	4.4
Duck	336	33.0	54.0	11,000	18,000	0.6
Sheep	1,130	16.0	38.0	18,000	43,000	0.4
Chicken	182	1.3	3.4	240	620	0.4
Cow	23,600	0.23	1.3	5,400	31,000	0.2
Turkey	448	0.29	2.8	130	1,300	0.1
Pig	2,700	3.3	84.0	8,900	230,000	0.04

*Publication WP-20-3, P. 102

B Species differences are the main cause of different fecal coliform-fecal streptococci ratios. Table 3 compares fecal streptococcus and fecal coliform counts for different species. Even though individuals vary widely, masses of individuals in a species have characteristic proportion of indicators. In intestinal wastes of human origin, the ratio of number of fecal coliforms to number of fecal streptococci tends to be considerably greater than two-to-one. In comparison, when such ratios are determined for intestinal wastes from non-human animal sources, the values tend to be markedly less than one-to-one.

Current interpretative use of this ratio is as follows:

Domestic Wastes (Predominately Human)	- > 4.0
Animal Wastes	- < 0.7

C Determination of specific strains of fecal streptococci sometimes is useful. Besides ratio of indicators, the specific types of fecal streptococci are more typical of some animals. Humans carry 80% or over of S. fecalis group, and characteristically the S. salivarius-mitis group. Cows and horses carry a majority of S. bovis and S. equinus groups. Other farm animals have mixtures of appreciable numbers of several groups. Fowl, however, resemble humans in their streptococcal flora. The S. salivarius group, besides being uniquely human, dies rapidly in water, giving an index of recentness of pollution.

VI INTERPRETATION

The presence of fecal streptococci in untreated water indicates the presence of fecal pollution by warm-blooded animals.

In samples where the source and significance of the coliform group has been questioned, the presence of the streptococcus group should be interpreted as indicating at least a portion of the coliform group were derived from fecal sources.

Because of the uncertainties in die-off rates, the absence of fecal streptococci does not necessarily mean that the water is bacteriologically safe.

Part 4. Other Bacterial Indicators of Pollution

I TOTAL BACTERIAL COUNTS

A Historical

1 The early studies of Robert Koch led him to develop tentative standards of water quality based on a limitation of not more than 100 bacterial colonies per ml on a gelatin plating medium incubated 3 days at 20° C.

2 Later developments led to inoculation of samples on duplicate plating media, with one set incubated at 37° C and the other at 20° C.

a Results were used to develop a ratio between the 37° C counts and the 20° C counts.

b Waters having a predominant count at 37° C were regarded as being of probable sanitary significance, while those giving predominant counts at 20° C were considered to be of probable soil origin, or natural inhabitants of the water being examined.

B Groups Tested

There is no such thing as "total" bacterial count in terms of a laboratory determination.

1 Direct microscopic counts do not differentiate between living and dead cells.

2 Plate counting methods enumerate only the bacteria which are capable of using the culture medium provided, under the temperature and other growth conditions used as a standard procedure. No one culture medium and set of growth conditions can provide, simultaneously, an acceptable environment for all the heterogeneous, often conflicting, requirements of the total range of bacteria which may be recovered from waters.

C Utilization of Total Counts

1 Total bacterial counts, using plating methods, are useful for:

a Detection of changes in the bacterial composition of a water source

b Process control procedures in treatment plant operations

c Determination of sanitary conditions in plant equipment or distributional systems

2 Serious limitations in total bacterial counts exist because

a No information is given regarding possible or probable fecal origin of bacterial changes. Large numbers of bacteria can sometimes be cultivated from waters known to be free of fecal pollution.

b No information of any kind is given about the species of bacteria cultivated.

c There is no differentiation between harmless or potentially dangerous forms.

3 Status of total counts

a There is no total bacterial count standard for any of the following:

Interstate Quarantine Drinking Water Standards

PHS regulations for water potability (as shown in "Standard Methods" Public Health Service Drinking Water Standards of 1962.)

b The most widely used current application of total bacterial counts in water bacteriology today is in

water treatment plants, where some workers use standard plate counts for process control and for determination of the bacterial quality of distribution systems and equipment.

c Total bacterial counts are not used in PHS water quality studies, though extensively used until the 1940's.

B Spore-Forming Bacteria (Clostridium perfringens, or C. welchii)

1 Distribution

This is one of the most widely distributed species of bacteria. It is regularly present in the intestinal tract of warm-blooded animals.

2 Nature of organism

C. perfringens is a Gram-positive, spore-forming rod. The spores cause a distinct swelling of the cell when formed. The organism is extremely active in fermentation of carbohydrates, and produces the well-known "stormy fermentation" of milk.

3 Status

The organism, when present, indicates that pollution has occurred at some time. However, because of the extremely extended viability of the spores, it is impossible to obtain even an approximation of the recency of pollution based only on the presence of C. perfringens.

The presence of the organism does not necessarily indicate an unsafe water.

C Tests for Pathogenic Bacteria of Intestinal Origin

1 Groups considered include Salmonella sp, Shigella sp, Vibrio comma, Mycobacterium sp, Pasteurella sp, Leptospira sp, and others.

2 Merits of direct tests

Demonstration of any pathogenic species would demonstrate an unsatisfactory water quality, hazardous to persons consuming or coming into contact with that water.

3 Limitations

a There is no available routine procedure for detection of the full range of pathogenic bacteria cited above.

b Quantitative methods are not available for routine application to any of the above.

c The intermittent release of these pathogens makes it impossible to regard water as safe, even in the absence of pathogens.

d After detection, the public already would have been exposed to the organism, thus, there is no built-in margin of safety, as exists with tests for the coliform group.

4 Applications

a In tracing the source of pathogenic bacteria in epidemiological investigations

b In special research projects

c In water quality studies concerned with enforcement actions against pollution, increasing attention is being given to the demonstration of enteric pathogenic bacteria in the presence of the bacterial indicators of pollution.

D Miscellaneous Indicators

It is beyond this discussion to explore the total range of microbiological indicators of pollution that have been proposed and

investigated to some extent. Mention can be made, however, of consideration of tests for the following:

1 Bacteriophages specific for any of a number of kinds of bacteria

2 Serological procedures for detection of coliforms and other indicators, a certain amount of recent attention has been given to applications of fluorescent antibodies in such tests

3 Tests for Pseudomonas aeruginosa

4 Tests for viruses, which may persist in waters even longer than members of the coliform group.

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EXAMINATION OF WATER FOR COLIFORM AND FECAL STREPTOCOCCUS GROUPS
(Multiple Dilution Tube (MPN) Methods)

I INTRODUCTION

The subject matter of this outline is contained in three parts, as follows:

A Part 1

1 Fundamental aspects of multiple dilution tube ("most probable numbers") tests, both from a qualitative and a quantitative viewpoint.

2 Laboratory bench records.

3 Useful techniques in multiple dilution tube methods.

4 Standard supplies, equipment, and media in multiple dilution tube tests.

B Part 2

Detailed, day-by-day, procedures in tests for the coliform group and subgroups within the coliform group.

C Part 3

Detailed, day-by-day, procedures in tests for members of the fecal streptococci.

D Application of Tests to Routine Examinations

The following considerations (Table 1) apply to the selection of the Presumptive Test, the Confirmed Test, and the Completed Test. Termination of testing at the Presumptive Test level is not practiced by laboratories of this agency. It must be realized that the Presumptive Test alone has limited use when water quality is to be determined.

TABLE 1

	Examination Terminated at -		
Type of Receiving Water	Presumptive Test	Confirmed Test	Completed Test
Sewage Receiving	Applicable	Applicable	Important where results are to be used for control of raw or finished water. Application to a statistically valid number of samples from the Confirmed Test to establish its validity in determining the sanitary quality.
Treatment Plant - Raw	Applicable	Applicable	
Chlorinated	Not Done	Applicable	
Bathing	Not Done	Applicable	
Drinking	Not Done	Applicable	
Other Information		Applicable in all cases where Presumptive Test alone is unreliable.	

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Environmental Protection Agency.

II BASIS OF MULTIPLE TUBE TESTS

A Qualitative Aspects

1 For purely qualitative aspects of testing for indicator organisms, it is convenient to consider the tests applied to one sample portion, inoculated into a tube of culture medium, and the follow-up examinations and tests on results of the original inoculation. Results of testing procedures are definite: positive (presence of the organism-group is demonstrated) or negative (presence of the organism-group is not demonstrated.)

2 Test procedures are based on certain fundamental assumptions:

a First, even if only one living cell of the test organism is present in the sample, it will be able to grow when introduced into the primary inoculation medium,

b Second, growth of the test organism in the culture medium will produce a result which indicates presence of the test organism, and,

c Third, extraneous organisms will not grow, or if they do grow, they will not limit growth of the test organism, nor will they produce growth effects that will be confused with those of the bacterial group for which the test is designed.

3 Meeting these assumptions usually makes it necessary to conduct the tests in a series of stages (for example, the Presumptive, Confirmed, and Completed Test stages, respectively, of standard tests for the coliform group).

4 Features of a full, multi-stage test

a First stage: The culture medium usually serves primarily as an enrichment medium for the group tested. A good first-stage growth medium should support growth of all the living cells of the group tested, and it should include provision for indicating the presence of the test organism being studied. A first-stage medium may include some component which inhibits growth of extraneous bacteria, but this feature never should be included if it also inhibits growth of any cells of the group for which the test is designed. The Presumptive Test for the coliform group is a good example. The medium supports growth, presumably, of all living cells of the coliform group, the culture container has a fermentation vial for demonstration of gas production resulting from lactose fermentation by coliform bacteria, if present, and sodium lauryl sulfate may be included in one of the approved media for suppression of growth of certain noncoliform bacteria. This additive apparently has no adverse effect on growth of members of the coliform group in the concentration used. If the result of the first-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. No further study is made of negative tests. If the result of the first-stage test is positive, the culture may be subjected to further study to verify the findings of the first stage.

b Second stage: A transfer is made from positive cultures of the first-stage test to a second culture medium. This test stage emphasizes provision to reduce confusion of results due to growth effects of extraneous bacteria, commonly achieved by addition of selective inhibitory agents. (The Confirmed Test for coliforms meets these requirements. Lactose and fermentation vials are provided for demonstration of coliforms in the medium. Brilliant green dye and bile salts are included as inhibitory agents which tend to suppress growth of practically all kinds of noncoliform bacteria, but do not suppress growth of coliform bacteria when used as directed).

If result of the second-stage test is negative, the study of the culture is terminated, and the result is recorded as a negative test. A negative test here means that the positive results of the first-stage test were "false positive," due to one or more kinds of extraneous bacteria. A positive second-stage test is partial vertification of the positive results obtained in the first-stage test, the culture may be subjected to final identification through application of still further testing procedures. In routine practice, most sample examinations are terminated at the end of the second stage, on the assumption that the result would be positive if carried to the third, and final stage. This practice should be followed only if adequate testing is done to demonstrate that the assumption is valid. Some workers recommend continuing at least 5% of all sample examinations to the third stage to demonstrate the reliability of the second-stage results.

B Quantitative Aspects of Tests

1 These methods for determining bacterial numbers are based on the assumption that the bacteria can be separated from one another (by shaking or other means) resulting in a suspension of individual bacterial cells, uniformly distributed through the original sample when the primary inoculation is made.

2 Multiple dilution tube tests for quantitative determinations apply a Most Probable Number (MPN) technique. In this procedure one or more measured portions of each of a stipulated series of decreasing sample volumes is inoculated into the first-stage culture medium. Through decreasing the sample increments, eventually a volume is reached where only one cell is introduced into some tubes, and no cells are introduced into other tubes. Each of the several tubes of sample-inoculated first-stage medium is tested independently, according to the principles previously described, in the qualitative aspects of testing procedures.

3 The combination of positive and negative results is used in an application of probability mathematics to secure a single MPN value for the sample.

4 To obtain MPN values, the following conditions must be met:

a The testing procedure must result in one or more tubes in which the test organism is demonstrated to be present; and

b The testing procedure must result in one or more tubes in which the test organism is not demonstrated to be present.

5 The MPN value for a given sample is obtained through the use of MPN Tables. It is emphasized that the precision of an individual MPN value is not great when compared with most physical or chemical determinations.

6 Standard practice in water pollution surveys conducted by this organization, is to plant five tubes in each of a series of sample increments, in sample volumes decreasing at decimal intervals. For example, in testing known polluted waters, the initial sample inoculations might consist of 5 tubes each in volumes of 0.1, 0.01, 0.001, and 0.0001 ml, respectively This series of sample volumes will yield determinate results from a low of 200 to a high of 1,600,000 organisms per 100 ml.

III LABORATORY BENCH RECORDS

A Features of a Good Bench Record Sheet

1 Provides complete identification of the sample.

2 Provides for full, day-by-day information about all tests performed on the sample.

3 Provides easy step-by-step record applicable to any portion of the sample.

4 Provides for recording of the quantitative result which will be transcribed to subsequent reports.

5 Minimizes the amount of writing by the analyst.

6 Identifies the analyst(s)

B There is no such thing as "standard" bench sheet for multiple tube tests; there are many versions of bench sheets. Some are prescribed by administrative authority (such as the Office of a State Sanitary Engineer), others are devised by laboratory or project personnel to meet specific needs.

It is not the purpose of this discussion to recommend an "ideal" bench form; however, the form used in this training course manual is essentially similar to that used in certain research laboratories of this organization. The student enrolled in the course for which this manual is written should make himself thoroughly familiar with the bench sheet and its proper use. See Figure 1.

IV NOTES ABOUT WORKING PROCEDURES IN THE LABORATORY

A Each bacteriological examination of water by multiple dilution tube methods requires a considerable amount of manipulation; much is quite repetitious. Laboratory workers must develop and maintain good routine working habits, with constant alertness to guard against lapses into careless, slip-shod laboratory procedures and "short cuts" which only can lead to lowered quality of laboratory work.

The student reader is urged to review the form for laboratory surveys (PHS-875, Rev. 1966) used by Public Health Service personnel charged with responsibility for accreditation of laboratories for examination of water under Interstate Quarantine regulations.

B Specific attention is brought to the following by no means exhaustive, critical aspects of laboratory procedures in multiple dilution tube tests:

1 Original sample

a Follow prescribed care and handling procedures before testing.

b Maintain absolute identification of sample at all stages in testing.

c Vigorously shake samples (and sample dilutions) before planting in culture media.

2 Sample measurement into primary culture medium

a Sample portions must be measured accurately into the culture medium for reliable quantitative tests to be made. Standard Methods prescribes that calibration errors should not exceed $\pm$ 2.5%.

BACTERIOLOGY BENCH SHEET

Multiple Dilution Tube Tests

Project Ohio River Survey

Sample Station Broadway Landing

Collection Data

Date 2/6/67 Time 8:50 By KJ

Temperature 8 °C pH 7.3

Other Observations

Analytical Record

Bench Number of Sample 2

Analyst Zobel-Jeter

Test started at 11:45 By HLJ

ml sample	Coliform Test								Fecal coliform	Fecal Streptococcus				Remarks
	LTB		BGLB		EMB	LSTB		Gram stain		A - D		EVA		
	24	48	24	48	24	24	48		24	24	48	24	48	
10														
1.0														
0.1														
0.01														
0.001														
0.001														

Coliform MPN/100 ml

Confirmed:

Completed:

Fecal Coliform MPN:

Fecal Streptococcus MPN/100 ml

A - D - EVA:

Figure 1. SAMPLE BENCH SHEET

Suggested sample measuring practices are as follows: Mohr measuring pipets are recommended. 10 ml samples are delivered at the top of the culture tube, using 10 ml pipets. 1.0 ml samples are delivered down into the culture tube, near the surface of the medium, and "touched off" at the side of the tube when the desired amount of sample has been delivered. 1.0 ml or 2.0 ml pipets are used for measurement of this volume. 0.1 ml samples are delivered in the same manner as 1.0 ml samples, using great care that the sample actually gets into the culture medium. Only 1.0 ml pipets are used for this sample volume. After delivery of all sample increments into the culture tubes, the entire rack of culture tubes may be shaken gently to carry down any of the sample adhering to the wall of the tube above the medium.

Workers should demonstrate by actual tests that the pipets and the technique in use actually delivers the rated volumes within the prescribed limits of error.

b Volumes as small as 0.1 ml routinely can be delivered directly from the sample with suitable pipets. Lesser sample volumes first should be diluted, with subsequent delivery of suitable volumes of diluted sample into the culture medium. A diagrammatic scheme for making dilutions is shown in Figure 2.

3 Reading of culture tubes for gas production

a On removal from the incubator, shake culture rack gently, to encourage release of gas which may be supersaturated in the culture medium.

b Gas in any quantity is a positive test. It is necessary to work in conditions of suitable lighting for easy recognition of the extremely small amounts of gas inside the tops of some fermentation vials.

4 Reading of liquid culture tubes for growth as indication of a positive test requires good lighting. Growth is shown by any amount of increased turbidity or opalescence in the culture medium, with or without deposit of sediment at the bottom of the tube.

5 Transfer of cultures with inoculation loops and needles

a Always sterilize inoculation loops and needles in flame immediately before transfer of culture; do not lay it down or touch it to any non-sterile object before making the transfer.

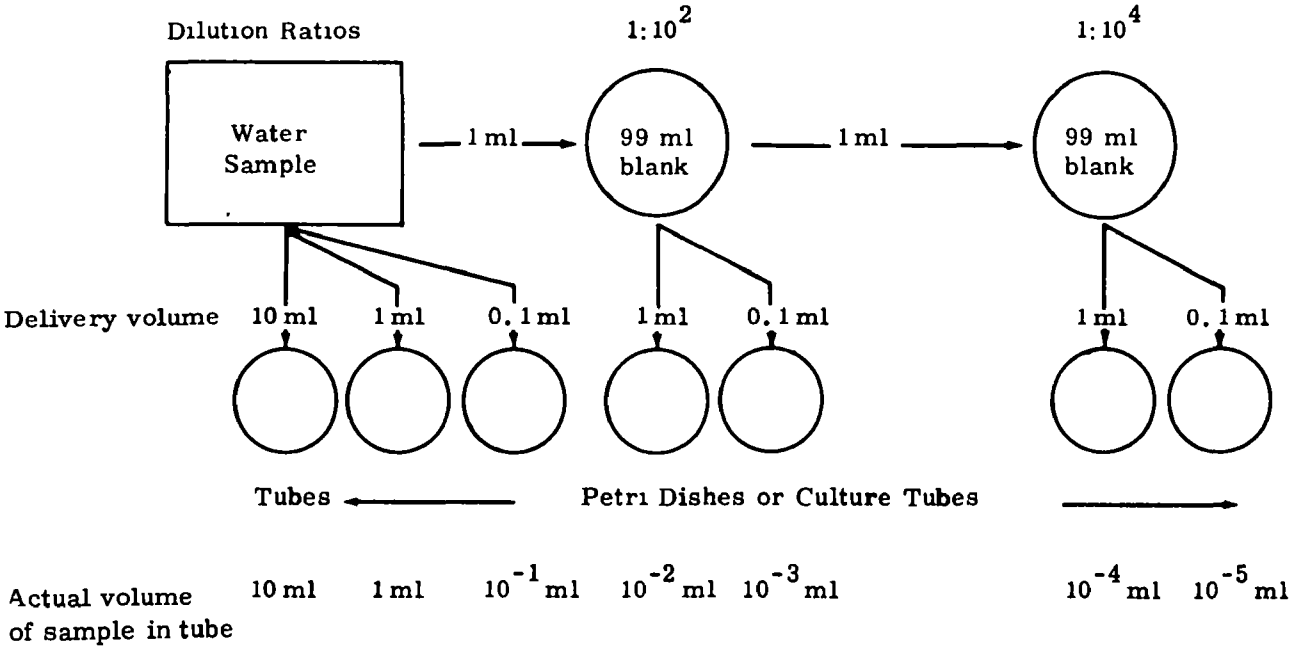
b After sterilization, allow sufficient time for cooling, in the air, to avoid heat-killing bacterial cells on the hot wire.

c Loops should be 3 mm in inside diameter, with a capability of holding a drop of water or culture.

For routine standard transfers requiring transfer of 3 loopsful of culture, many workers form three 3-mm loops on the same length of wire.

6 As an alternative to use of standard inoculation loops, the use of "applicator sticks" have now been sanctioned by the 13th Edition of Standard Methods.

Figure 2. PREPARATION OF DILUTIONS



The applicator sticks are dry heat sterilized (autoclave sterilization is not acceptable because of possible release of phenols if the wood is steamed) and are used on a single-service basis. Thus, for every culture tube transferred, a new applicator stick is used.

This use of applicator sticks is particularly attractive in field situations where it is inconvenient or impossible to provide a gas burner suitable for sterilization of the inoculation loop. In addition, use of applicator sticks is favored in laboratories where room temperatures are significantly elevated by use of gas burners.

7 Streaking cultures on agar surfaces

a All streak-inoculations should be made without breaking the surface of the agar. Learn to use a light touch with the needle, however, many inoculation needles are so sharp that they are virtually useless in this respect. When the needle is platinum or platinum-iridium wire, it sometimes is beneficial to fuse the working tip into a small sphere. This can be done by momentary insertion of a well-insulated (against electricity) wire into a carbon arc, or some other extremely hot environment. The sphere should not be more than twice the diameter of the wire from which it is formed, otherwise it will be entirely too heat-retentive to be useful.

When the needle is nichrome resistance wire, it cannot be heat-fused; the writer prefers to bend the terminal 1/16 - 1/8" of the wire at a slight angle to the overall axis of the needle. The side of the terminal bent portion of the needle then is used for inoculation of agar surfaces.

b When streaking for colony isolation, avoid using too much inoculum. The streaking pattern is somewhat variable according to individual preference. The procedure favored by the writer is shown in the accompanying figures. Note particularly that when going from any one stage of the streaking to the next, the inoculation needle is heat-sterilized.

8 Preparation of cultures for Gram stain

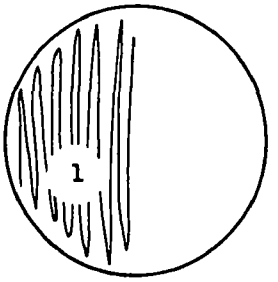
a The Gram stain always should be made from a culture grown on a nutrient agar surface (nutrient agar slants are used here) or from nutrient broth.

b The culture should be young, and should be actively growing. Many workers doubt the validity of the Gram stain made on a culture more than 24 hours old.

c Prepare a thin smear for the staining procedure. Most beginning workers tend to use too much bacterial suspension in preparing the dried smear for staining. The amount of bacteria should be so small that the dried film is barely visible to the naked eye.

V EQUIPMENT AND SUPPLIES

Consolidated lists of equipment, supplies, and culture media required for all multiple dilution tube tests described in this outline are shown in Table 2. Quantitative information is not presented; this is variable—according to the extent of the testing procedure, the number of dilutions used, and the number of replicate tubes per dilution. It is noted that requirements for alternate procedures are fully listed and choices are made in accordance to laboratory preference.

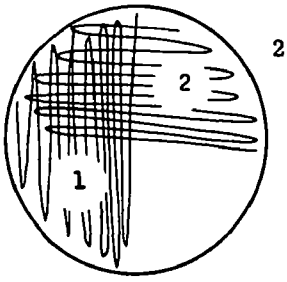


1 a Flame-sterilize an inoculation needle and air-cool.

b Dip the tip of the inoculation needle into the bacterial culture being studied.

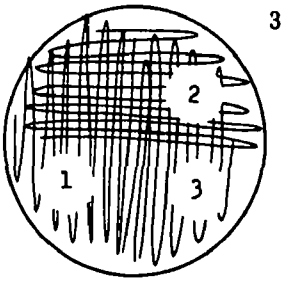
c Streak the inoculation needle tip lightly back and forth over half the agar surface, as in (1), avoiding scratching or breaking the agar surface.

d Flame-sterilize the inoculation needle and air-cool.



2 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (1) into one-half the unstreaked area of the agar.

b Flame-sterilize the inoculation needle and air-cool.



3 a Turn the Petri dish one-quarter-turn and streak the inoculation needle tip lightly back and forth over one-half the agar surface, working from area (2) into area (3), the remaining unstreaked area.

b Flame-sterilize the inoculation needle and set it aside.

c Close the culture container and incubate as prescribed.

Figure 3. A SUGGESTED PROCEDURE FOR COLONY ISOLATION BY A STREAK-PLATE TECHNIQUE

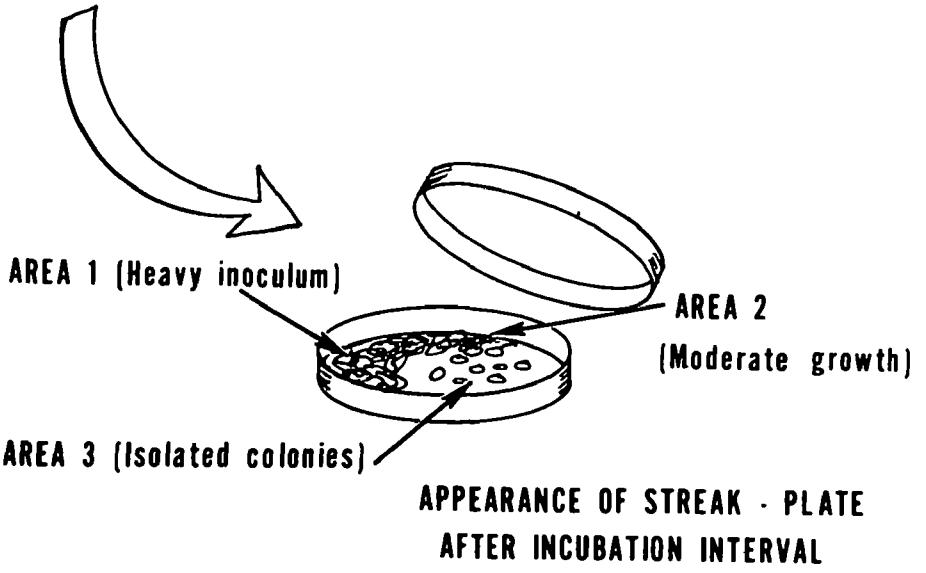


TABLE 2. APPARATUS AND SUPPLIES FOR STANDARD FERMENTATION TUBE TESTS

Description of Item	Total Coliform Group			Fecal Coliform	
	Presumptive Test	Confirmed Test	Completed Test	(BALB)	(EC broth)
Lauryl tryptose broth or Lactose broth 20 ml amounts of 1 5 × concentration medium, in 25 × 150 mm culture tubes with inverted fermentation vials, suitable caps	X				
Lauryl tryptose broth or Lactose broth 10 ml amounts of single strength medium in 20 × 150 mm culture tubes with inverted fermentation vials suitable caps	X		X		
Brilliant green lactose bile broth 2% in 10 ml amounts, single strength, in 20 × 150 mm culture tubes with inverted fermentation vials, suitable caps		X	X		
Eosin methylene blue agar, poured in 100 × 15 mm Petri dishes		X	X		
Endo Agar, poured in 100 × 15 mm dishes		X			
Nutrient agar slant, screw cap tube			X		
Boric acid lactose broth, 10 ml amounts of single strength medium in fermentation tubes				X	
EC Broth, 10 ml amounts of single strength medium in fermentation tubes					X
Formate ricinoleate broth (provisional)			X		
Culture tube racks, 10 × 5 openings, each opening to accept 25 mm diameter tubes	X	X	X	X	X
Pipettes, 10 ml, Mohr type, sterile, in suitable cans	X				
Pipettes, 2 ml (optional), Morh type, sterile, in suitable cans	X				
Pipettes, 1 ml Mohr type, sterile in metal suitable cans	X				
Standard buffered dilution water, sterile, 99-ml amounts in screw-capped bottles	X				
Gas burner, Bunsen type		X	X	X	X
Inoculation loop, loop 3 mm diameter of nichrome or platinum-iridium wire, 26 B & S gauge, in suitable holder (or sterile applicator stick)		X	X	X	X
Inoculation needle, nichrome, or platinum-iridium wire, 26 B & S gauge, in suitable holder		X	X		
Incubator adjusted to 35 ± 0 5° C	X	X	X		
Waterbath incubator, adjusted to 43 ± 0 2°C				X	
Waterbath incubator, adjusted to 44 5 ± 0 2°C					X
Glass microscopic slides, 1" × 3"			X		
Slide racks (optional)			X		
Gram-stain solutions, complete set			X		
Compound microscope, oil immersion lens, Abbe' condenser			X		
Basket for discarded cultures	X	X	X	X	X
Container for discarded pipettes	X				

Part 2

DETAILED TESTING PROCEDURES FOR MEMBERS OF THE COLIFORM GROUP BY MULTIPLE DILUTION TUBE METHODS

I SCOPE

A Tests Described

1 Presumptive Test

2 Confirmed Test

3 Completed Test

4 Fecal Coliform Test

B Form of Presentation

The Presumptive, Confirmed, and Completed Tests are presented as total, independent procedures. It is recognized that this form of presentation is somewhat repetitious, inasmuch as the Presumptive Test is preliminary to the Confirmed Test, and both the Presumptive Test and the Confirmed Test are preliminary to the Completed Test for total coliforms.

In using these procedures, the worker must know at the outset what is to be the stage at which the test is to be ended, and the details of the procedures throughout, in order to prevent the possibility of discarding gas-positive tubes before proper transfer procedures have been followed.

Thus, if the worker knows that the test will be ended at the Confirmed Test, he will turn at once to Section III, TESTING TO THE CONFIRMED TEST STAGE, and will ignore Sections II and IV.

The Fecal Coliform Test is described separately, in Section V, as an adjunct to the Confirmed Test and to the Completed Test.

II TESTING TO PRESUMPTIVE TEST STAGE

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample (Table 3). The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

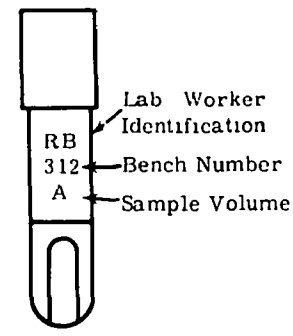
NOTE: Be sure to use tubes containing the correct concentrations of culture medium for the inoculum/tube volumes. (See the chapter on media and solutions for multiple dilution tube methods or refer to the current edition of Standard Methods for Water and Wastewater).

Table 3. SUGGESTED LABELING SCHEME FOR ORIGINAL CULTURES AND SUBCULTURES IN MULTIPLE DILUTION TUBE TESTS

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Sample volume represented
Bench number Volume & tube	312 A	312 B	312 C	312 D	312 E	Tubes with 10 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 1 ml of sample
Bench number Volume & tube	312 a	312 b	312 c	312 d	312 e	Tubes with 0.1 ml of sample
Bench number Volume & tube	312 1a	312 1b	312 1c	312 1d	312 1e	Tubes with 0.01 ml of sample
Bench number Volume & tube	312 2a	312 2b	312 2c	312 2d	312 2e	Tubes with 0.001 ml of sample

The labeling of cultures can be reduced by labeling only the first tube of each series of identical sample volumes in the initial planting of the sample. All subcultures from initial plantings should be labeled completely.

Typical Example



Tube of Culture Medium

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an arc of one foot within seven seconds and withdraw the sample portion at once.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10 ml pipet for 10 ml sample portions, and 1 ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample; otherwise sample running down the outside of the pipet will make measurements inaccurate.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Discard all gas-positive tubes of lauryl tryptose broth, and return all the gas-negative tubes to the 35°C incubator for an additional 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Be sure to record all results under the 48-hour LTB column on the data sheet. Discard all tubes. The Presumptive Test is concluded at this point, and Presumptive coliforms per 100 ml can be computed according to the methods described elsewhere in this manual.

III TESTING TO CONFIRMED TEST STAGE

Note that the description starts with the sample inoculation and includes the Presumptive Test stage. The Confirmed Test preferred in Laboratories of this agency is accomplished by means of the brilliant green lactose bile broth (BGLB) and the acceptable alternate tests are mentioned in III F. In addition, the Fecal Coliform Test is included as an optional adjunct to the procedure

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated.

NOTE: If 10-ml samples are being planted, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted in II A-2.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample, otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube, too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 1 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile borth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB. Place each newly inoculated culture into BGLB in the position of the original gas-positive tube.

7 After making the transfers, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose broth and the newly inoculated BGLB.

8 If the Fecal Coliform Test is included in the testing procedures, consult Section V of this part of the outline of testing procedures.

9 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB an additional 24 ± 2 hours at 35° ± 0.5°C.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred. The option described above for 24-hour cultures does not apply at 48 hours.

4 If the Fecal Coliform Test is included in the testing procedure, consult Section V of the part of the outline of testing procedures.

5 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5°C.

6 Discard all tubes of LTB and all 24-hour gas-positive BGLB cultures.

D 72-hour Procedures

1 If any cultures remain to be examined, all will be BGLB. Some may be 24 hours old and some may be 48 hours old. Remove such cultures from the incubator, examine each tube for gas production, and record results on the data sheet.

2 Be sure to record the results of 24-hour BGLB cultures in the "24" column under BGLB and the 48-hour results under the "48" column of the data sheet

3 Return any 24-hour gas-negative cultures for incubation 24 ± 2 hours at 35 ± 0.5°C.

4 Discard all gas-positive BGLB cultures and all 48-hour gas-negative cultures from BGLB.

5 It is possible that all cultural work and results for the Confirmed Test have been finished at this point. If so, codify results and determine Confirmed Test coliforms per 100 ml as described in the outline on use of MPN Tables.

E 96-hour Procedures

At most only a few 48-hour cultures in BGLB may be present. Read and record gas production of such cultures in the "48" column under BGLB on the data sheet. Codify results and determine Confirmed Test coliforms per 100 ml.

F Streak-plate methods for the Confirmed Test, using eosin methylene blue agar or Endo agar plates, are accepted procedures in Standard Methods. The worker who prefers to use one of these media in preference to BGLB (also approved in Standard Methods) is advised to refer to the current edition of "Standard Methods for the Examination of Water and Wastewater" for procedures.

IV TESTING TO COMPLETED TEST STAGE

(Note that this description starts with the sample inoculation and proceeds through the Presumptive and the Confirmed Test stages. In addition, the Fecal Coliform Test is referred to as an optional adjunct to the procedure.)

A First-Day Procedures

1 Prepare a laboratory data sheet for the sample. Record the following information: assigned laboratory number, source of sample, date and time of collection, temperature of the source, name of sample collector, date and time of receipt of sample in the laboratory. Also show the date and time of starting tests in the laboratory, name(s) of worker(s) performing the laboratory tests, and the sample volumes planted.

2 Label the tubes of lauryl tryptose broth required for the initial planting of the sample. The label should bear three identifying marks. The upper number is the identification of the worker(s) performing the test (applicable to personnel in training courses), the number immediately below is the assigned laboratory number, corresponding with the laboratory record sheet. The lower number is the code to designate the sample volume and which tube of a replicate series is indicated. Guidance on labeling for laboratory data number and identification of individual tubes is described elsewhere in this outline.

NOTE: If 10-ml samples are being plated, it is necessary to use tubes containing the correct concentration of culture medium. This has previously been noted elsewhere in this outline and referral is made to tables.

3 Place the labeled culture tubes in an orderly arrangement in a culture tube rack, with the tubes intended for the largest sample volumes in the front row, and those intended for smaller volumes in the succeeding rows.

4 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

5 Measure the predetermined sample volumes into the labeled tubes of lauryl tryptose broth, using care to avoid introduction of any bacteria into the culture medium except those in the sample.

a Use a 10-ml pipet for 10 ml sample portions, and 1-ml pipets for portions of 1 ml or less. Handle sterile pipets only near the mouthpiece, and protect the delivery end from external contamination. Do not remove the cotton plug in the mouthpiece as this is intended to protect the user from ingesting any sample.

b When using the pipet to withdraw sample portions, do not dip the pipet more than 1/2 inch into the sample, otherwise sample running down the outside of the pipet will make measurements inaccurate.

c When delivering the sample into the culture medium, deliver sample portions of 1 ml or less down into

the culture tube near the surface of the medium. Do not deliver small sample volumes at the top of the tube and allow them to run down inside the tube; too much of the sample will fail to reach the culture medium.

d Prepare preliminary dilutions of samples for portions of 0.01 ml or less before delivery into the culture medium. See Table 2 for preparation of dilutions. NOTE: Always deliver diluted sample portions into the culture medium as soon as possible after preparation. The interval between preparation of dilution and introduction of sample into the medium never should be as much as 30 minutes.

6 After measuring all portions of the sample into their respective tubes of medium, gently shake the rack of inoculated tubes to insure good mixing of sample with the culture medium. Avoid vigorous shaking, as air bubbles may be shaken into the fermentation vials and thereby invalidate the test.

7 Place the rack of inoculated tubes in the incubator at 35° ± 0.5°C for 24 ± 2 hours.

B 24-hour Procedures

1 Remove the rack of lauryl tryptose broth cultures from the incubator, and shake gently. If gas is about to appear in the fermentation vials, the shaking will speed the process.

2 Examine each tube carefully. Record, in the column "24" under LST on the laboratory data sheet, each tube showing gas in the fermentation vial as a positive (+) test and each tube not showing gas as a negative (-) test. GAS IN ANY QUANTITY IS A POSITIVE TEST.

3 Retain all gas-positive tubes of lauryl tryptose broth culture in their place in the rack, and proceed.

4 Select the gas-positive tubes of lauryl tryptose broth culture for the Confirmed Test procedures. Confirmed Test procedures may not be required for all gas-positive cultures. If, after 24-hours of incubation, all five replicate cultures are gas-positive for two or more consecutive sample volumes, then select the set of five cultures representing the smallest volume of sample in which all tubes were gas-positive. Apply Confirmed Test procedures to all these cultures and to any other gas-positive cultures representing smaller volumes of sample, in which some tubes were gas-positive and some were gas-negative.

5 Label one tube of brilliant green lactose bile broth (BGLB) to correspond with each tube of lauryl tryptose broth selected for Confirmed Test procedures.

6 Gently shake the rack of Presumptive Test cultures. With a flame-sterilized inoculation loop transfer one loopful of culture from each gas-positive tube to the corresponding tube of BGLB Place each newly inoculated culture into BGLB in the position of the original gas-positive tube

7 If the Fecal Coliform Test is included in the testing procedure, consult Section V of this outline for details of the testing procedure.

8 After making the transfer, the rack should contain some 24-hour gas-negative tubes of lauryl tryptose borth and the newly inoculated BGLB. Incubate the rack of cultures at 35° C ± 0.5° C for 24 ± 2 hours.

C 48-hour Procedures

1 Remove the rack of culture tubes from the incubator, read and record gas production for each tube.

2 Some tubes will be lauryl tryptose broth and some will be brilliant green lactose

bile broth (BGLB). Be sure to record results from LTB under the 48-hour LTB column and the BGLB results under the 24-hour column of the data sheet.

3 Label tubes of BGLB to correspond with all (if any) 48-hour gas-positive cultures in lauryl tryptose broth. Transfer one loopful of culture from each gas-positive LTB culture to the correspondingly-labeled tube of BGLB. NOTE: All tubes of LTB culture which were negative at 24 hours and became positive at 48 hours are to be transferred. The Option described above for 24-hour LTB cultures does not apply at 48 hours.

4 Incubate the 24-hour gas-negative BGLB tubes and any newly-inoculated tubes of BGLB 24 ± 2 hours at 35° ± 0.5° C. Retain all 24-hour gas-positive cultures in BGLB for further test procedures.

5 Label a Petri dish preparation of eosin methylene blue agar (EMB agar) to correspond with each gas-positive culture in BGLB.

6 Prepare a streak plate for colony isolation from each gas-positive culture in BGLB on the correspondingly-labeled EMB agar plate.

Incubate the EMB agar plates 24 ± 2 hours at 35° ± 0.5° C.

D 72-hour Procedures

1 Remove the cultures from the incubator. Some may be on BGLB; several EMB agar plates also can be expected.

2 Examine and record gas production results for any cultures in BGLB.

3 Retain any gas-positive BGLB cultures and prepare streak plate inoculations for colony isolation in EMB agar. Incubate the EMB agar plates 24 ± 2 hours at 35 ± 0.5° C. Discard the gas-positive BGLB cultures after transfer.

4 Reincubate any gas-negative BGLB cultures 24 ± 2 hours at 35° ± 0.5° C.

5 Discard all 48-hour gas-negative BGLB cultures.

6 Examine the EMB agar plates for the type of colonies developed thereon. Well-isolated colonies having a dark center (when viewed from the lower side, held toward a light) are termed "nucleated or fisheye" colonies, and are regarded as "typical" coliform colonies. A surface sheen may or may not be present on "typical" colonies. Colonies which are pink or opaque but are not nucleated are regarded as "atypical colonies." Other colony types are considered "noncoliform." Read and record results as + for "typical" (nucleated) colonies ± for "atypical" (non-nucleated pink or opaque colonies), and - for other types of colonies which might develop.

7 With plates bearing "typical" colonies, select at least one well-isolated colony and transfer it to a correspondingly-labeled tube of lactose broth and to an agar slant. As a second choice, select at least two "atypical" colonies (if typical colonies are not present) and transfer them to labeled tubes of lactose broth and to agar slants. As a third choice, in the absence of typical or atypical coliform-like colonies, select at least two well-isolated colonies representative of those appearing on the EMB plate, and transfer them to lactose broth and to agar slants.

8 Incubate all cultures transfered from EMB agar plates 24 ± 2 hours at 35 ± 0.5° C.

E 96-hour Procedures

1 Subcultures from the samples being studied may include: 48-hour tubes of BGLB, EMB agar plates, lactose broth tubes, and agar slant cultures.

2 If any 48-hour tubes of BGLB are present, read and record gas production in the "48" column under BGLB. From any gas-positive BGLB cultures prepare streak plate inoculations for colony isolation on EMB agar. Discard all tubes of BGLB, and incubate EMB agar plates 24 ± 2 hours at 35 ± 0.5° C.

3 If any EMB plates are present, examine and record results in the "EMB" column of the data sheet. Make transfers to agar slants and to lactose broth from all EMB agar plate cultures. In decreasing order of preference, transfer at least one typical colony, or at least two atypical colonies, or at least two colonies representative of those on the plate.

4 Examine and record results from the lactose broth cultures.

5 Prepare a Gram-stained smear from each of the agar slant cultures, as follows:

NOTE: Always prepare Gram stain from an actively growing culture, preferably about 18 hours old, and never more than 24 hours old. Failure to observe this precaution often results in irregular staining reactions.

a Thoroughly clean a glass slide to free it of any trace of oily film.

b Place one drop of distilled water on the slide.

c Use the inoculation needle to suspend a tiny amount of growth from the nutrient agar slant culture in the drop of water.

d Mix the thin suspension of cells with the tip of the inoculation needle, and allow the water to evaporate.

e "Fix" the smear by gently warming the slide over a flame.

f Stain the smear by flooding it for 1 minute with crystal violet solution.

g Flush the excess crystal violet solution off in gently running water, and gently blot dry with filter paper or with other clean absorbent paper.

h Flood the smear with Lugol's iodine for 1 minute.

i Wash the slide in gently running water and blot dry with filter paper.

j Decolorize the smear with 95% alcohol solution with gentle agitation for 10-30 seconds, depending upon extent of removal of crystal violet dye, then blot dry.

k Counterstain for 10 seconds with safranin solution, then wash in running water and blot dry.

l Examine the slide under the microscope, using the oil immersion lens. Coliform bacteria are Gram-negative, nonspore-forming, rod-shaped cells, occurring singly, in pairs, or rarely in short chains.

m If typical coliform staining reaction and morphology are observed, record + in the appropriate space under the "Gram Stain" column of the data sheet. If typical morphology and staining reaction are not observed, then mark it ± or -, and make suitable comment in the "remarks" column at the right-hand side of the data sheet.

n If spore-forming bacteria are observed, it will be necessary to repurify the culture from which the observations were made. Consult the instructor, or refer to Standard Methods, for procedures.

At this point, it is possible that all cultural work for the Completed Test has been finished. If so, codify results and determine Completed Test coliforms per 100 ml.

F 120-hour Procedures and following:

1 Any procedures to be undertaken from this point are "straggler" cultures on media already described, and requiring step-by-step methodology already given in detail. Such cultures may be on: EMB plates, agar slants, or lactose broth. The same time-and-temperature of incubation required for earlier studies applies to the "stragglers" as do the observations, staining reactions, and interpretation of results. On conclusion of all cultural procedures, codify results and determine Completed Test coliforms per 100 ml.

V FECAL COLIFORM TEST

A General Information

1 The procedure described is an elevated temperature test for fecal coliform bacteria.

2 Equipment required for the tests are those required for the Presumptive Test of Standard Methods, a water-bath incubator, and the appropriate culture media.

B Fecal Coliform Test with EC Broth

1 Sample: The test is applied to gas-positive tubes from the Standard Methods Presumptive Test (lauryl tryptose broth), in parallel with Confirmed Test procedures.

2 24-hour Operations. Initial procedures are the planting procedures described for the Standard Methods Presumptive Coliform test.

a After reading and recording gas-production on lauryl tryptose broth, temporarily retain all gas-positive tubes.

b Label a tube of EC broth to correspond with each gas-positive tube of lauryl tryptose broth. The option regarding transfer of only a limited number of tubes to the Confirmed Test sometimes can be applied here. However, the worker is urged to avoid exercise of this option until he has assured the applicability of the option by preliminary tests on the sample source.

c Transfer one loopful of culture from each gas-positive culture in lauryl tryptose broth to the correspondingly labeled tube of EC broth.

d Incubate EC broth tubes 24 ± 2 hours at 44.5 ± 0.2°C in a waterbath with water depth sufficient to come up at least as high as the top of the culture medium in the tubes. Place in waterbath as soon as possible after inoculation and always within 30 minutes after inoculation.

3 48-hour operations

a Remove the rack of EC cultures from the waterbath, shake gently, and record gas production for each tube. Gas in any quantity is a positive test.

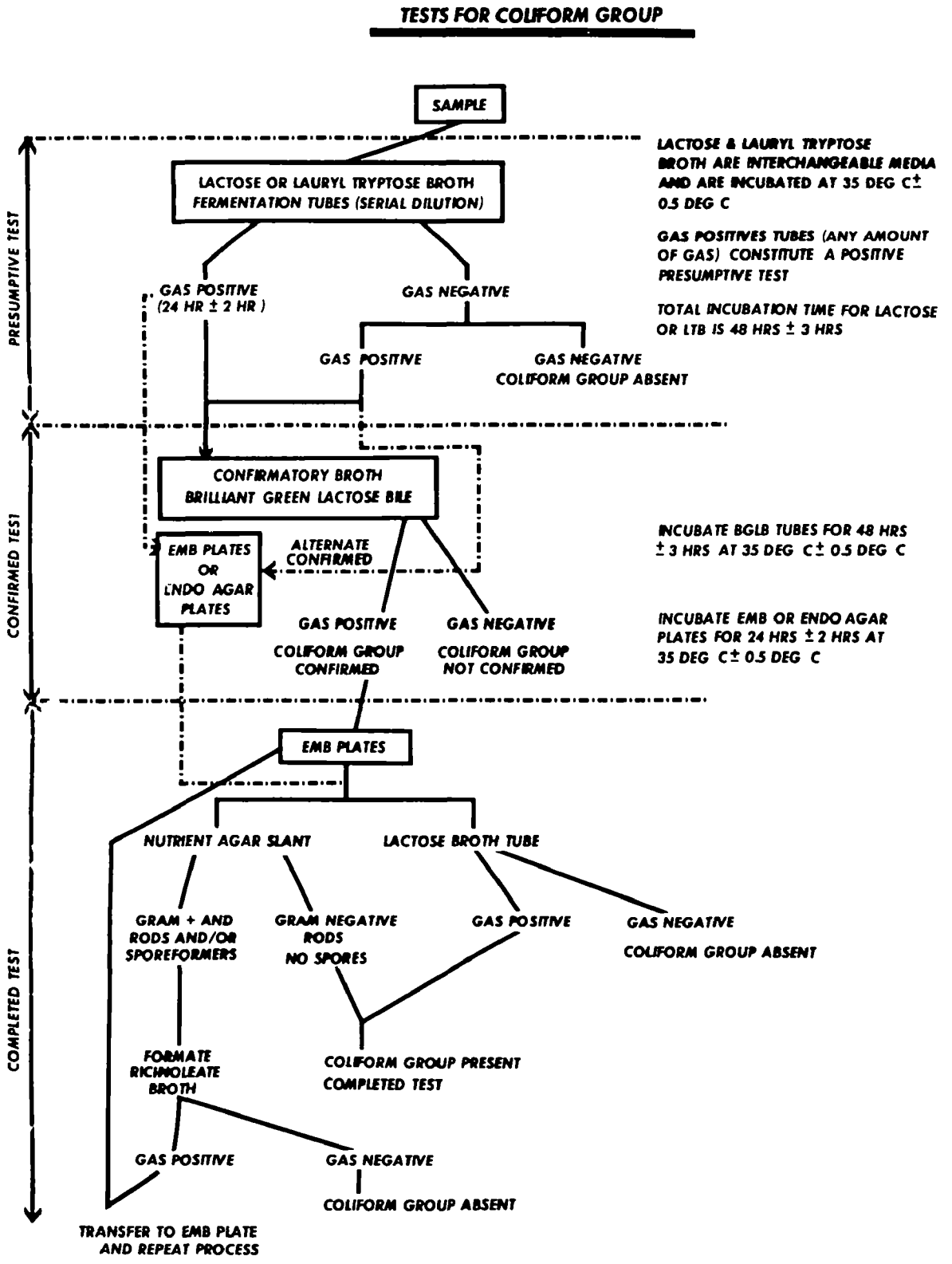
b As soon as results are recorded, discard all tubes. (This is a 24-hour test for EC broth inoculations and no a 48-hour test.)

c Transfer any additional 48-hour gas-positive tubes of lauryl tryptose broth to correspondingly labeled tubes of EC broth Incubate 24 ± 2 hours at 44.5 ± 0.2°C.

4 72-hour operations

a Read and record gas production for each tube. Discard all cultures.

b Codify results and determine fecal coliform count per 100 ml of sample.


TESTS FOR COLIFORM GROUP
SAMPLE
LACTOSE OR LAURYL TRYPTOSE BROTH FERMENTATION TUBES (SERIAL DILUTION)
LACTOSE & LAURYL TRYPTOSE BROTH ARE INTERCHANGEABLE MEDIA AND ARE INCUBATED AT 35 DEG C± 0.5 DEG C
GAS POSITIVES TUBES (ANY AMOUNT OF GAS) CONSTITUTE A POSITIVE PRESUMPTIVE TEST
TOTAL INCUBATION TIME FOR LACTOSE OR LTB IS 48 HRS ± 3 HRS
PRESUMPTIVE TEST
GAS POSITIVE (24 HR ± 2 HR)
GAS NEGATIVE
GAS POSITIVE
GAS NEGATIVE COLIFORM GROUP ABSENT
CONFIRMATORY BROTH BRILLIANT GREEN LACTOSE BILE
INCUBATE BGLB TUBES FOR 48 HRS ± 3 HRS AT 35 DEG C± 0.5 DEG C
CONFIRMED TEST
EMB PLATES OR ENDO AGAR PLATES
ALTERNATE CONFIRMED
GAS POSITIVE COLIFORM GROUP CONFIRMED
GAS NEGATIVE COLIFORM GROUP NOT CONFIRMED
INCUBATE EMB OR ENDO AGAR PLATES FOR 24 HRS ± 2 HRS AT 35 DEG C± 0.5 DEG C
EMB PLATES
NUTRIENT AGAR SLANT
LACTOSE BROTH TUBE
GRAM + AND RODS AND/OR SPOREFORMERS
GRAM NEGATIVE RODS NO SPORES
GAS POSITIVE
GAS NEGATIVE COLIFORM GROUP ABSENT
COMPLETED TEST
FORMATE RICINOLEATE BROTH
COLIFORM GROUP PRESENT COMPLETED TEST
GAS POSITIVE
GAS NEGATIVE
COLIFORM GROUP ABSENT
TRANSFER TO EMB PLATE AND REPEAT PROCESS

Part 3
LABORATORY METHODS FOR FECAL STREPTOCOCCUS
(Day-By-Day Procedures)

I GENERAL INFORMATION

A The same sampling and holding procedures apply as for the coliform test.

B The number of fecal streptococci in water generally is lower than the number of coliform bacteria. It is good practice in multiple dilution tube tests to start the sample planting series with one sample increment larger than for the coliform test. For example: If a sample planting series of 1.0, 0.1, 0.01, and 0.001 ml is planned for the coliform test, it is suggested that a series of 10, 1.0, 0.1, and 0.01 ml be planted for the fecal streptococcus test.

C Equipment required for the test is the same as required for the Standard Methods Presumptive and Confirmed Tests, except for the differences in culture media.

II STANDARD METHODS (Tentative) PROCEDURES

A First-Day Operations

1 Prepare the sample data sheet and labeled tubes of azide dextrose broth in the same manner as for the Presumptive Test. NOTE: If 10-ml samples are included in the series, be sure to use a special concentration (ordinarily double-strength) of azide dextrose broth for these sample portions.

2 Shake the sample vigorously, approximately 25 times, in an up-and-down motion.

3 Measure the predetermined sample volumes into the labeled tubes of azide dextrose broth, using the sample measurement and delivery techniques used for the Presumptive Test.

4 Shake the rack of tubes of inoculated culture media, to insure good mixing of sample with medium.

5 Place the rack of inoculated tubes in the incubator at 35° ± 0.5° C for 24 ± 2 hours.

B 24-hour Operations

1 Remove the rack of tubes from the incubator. Read and record the results from each tube. Growth is a positive test with this test. Evidence of growth consists either of turbidity of the medium, a "button" of sediment at the bottom of the culture tube, or both.

2 Label a tube of ethyl violet azide broth to correspond with each positive culture of azide dextrose broth. It may be permissible to use the same confirmatory option as described for the coliform Confirmed Test, in this outline.

3 Shake the rack of cultures gently, to resuspend any living cells which have settled out to the bottom of the culture tubes

4 Transfer three loopfuls of culture from each growth-positive tube of azide dextrose broth to the correspondingly labeled tube of ethyl violet azide broth.

5 As transfers are made, place the newly inoculated tubes of ethyl violet azide broth in the positions in the rack formerly occupied by the growth-positive tubes of azide dextrose broth. Discard the tubes of azide dextrose broth culture.

6 Return the rack, containing 24-hour growth-negative azide dextrose broth tubes and newly-inoculated tubes of ethyl violet azide broth, to the incubator. Incubate 24 ± 2 hours at 35° ± 0.5° C.

C 48-hour Operations

1 Remove the rack of tubes from the incubator. Read and report results. Growth, either in azide dextrose broth or in ethyl violet azide broth, is a positive test. Be sure to report the results of the azide dextrose broth medium under the "48" column for that medium and the results of the ethyl violet azide broth cultures under the "24" column for that medium.

2 Any 48-hour growth-positive cultures of azide dextrose broth are to be transferred (three loopfulls) to ethyl violet azide broth. Discard all 48-hour growth-negative tubes of azide dextrose broth and all 24-hour growth-positive tubes of ethyl violet azide broth

3 Incubate the 24-hour growth-negative and the newly-inoculated tubes of ethyl violet azide broth 24 $\pm$ 2 hours at 35° $\pm$ 0.5°C.

D 72-hour Operations

1 Read and report growth results of all tubes of ethyl violet azide broth.

2 Discard all growth-positive cultures and all 48-hour growth-negative cultures.

3 Reincubate any 24-hour growth-negative cultures in ethyl violet azide broth 24 $\pm$ 2 hours at 35° $\pm$ 0.5°C.

E 96-hour Operations

1 Read and report growth results of any remaining tubes of ethyl violet azide broth.

2 Codify results and determine fecal streptococci per 100 ml.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater (13th Ed). Prepared and published jointly by American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1971.

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3 Geldreich, E.E., Bordner, R.H., Huff, C.B., Clark, H.F. and Kabler, P.W. Type Distribution of Coliform Bacteria in the Feces of Warm-Blooded Animals. J. Water Pollution Control Federation. 34:295-301. 1962.

4 Recommend Proc. for the Bacteriological Examination of Sea Water and Shellfish 3rd Edition, American Public Health Association. 1962.

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USE OF TABLES OF MOST PROBABLE NUMBERS

Part 1

I INTRODUCTION

A Using probability mathematics, it is possible to estimate the number of bacteria producing the observed result for any combination of positive and negative results in dilution tube tests. Because the computations are so repetitious and time-consuming, it is common laboratory practice to use Tables of Most Probable Numbers. These tables are orderly arrangements of the possible cultural results obtainable from inoculating various sample increments in differential culture media. Each possible combination of positive and negative tube results is accompanied by the result (MPN) of the calculated estimate and the 95% confidence limits of the MPN.

B The Tables of Most Probable Numbers used in the current (13th) edition of Standard Methods for the Examination of Water and Wastewater were developed by Swaroop.[1] Previous editions of Standard Methods have used the tables prepared by Hoskins.[2]

1 Most of the tables are based on using 3 sample volumes in decreasing decimal increments. Thus, the systems are based on using volumes of 10 ml, 1.0 ml, and 0.1 ml, etc. Other quantity relationships can be used, such as 50 ml, 10 ml, and 1.0 ml in a table. Tables of Most Probable Numbers can be prepared for any desired series of sample increments.

2 In addition, tables can be devised for different numbers of replicate inoculations of individual sample volumes. For example, the MPN Table most commonly used in the laboratories of this agency is based on five replicate 10 ml portions, five 1.0 ml portions, and five 0.1 ml portions A separate table is required for another combination of sample volumes, consisting of five replicate 10 ml portions, one 1.0 ml portion, and one 0.1 ml portion. This is popular in bacteriological potability tests on water. MPN Tables can be prepared for any desired combinations of replicates of the sample increments used in a dilution tube series.

3 An approximation of the MPN values shown in the Tables can be obtained by a simple calculation, developed by Thomas.[3] The formula and application of this calculation is shown on a later page of this chapter.

C The method of using a Table of Most Probable Numbers is described here, based on the table for five 10 ml portions, five 1.0 portions, and five 0.1 portions. The principles apply equally to the other tables presented in the current edition of Standard Methods for the Examination of Water and Wastewater.

II DETERMINING THE MOST PROBABLE NUMBER

A Codifying Results of the Dilution Tube Series

If five 10 ml portions, five 1.0 ml portions, and five 0.1 ml portions are inoculated initially, and positive results are secured from five of the 10 ml portions, three of the 1.0 ml portions and none of the 0.1 ml portions, then the coded result of the test is 5-3-0. The code can be looked up in the MPN Table, and the MPN per 100 ml is recorded directly. If more than the above three sample volumes are to be considered, then the determination of the coded result may be more complex. The examples described in Table 1 are useful guides for selection of the significant series of three sample volumes.

Table 1. EXAMPLES OF CODED RESULTS

No. ml sample per tube →	100	10	1.0	0.1	0.01	0.001	Code	See Below
No. tubes per sample vol. →	5	5	5	5	5	5		
No. tubes in sample giving positive results in test		5	4	1			5-4-1	
	5	5	4	0	0	0	5-4-0	(1)
		4	1	0	0	0	4-1-0	(2)
	5	5	4	1	1	0	5-4-2	(3)
		5	5	5	4		5-5-4	(4)
		5	5	5	5		5-5-5	(5)
		0	0	0	0		0-0-0	(6)
		0	1	0	0		0-1-0	(7)
		1	0	0	0		1-0-0	(8)

Discussion of examples:

1 When all the inoculated tubes of more than one of the decimal series give positive results, then it is customary to select the smallest sample volume (here, 10 ml) in which all tubes gave positive results. The results of this volume and the next lesser volumes are used to determine the coded result.

2 When none of the sample volumes give positive results in all increments of the series, then the results obtained are used to designate the code. Note that it is not permissible to assume that if the next larger increment had been inoculated, all tubes probably would have given positive results and therefore assign a 5-4-1 code to the results.

3 Here the results are spread through four of the sample volumes. In such cases, the number of positive tubes in the smallest sample volume is added to the number of tubes in the third sample volume (counting down from the smallest sample volume in which all tubes gave positive results).

4 Here it is necessary to use the 5-5-4 code, because inoculations were not made of 0.001 ml sample volumes, and it is not permissible to assume that if such sample volumes had been inoculated, they would have given negative results, or any other arbitrarily-designated result.

5 This is an indeterminate result. Many MPN tables do not give a value for such a result. If the table used does not have the code, then look up the result for code 5-5-4, and report the result "greater than" the value shown for the 5-5-4 code. The first number of the 5-5-4 code is based on the 1.0 ml sample volume.

6 Like (5), this is an indeterminate result. If the code does not appear in the table being used, then look up the result for code 1-0-0, and report the MPN as "less than" the value shown for the 1-0-0 code.

7 The current edition of Standard Methods stipulates this type of code designation when unusual results such as this occur.

8 Note the difference from (7) above. Inoculations of 100 ml portions were not made, and it cannot be assumed that the result would have called for code 0-1-0.

B Computing and Recording the MPN

When the dilution tube results have been codified, they are read and recorded from the appropriate MPN Table.

1 If, as in the first four of the examples shown under (A) the first number in the coded result represents a 10 ml sample volume, then the MPN per 100 ml is read and recorded directly from the appropriate column in the table.

2 On the other hand, if the first number in the coded result represents a sample volume other than 10 ml, then a calculation is required to give the corrected MPN. For example (4) under (A) above, the first "5" of the 5-5-4 code represents a sample volume of 1.0 ml. Look up the 5-5-4 code as if the 1.0 ml volume actually were 10 ml, as if the 0.1 ml volume actually were 1.0 ml and as if the 0.01 ml volume actually were 0.1 ml. The MPN obtained (1600) then is multiplied by a factor of 10 to give the corrected value. A simple formula for this type of correction is shown on a later page of this chapter.

III PRECISION OF THE MPN VALUE

A The current edition of Standard Methods shows for each MPN value, the 95% confidence limits for that value. This draws attention to the fact that a given MPN value is not a precise measurement, but an estimate. The 95% confidence limits means that the observer will be correct 95% of the time when he considers that the actual number of cells producing the observed combination of positive and negative tubes was somewhere between the stated upper limit and the stated lower limit.

B The greater the number of replicates of each sample volume in a dilution series, the greater the precision (in other words, the narrower the limits of the 95% confidence range) of the test. The precision of results, based on numbers of tubes inoculated per sample volume, is shown in Table 2.

C Woodward [4] and other workers have studied the precision of the MPN in detail. Such reports should be studied by those desiring further information regarding the precision of the MPN test.

Table 2. Approximate Confidence Limits for Bacterial Densities as Per Cent of MPN as Determined from Various Numbers of Tubes in Three Decimal Dilutions*

Number of tubes in each dilution	50%		75%		80%		90%		95%	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
1	33	186	18	340	15	402	10	637	6.5	955
2	47	160	31	246	27	276	20	383	15	511
3	53	150	38	215	34	237	26	311	21	395
5	64	139	49	182	46	196	37	241	31	289
10	76	127	63	152	60	160	52	184	46	208

*The interpretation of these figures is as follows: When MPN estimates are made on the basis of dilution tests using one tube in each of three decimal dilutions, you will be right 50% of the time if you say that the true bacterial density is between 33% and 186% of the MPN. If you had used 5 tubes in each dilution you could reduce this interval to from 64% to 139% of the MPN and still be right 50% of the time. If a greater certainty were desired, say 95%, you would have to widen this interval to from 31% to 289%.

IV OCCURRENCE OF IMPROBABLE TUBE RESULTS

A Many of the theoretically possible tube results are omitted from the MPN Table. For example, codes 0-0-3, 0-0-4, and 0-0-5 are not included as well as many others. These are omitted, because, in the opinion of the authors of the tables, the probability of occurrence of such results is so low as to exclude them from practical consideration.

B The frequency of occurrence of various code results is shown in the Table 2 both on a theoretical basis and on the basis of actual laboratory experience.

C From the MPN tables, it can be inferred that the codes omitted from the MPN Table can be expected to occur up to 1% of the time. If, in reviewing laboratory data, the theoretically unlikely codes occur appreciably more than 1% of the time, there is an indication for inquiry into the causes. Such results can occur (1) as a consequence of faulty laboratory procedures, or (2) as a result of extraneous influences in the samples.

D The current edition of Standard Methods does not include MPN values for many rare combinations listed in previous editions. By pruning out those codes listed as Group IV in Table 3, the table has been considerably condensed. Table 4 suggests maximum permissible numbers of samples for various numbers of samples tested.

Table 3

FIVE-TUBE AND THREE-TUBE CODES THAT INCLUDE 99 PER CENT OF ALL RESULTS

Group	Theoretically Expected Percentage of Results	Theoretically Expected Cumulative Percentage	Observed Percentage of 360 Samples
	Five-Tube-Test		
Class 1 codes 550, 551, 552, 553, 554, 500, 510, 520, 530, 540, 100, 200, 300, 400	67 5	67.5	68 0
Class 2 codes 511, 521, 531, 541, 542, 110, 210, 310, 410, 420	23.6	91 1	23 1
Class 3 codes 501, 010, 532, 320, 522, 220, 543, 430, 120, 533, 330, 502, 020, 544, 440, 301, 401, 431, 201, 411, 101, 311, 421, 211, 001	7 9	99 0	7.5
Improbable codes	1 0	100 0	1 4
	Three-Tube Test		
Class 1 codes 330, 331, 332, 300, 310, 320, 100, 200	81 5	81.5	81 7
Class 2 codes 321, 311, 301, 210, 110, 010.	14.9	96.4	14 1
Class 3 codes 322, 220, 201, 101 312, 120	2 7	99.1	3 7
Improbable codes	0.9	100.0	0.6

Table 4

MAXIMUM PERMISSIBLE NUMBERS OF IMPROBABLE CODES FOR VARIOUS NUMBERS OF SAMPLES TESTED

Number of Samples	Maximum Number of Improbable Codes
1 - 15	1
16 - 45	2
46 - 83	3
84 - 130	4
131 - 180	5
181 - 233	6
234 - 290	7
291 - 350	8
351 - 413	9
414 - 477	10
478 - 543	11

E Table 5 is from International Standards for Drinking-Water, published by the World Health Organization, Geneva (1958). The last three values, not shown in the WHO publication, are from Woodward, "How Probable is the Most Probable Number."(4)

F Several theoretically possible combinations of positive tube results are omitted in Table 5. These combinations are omitted because the statistical probability of occurrence of any of the missing results is less than 1%. If such theoretically unlikely tube combinations occur in more than 1% of samples, there is need for review of the laboratory procedures and of the nature of the samples being tested.

When the series of decimal dilutions is other than 10, 1.0 and 0.1 ml, use the MPN in Table 5 according to the following formula:

$$\text{MPN (from table)} \times \frac{10}{\text{Largest quantity tested}}$$

$$= \text{MPN/100 ml}$$

Example: From a sample of water, 5 out of five 0.01 - ml portions, 2 out of five 0.001 - ml portions, and 0 out of five 0.0001 - ml portions, gave positive reactions.

From the code 5-2-0 in the MPN table, the MPN index is 49

$$\underset{\text{(from table)}}{49} \times \frac{10}{0.01} = 49,000$$

MPN Index = 49,000

A simple approximation of the most probable number may be obtained from the following formula (after Thomas):

$$\text{MPN/100 ml} = \frac{\text{No. of Positive Tubes} \times 100}{\sqrt{\text{No. of ml in negative tubes)} \times \text{(No. of ml in all tubes)}}}$$

Example: From a sample of water, 5 out of five 10 - ml portions, 2 out of five 1.0 ml portions, and 0 out of five 0.1 ml portions gave positive results.

$$\text{MPN/100 ml} = \frac{7 \times 100}{\sqrt{(3.5) \times (55.5)}} = 50.22$$

MPN/100 ml = 50

Note that the MPN obtained from the table on the preceding pages with these tube results is 49. "Most probable numbers computed by the above formula deviate from values given by the usual methods by amounts which ordinarily are insignificant. The formula is not restricted as to the number of tubes and dilutions used ---" (Thomas)

Table 5. MPN AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS IN A PLANTING SERIES OF FIVE 10-ml, FIVE 1-ml AND FIVE 0.1-ml PORTIONS OF SAMPLE

Number of tubes giving positive reaction out of			MPN Index (organisms per 100 ml)	Confidence Limits (95%)	
Five 10-ml portions	Five 1-ml portions	Five 0.1-ml portions		Lower limit	Upper limit
0	0	0	<2		
0	0	1	2	<0.5	7
*0	0	2	4	<0.5	11
0	1	0	2	<0.5	7
*0	1	1	4	<0.5	11
*0	1	2	6	<0.5	15
0	2	0	4	<0.5	11
*0	2	1	6	<0.5	15
*0	3	0	6	<0.5	15
1	0	0	2	<0.5	7
1	0	1	4	<0.5	11
*1	0	2	6	<0.5	15
*1	0	3	8	1	19
1	1	0	4	<0.5	11
1	1	1	6	<0.5	15
*1	1	2	8	1	19
1	2	0	6	<0.5	15
*1	2	1	8	1	19
*1	2	2	10	2	23
*1	3	0	8	1	19
*1	3	1	10	2	23
*1	4	0	11	2	25
2	0	0	5	<0.5	13
2	0	1	7	1	17
*2	0	2	9	2	21
*2	0	3	12	3	28
2	1	0	7	1	17
2	1	1	9	2	21
*2	1	2	12	3	28
2	2	0	9	2	21
*2	2	1	12	3	28
*2	2	2	14	4	34
2	3	0	12	3	28
*2	3	1	14	4	34
*2	4	0	15	4	37
3	0	0	8	1	19
3	0	1	11	2	25
*3	0	2	13	3	31
3	1	0	11	2	25
3	1	1	14	4	34
*3	1	2	17	5	46
*3	1	3	20	6	60
3	2	0	14	4	34
3	2	1	17	5	46
*3	2	2	20	6	60
3	3	0	17	5	46
*3	3	1	21	7	63
*3	4	0	21	7	63
*3	4	1	24	8	72

*Omitted from 12th Edition Standard Methods for Water and Wastewater

Table 5. (Cont'd) MPN AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS IN A PLANTING SERIES OF FIVE 10-ml, FIVE 1-ml AND FIVE 0.1-ml PORTIONS OF SAMPLE

Number of tubes giving positive reaction out of			MPN Index (organisms per 100 ml)	Confidence Limits 95%	
Five 10-ml portions	Five 1-ml portions	Five 0.1-ml portions		Lower limit	Upper limit
*3	5	0	25	8	75
4	0	0	13	3	31
4	0	1	17	5	46
*4	0	2	21	7	63
*4	0	3	25	8	75
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
*4	2	2	32	11	91
4	3	0	27	9	80
4	3	1	33	11	93
*4	3	2	39	13	106
4	4	0	34	12	96
*4	4	1	40	14	108
*4	5	0	41	14	110
*4	5	1	48	16	124
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	114
*5	0	3	58	19	144
*5	0	4	76	24	180
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	154
*5	1	3	84	26	197
5	2	0	49	17	126
5	2	1	70	23	168
5	2	2	94	28	219
*5	2	3	120	33	284
*5	2	4	148	38	366
*5	2	5	177	44	515
5	3	0	79	25	187
5	3	1	109	31	253
5	3	2	141	37	343
5	3	3	175	44	503
*5	3	4	212	53	669
*5	3	5	253	77	788
5	4	0	130	35	302
5	4	1	172	43	486
5	4	2	221	57	698
5	4	3	278	90	849
5	4	4	345	117	999
*5	4	5	426	145	1161
5	5	0	240	68	754
5	5	1	348	118	1005
5	5	2	542	180	1405
5	5	3	920	210	3000
5	5	4	1600	350	5300
*5	5	5	>2400	800	--

*Omitted from 12th Edition Standard Methods for Water and Wastewater

Table 6. MPN AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS IN A PLANTING SERIES OF FIVE 10-ml PORTIONS OF SAMPLE

No. of Positive Tubes Out of: Five 10-ml Tubes	MPN per 100 ml	Limits of MPN	
		Lower	Upper
0	2.2	0	6.0
1	2.2	0.1	12.6
2	5 1	0.5	19.2
3	9.2	1.6	29.4
4	16 0	3.3	52.9
5	> 16	8.0	

Table 7. MPN AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS IN A PLANTING SERIES OF FIVE 10-ml, ONE 1-ml, AND ONE 0 1-ml PORTIONS OF SAMPLE

No of Positive Tubes Out of·			MPN per 100 ml	Limits of MPN	
Five 10-ml Tubes	One 1-ml Tube	One 0.1-ml Tube		Lower	Upper
0	0	0	<2		5.9
0	1	0	2	0 050	13
1	0	0	2 2	0.050	13
1	1	0	4.4	0.52	14
2	0	0	5	0.54	19
2	1	0	7.6	1.5	19
3	0	0	8.8	1.6	29
3	1	0	12	3.1	30
4	0	0	15	3.3	46
4	0	1	20	5.9	48
4	1	0	21	6.0	53
5	0	0	38	6.4	330
5	0	1	96	12	370
5	1	0	240	12	3700
5	1	1	>240	88	

IV TABLES OF MOST PROBABLE NUMBERS

These tables consist of the MPN indices and 95% confidence limits, within which the actual number of organisms can lie, for various combinations of positive and negative tubes. Three MPN tables are presented. Table 5 is based on five 10 ml five 1.0 ml and five 0.1 ml sample portions. Table 6 is based on five 10 ml sample portions; and Table 7 is based on five 10 ml, one 1 ml and one 0.1 ml sample portion.

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5 Standard Methods for the Examination of Water and Wastewater. 13th Edition. Prepared and Published Jointly by American Public Association. American Water Works Association, and Water Pollution Control Federation.

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MEMBRANE FILTER EQUIPMENT AND ITS PREPARATION FOR LABORATORY USE

I Some equipment and supplies used in the bacteriological examination of water with membrane filters are specific for the method Other items are standard in most well-equipped bacteriological laboratories and are readily adapted to membrane filter work. This chapter describes needed equipment and methods for its preparation for laboratory use Where more than one kind of item is available or acceptable for a given function, sufficient descriptive information is provided to aid the worker in selecting the one best suited to his own needs

II EQUIPMENT FOR SAMPLE FILTRATION AND INCUBATION

A Filter Holding Unit

1 The filter holding unit is a device for supporting the membrane filter and for holding the sample until it passes through the filter. During filtration the sample passes through a circular area, usually about 35 mm in diameter, in the center of the filter The outer part of the filter disk is clamped between the two essential components of the filter holding unit (See Plate 1)

a The lower element, called the filter base, or receptacle, supports the membrane filter on a plate about 50 mm in diameter The central part of this plate is a porous disk to allow free passage of liquids. The outer part of the plate is a smooth nonporous surface. The lower element includes fittings for mounting the unit in a suction flask or other container suitable for filtration with vacuum

b The upper element, usually called the funnel, holds the sample until it is drawn through the filter. Its lower portion is a flat ring that rests on the outer part of the membrane filter disk, directly over the non-porous part of the filter support plate.

c The assembled filter holding unit is joined by a locking ring or by one or more clamps

2 Characteristics of filter holding units should include·

a The design of filter holding units should provide for filtration with vacuum.

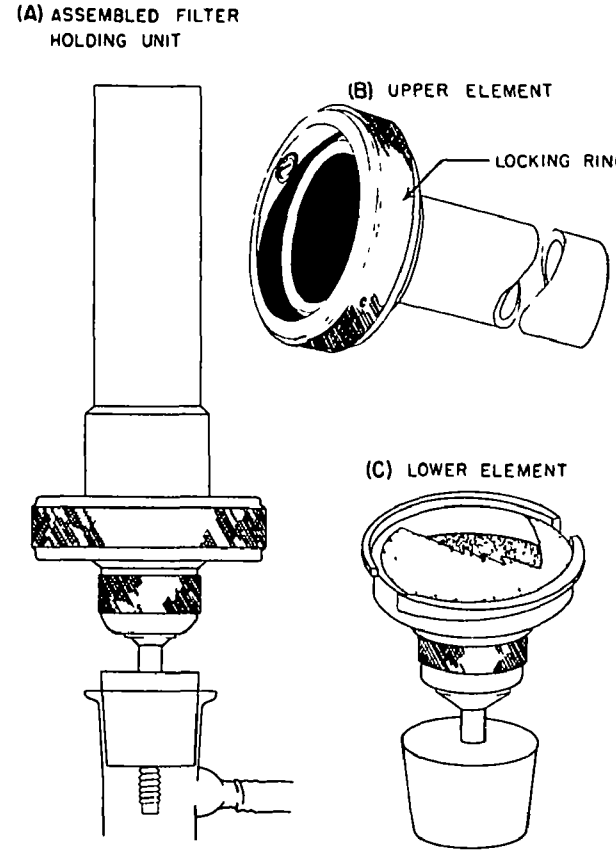

PLATE 1

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the OWP and the Environmental Protection Agency.

b Filter holding units may be made of glass, porcelain, plastic, non-corrosive metal, or other impervious material.

c Filter holding units should be made of bacteriologically inert materials.

d All surfaces of the filter holding assembly in contact with the water sample prior to its passage through the membrane filter should be uniformly smooth and free from corrugations, seams, or other surface irregularities that could become lodging places for bacteria.

e Filter holding units should be easily sterilized by routine methods.

f The filter holding unit should be easily and quickly assembled and disassembled in routine operational use.

g Filter holding units should be durable and inexpensive Maintenance should be simple.

3 Several forms of filter holding units have been developed for use with aqueous suspensions.

a SS 47 Membrane Filter Holder (Plate 2, Figure 1)

Conical-shape funnel with a 500 ml capacity. The base section includes a wirescreen membrane support. Funnel and base section are evenly joined by a locking ring mechanism. This assembly is designed to hold a 47 mm diameter membrane firmly in place allowing an effective filter area of approximately 9.6 square centimeters. The entire filter unit is made of stainless steel with the funnel interior having a mirror-like finish

b The Millipore Pyrex Filter Holder (Plate 2, Figure 2)

The unit is made of pyrex glass with coarse grade fitted support in base for filter The upper element of early models of glass filter holders had a capacity of 1 liter. Currently available units are supplied with upper elements having 300 ml capacity The assembled filter holder is joined with a spring clamp which engages on flat surfaces encircling the upper and lower elements.

c Millipore Standard Hydrosol Filter Holder (Plate 2, Figure 3)

Most components of this unit are made of stainless metal. The porous membrane support plate is fine-mesh stainless steel screening The upper element is a straight-sided cylinder 4 to 5 inches in diameter, constricted to a narrow cylinder at the bottom, to fit the lower element Capacity of the funnel element is about 1 liter The assembled filter holding unit is joined by a bayonet joint and locking ring. Accessories may be obtained for collection of small amounts of filtrate and for anhydrous sterilization of the filter holding assembly.

d Gelman "Parabella Vacuum Funnel" (Plate 2, Figure 5)

The unit is made of spun stainless steel The locking ring is a bayonet-type fitting, and is spring-loaded. The funnel element has a 1-liter capacity

e The Sabro Membrane Filter Holder (Plate 2, Figure 4)

The unit is mostly of stainless steel construction. The lower element is a combination vacuum chamber, filtrate receiver, and filter supporting element It consists of a stainless steel cup with a metal cover The cover is fitted with a rubber gasket permitting airtight fit of the cover into the top of the cup. A porous sintered stainless steel membrane support disk is mounted in the center of the cover At the side

of the beaker is a valve to which a pumping device can be fitted. The upper element is a stainless steel funnel with about 500 ml capacity. The assembled filter holding unit is joined by a locking ring at the base of the upper element. This engages on three spring clamps on the covering plate of the lower element.

f Millipore "Sterifil" filter unit (Plate 2, Figure 6)

A funnel and flask unit of polycarbonate with filter base and support of polypropylene. Manufacturers tables should be referred to regarding chemicals which may be present in the sample and their effect on the holder and flask elements. This unit can be safely sterilized under steam pressure.

FILTER HOLDING UNITS FOR AQUEOUS SUSPENSIONS

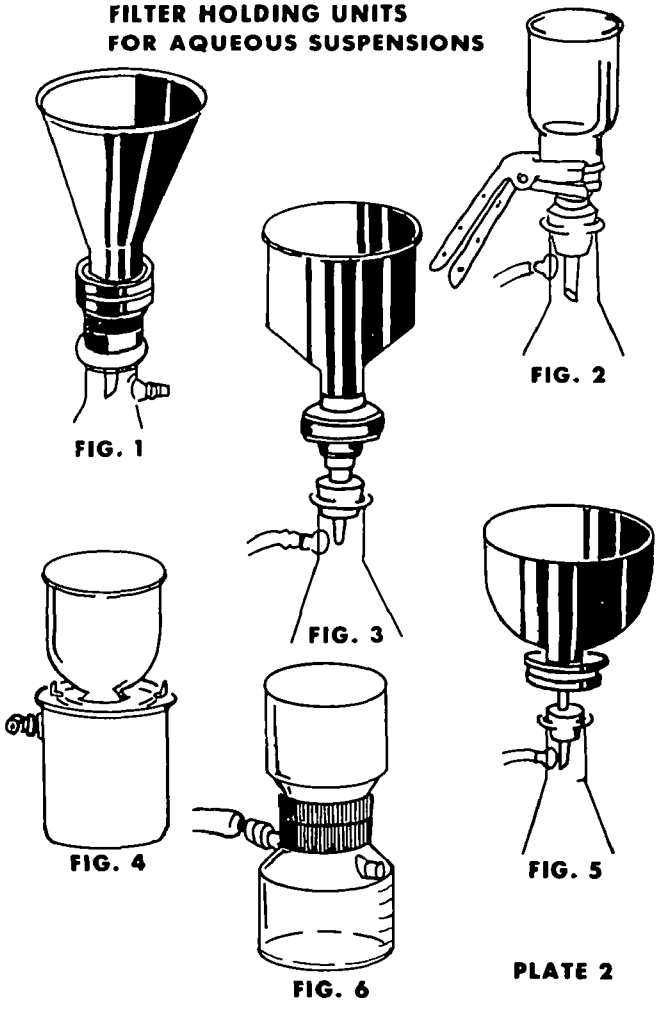

PLATE 2

4 Care and maintenance of filter holding units

a Filter holding units should be kept clean and free of accumulated foreign deposits.

b Metal filter holding units should be protected from scratches or other physical damage which could result in formation of surface irregularities. The surfaces in contact with membrane filters should receive particular care to avoid formation of shreds of metal or other irregularities which could cause physical damage to the extremely delicate filters.

c Some filter holding units have rubber components. The rubber parts may in time become worn, hardened, or cracked, necessitating replacement of the rubber part involved

d The locking rings used in some kinds of filter holders have two or more small wheels or rollers which engage on parts of the filter holding assembly Occasional adjustment or cleaning is necessary to insure that the wheels turn freely and function properly On some units, the wheels are plastic, and are not intended to turn. When worn flat, they should be loosened, turned a partial turn, and tightened again.

B Membrane Filters and Absorbent Pads

1 The desired properties of membrane filters have been discussed elsewhere Typical examples, commercially available include:

a Millipore Filters, Type HA, white, grid-marked, 47 mm in diameter

b S & S Type B-9, white, black-grid mark, 47 mm diameter

c Oxoid cellulose acetate membrane filters, 4.7 cm, grid-marked

2 An absorbent pad for nutrient is a paper filter disk, usually the same diameter as the membrane filter. Absorbent pads must be free of soluble chemical substances which could interfere with bacterial growth. They should be of such thickness that they will retain 1.8 - 2.2 ml of liquid culture medium. During incubation of cultures on membrane filters an absorbent pad saturated with liquid culture medium is the substrate for each filter. Absorbent pads are supplied with the purchase of membrane filters. Additional absorbent pads may be purchased separately. Sterilization in an autoclave is recommended for absorbent pads.

C Vacuum

Water can be filtered through a membrane filter by gravity alone, but the filtration rate would be too slow to be practical. For routine laboratory practice, two convenient methods are available for obtaining vacuum to hasten sample filtration.

1 An electric vacuum pump may be used connected to a filtration apparatus mounted in a suction flask. The pump need not be a high-efficiency type. For protection of the pump, a water trap should be included in the system, between the filtration apparatus and the vacuum pump

2 A water pump, the so-called "aspirator" gives a satisfactory vacuum, provided there is reasonably high water pressure.

3 In emergency, a rubber suction bulb, a hand pump, or a syringe, may be used for vacuum. It will be necessary to include some form of valve system to prevent return flow of air.

D Culture Containers (Plate 3)

Most membrane filter cultures are incubated in individual containers.

Almost any form of culture container is acceptable if it is made of impervious bacteriologically inert material. The culture container should, or course, be large enough to permit the membrane filters to lie perfectly flat. The following are widely used:

1 Glass petri dishes

Conventional borosilicate glass culture dishes are widely used in laboratory applications of membrane filters. For routine work, 60 mm X 15 mm petri dishes are recommended. The common 100 mm X 15 mm petri dishes are acceptable, but are subject to difficulties.

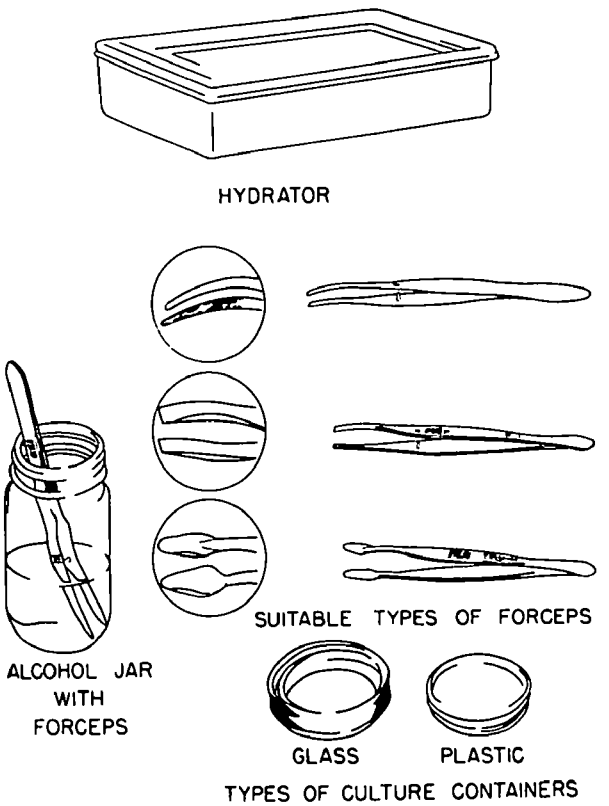

PLATE 3

2 Plastic petri dishes

Plastic containers have been developed for use with membrane filter cultures. Their cost is fairly low, and single-service use feasible. They cannot be heat-sterilized, but are supplied sterile. They must be free from soluble toxic substances. They can either be loose-fitting or of a tight lid to base friction fit.

E Other Equipment and Supplies Associated with Sample Filtration

1 Suction flask (Plate 4)

a Most types of filter holding apparatus are fitted in a conventional suction flask for sample filtration. While other sizes may be used, the 1-liter size is most satisfactory.

b The suction flask can be connected to the vacuum facility with thick-walled rubber tubing. Latex rubber tubing, 3/16" inside diameter, with wall thickness 3/32", is suggested. This tubing does not collapse under vacuum, yet it is readily closed with a pinch clamp.

c A pinch clamp on the rubber tubing is a convenient means of cutting off the vacuum from the suction flask during intervals when samples are not actually being filtered. It is most convenient to have the vacuum facility in continuous operation during sample filtration work.

d In laboratories conducting a high volume of filtration work, the suction flask may be dispensed. Filter-holding manifolds are available to receive up to three filtration units. The filtrate water is collected in a trap (in series with the vacuum source) which is periodically emptied.

e Another arrangement can be made for dispensing with the suction flask. In this case, the receptacle element of the filtration unit is mounted in the bench top. Instead of using a suction flask, the lower element of the filter holding unit has a dual connection with the vacuum source and with the laboratory drain. A solenoid-operated valve is used to determine whether the vacuum system or the drain line is in series with the filtration unit.

2 Ring stand with split ring (Optional) (See Plate 4)

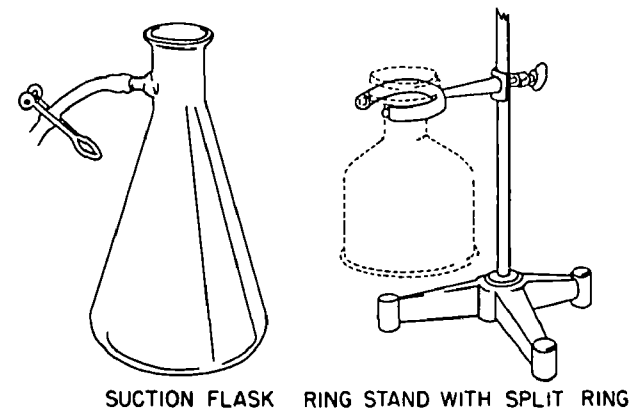

PLATE 4

When the filter holding unit is disassembled after sample filtration, the worker's hands must be free to manipulate the membrane filter. Upon disassembly of the filter holding unit, many workers place the funnel element, inverted, on the laboratory bench. Some workers, to prevent bacterial contamination, prefer a rack or a support to keep the funnel element from any possible source of contamination. A split ring on a ring stand is a convenient rack for this purpose.

3 Graduated cylinders

In laboratory practice, 100 ml graduated borosilicate glass cylinders are satisfactory for measurement of samples greater than 20 ml.

4 Pipettes and cans

a Graduated Mohr pipettes are needed for many procedures, such as measurement of small samples, and for preparing and dispensing culture media. Pipettes should be available in 1 ml and 10 ml sizes.

b Holding cans may be round or square but must not be made of copper. Aluminum or stainless steel are acceptable.

5 Alcohol jar with forceps (Plate 3)

a All manipulation of membrane filters is with sterile forceps. For sterilization, forceps are kept with their tips immersed in ethanol or methanol. When forceps are to be used, they are removed from the container and the alcohol is burned off.

b Forceps may be straight or curved. They should be designed to permit easy handling of filters without damage. Some forceps have corrugations on their gripping tips. It is recommended that such corrugations be filed off for membrane filter work.

6 A gas burner or alcohol burner is needed to ignite the alcohol prior to use of forceps.

7 Dilution water

The buffered distilled water described in "Standard Methods for the Examination of Water and Wastewater" for bacteriological examination of water is used in membrane filter methods. Dilution water is conveniently used in 99 ± 2 ml amounts stored in standard dilution bottles. Some workers prefer to use 9.0 ± 0.2 ml dilution blanks.

8 Culture medium

Bacteriological culture media used with membrane filter techniques are discussed at length in another part of this manual.

F Incubation Facilities

1 Requirements

a Temperature

For cultivation of a given kind of bacteria, the same temperature requirements apply with membrane filter methods as with any other method for cultivating the bacteria in question. For example, incubation temperature for coliform tests on membrane filters should be 35° C ± 0.5° C.

b Humidity

Membrane filter cultures must be incubated in an atmosphere maintained at or very near to 100% relative humidity Failure to maintain high humidity during incubation results in growth failure, or at best, in small or poorly differentiated colonies.

2 The temperature and humidity requirements can be satisfied in any of several types of equipment.

a A conventional incubator may be used. With large walk-in incubators, it is extremely difficult to maintain satisfactory humidity. With most conventional incubators, membrane filter cultures can be incubated in tightly closed containers, such as plastic petri dishes. In such containers, required humidity conditions are established with evaporation of some of the culture medium. Because the volume of air in a tightly closed container is small, this results in negligible change in the culture medium. If glass petri dishes or other loosely fitting containers are used, the containers should be placed in a tightly closed container, with wet paper or cloth inside to obtain the required humidity conditions. A vegetable crisper, such as used in most home refrigerators,

is useful for the purpose. (See Plate 3)

b A covered water bath maintaining 44.5°C ± 0.2°C is necessary for the fecal coliform test and this will necessitate the use of a water-bath having forced circulation of water.

III STERILIZATION OF MEMBRANE FILTER EQUIPMENT AND SUPPLIES

A Filter Holding Unit

1 When is sterilization necessary?

a The filter holding unit should be sterile at the beginning of each filtration series. A filtration series is considered to be interrupted if there is an interval of 30 minutes or longer between sample filtrations. After such interruption any further sample filtration is treated as a new filtration series and requires a sterile filter holding unit.

b It is not necessary to sterilize the filter holding unit between successive filtrations, or between successive samples, of a filtration series.

After each filtration the funnel walls are flushed with sterile water to free them of bacterial contamination. If properly done, the flushing procedure will remove bacteria remaining on the funnel walls and prevent contamination of later samples.

2 Methods for sterilization of filter holding unit

a Sterilization in the autoclave is preferred. Wrap the funnel and receptacle separately in Kraft paper and sterilize in the autoclave 15 minutes at 121°C. At the end of the 15 minutes holding period in the autoclave, release the steam pressure rapidly, to encourage drying of the filter holding unit.

b The unit may be sterilized by holding it 30 minutes in a flowing steam sterilizer.

c The unit may be immersed 2 to 10 minutes in boiling water. This method is recommended for emergency or field use.

d Some units (Millipore Stainless Unit) are available with accessories permitting anhydrous sterilization with formaldehyde. The method consists of introduction of methanol into a wick or porous plate in the sterilization accessory, assembly of the filter holding unit for formaldehyde sterilization, ignition of the methanol, and closure of the unit. The methanol is incompletely oxidized in the closed container, resulting in the generation of formaldehyde, which is bactericidal. The filter holding unit is kept closed for at least 15 minutes before use.

e Ultraviolet lamp sterilizers are convenient to use. A device now commercially available for ultraviolet sterilization of membrane filter funnel units.

B Sterilization of Membrane Filters and Absorbent Pads

1 Membrane filters

a Membranes are supplied in units of 10 in kraft envelopes, or in packages of 100 membranes. They may be sterilized conveniently in the packets of 10, but should be repackaged if supplied in units of 100. Large packages of filters can be distributed in standard 100 mm ×15 mm petri dishes, or they can be wrapped in kraft paper packets for sterilization.

b Sterilization in the autoclave is preferred. Ten minutes at 121° C or, preferably, at 116° C is recommended. After sterilization the steam pressure is released as rapidly as possible, and the filters are removed from the autoclave and dried at room temperature. Avoid excessive exposure to steam.

c In emergency, membrane filters may be sterilized by immersion in boiling distilled water for 10 minutes. The filters should first be separated from absorbent pads and paper separators which usually are included in the package. The boiling water method is not recommended for general practice, as the membranes tend to adhere to each other and must be separated from one another with forceps.

2 Absorbent pads for nutrient

a Unsterile absorbent pads can be wrapped in kraft paper or stacked loosely in petri dishes, and autoclaved with membrane filters (ten minutes or longer at 121° C or 116° C).

b After sterilization absorbent pads for nutrient should be dried before use.

C Glassware

1 Sterilization at 170° C for not less than 1 hour is preferred for most glassware (pipettes, graduated cylinders, glass petri dishes). Pipettes can be sterilized in aluminum or stainless steel cans, or they may be wrapped individually in paper. The opening of graduated cylinders should be covered with paper or metal foil prior to sterilization. Glassware with rubber fittings must not be sterilized at 170° C, as the rubber will be damaged.

2 Sterilization in the autoclave, 15 minutes at 121° C, is satisfactory, and preferred by many workers. When sterilizing pipettes it is important to exhaust the steam pressure rapidly and vent the containers momentarily. This allows the vapor to leave the can and prevents wet pipettes.

D Culture Containers

1 Glass petri dishes

a Petri dishes may be sterilized in aluminum or stainless steel cans, or wrapped in kraft paper or metal foil. They can be wrapped individually or, more conveniently, in rolls of up to 10 dishes.

b Preferably, sterilize glass petri dishes at 170° C for at least 1 hour.

c Alternately, they may be sterilized in the autoclave, 15 minutes at 121° C. After sterilization steam pressure should be released rapidly to facilitate drying of the dishes. Other suggested methods for sterilization of plastic dishes include exposure to ethylene oxide vapor (0.5 ml ethylene oxide per liter of container volume), or exposure to ultraviolet light. Ethylene oxide is a dangerous chemical being both toxic and explosive, and it should be used only when more convenient and safer methods are not available.

2 Plastic culture containers

a Because of the thermo-labile characteristics of the plastic, these containers cannot be heat sterilized. Manufacturers supply these in a sterile condition.

b For practical purposes, plastic dishes may be sterilized by immersion in a 70% solution of ethanol in water, for at least 30 minutes. Dishes must be allowed to drain and dry before use, as ethanol will influence the performance of culture media.

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MEMBRANE FILTER LABORATORY AND FIELD PROCEDURES

I BASIC PROCEDURES

A Introduction

Successful application of membrane filter methods requires development of good routine operational practices. The detailed basic procedures described in this Section are applicable to all membrane filter methods in water bacteriology for filtration, incubation, colony counting, and reporting of results. In addition, equipment and supplies used in all membrane filter procedures are described here and not repeated elsewhere in such detail.

Workers using membrane filter methods for the first time are urged to become thoroughly familiar with these basic procedures and precautions.

B General Supplies and Equipment List

Table 1 is a check list of materials.

C "Sterilizing" Media

Set tubes in a boiling waterbath for 10 minutes. This method suffices for medium in tubes up to 25 × 150 mm. Frequent agitation improves dissolving of the medium.

Alternately, coliform media can be directly heated on a hotplate to the first bubble of boiling. Stir the medium frequently if direct heat is used, to avoid charring the medium.

Do not autoclave.

D General Laboratory Procedures with Membrane Filters

1 Prepare data sheet

Minimum data required are: sample identification, test performed including media and methods, sample filtration volumes, and the bench numbers assigned to individual membrane filters.

2 Disinfect the laboratory bench surface.

Use a suitable disinfectant solution and allow the surface to dry before proceeding.

3 Set out sterile culture containers in an orderly arrangement.

4 Label the culture containers.

Numbers correspond with the filter numbers shown on the data sheet.

5 Place one sterile absorbent pad* in each culture container, unless an agar medium is being used.

Use sterile forceps for all manipulations of absorbent pads and membrane filters. Forceps sterility is maintained by storing the working tips in about 1 inch of methanol or ethanol. Because the alcohol deteriorates the filter, dissipate it by burning before using the forceps. Avoid heating the forceps in the burner as hot metal chars the filter.

*When an agar medium is used, absorbent pads are not used. The amount of medium should be sufficient to make a layer approximately 1/8" deep in the culture container. In the 50 mm plastic culture containers this corresponds to approximately 6-8 ml of culture medium.

NOTE: Mention of commercial products and manufacturers does not imply endorsement by the Office of Water Programs, Environmental Protection Agency.

Table 1. EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

Item	Standard Tests		Nonstandard Tests			
	M-Endo Broth	L. E. S Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
Half-round glass paper weights for colony counting, with lower half of a 2-oz metal ointment box	X	X	X	X	X	
Hand tally, single unit acceptable, hand or desk type	X	X	X	X	X	
Stereoscopic (dissection) microscope, magnification of 10X or 15X, preferable binocular wide field type	X	X	X	X	X	X
Bacteriological inoculating needle						X
Wire racks for culture tubes, 10 openings by five openings preferred, dimensions overall approximately 6" X 12"						X
Phenol Red Lactose Broth in 16 X 150 mm fermentation tubes with metal caps, 10 ml per tube						X
Eosin Methylene Blue Agar (Levine) in petri plates, prepared ready for use						X
Nutrient agar slants, in screw capped tubes, 16 X 126 mm						X
Gram stain solutions, 4 solutions per complete set						X
Microscope, compound, binocular, with oil immersion lens, microscope lamp and immersion oil						X
Microscope slides, new, clean, 1" X 3" size						X
Water proof plastic bags for fecal coliform culture dish incubation				X		
M-Endo medium, MF dehydrated medium in 25 X 95 mm flat bottomed screw-capped glass vials, 1.44 g per tube, sufficient for 30 ml of medium	X		X			
Ethanol, 95% in small bottles or screw-capped tubes, about 20 ml per tube	X	X	X			
Sodium benzoate solution, 12% aqueous, in 25 X 150 mm screw-capped tubes, about 10 ml per tube			X			
L. E. S. Endo Agar MF, dehydrated M-Endo medium, 0.36 g per 25 X 95 mm flat bottomed screw-capped glass vial, plus 0 45 g agar, for 30 ml		X				
Lactose Lauryl Sulfate Tryptose Broth in 25 X 150 mm test tube without included gas tube, about 25 ml, for enrichment in L. E. S method		X				

Table 1. EQUIPMENT, SUPPLIES AND MEDIA

Item	Standard Tests		Nonstandard Tests			
	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
Funnel unit assemblies	X	X	X	X	X	
Ring stand, with about a 3" split ring, to support the filtration funnel	X	X	X	X	X	
Forceps, curved-end round tipped, special type for MF work	X	X	X	X	X	
Methanol, in small wide-mouthed bottles, about 20 ml for sterilizing forceps	X	X	X	X	X	
Suction flasks, glass, 1 liter, mouth to fit No. 8 stopper	X	X	X	X	X	
Rubber tubing, 2-3 feet, to connect suction flask to vacuum services, latex rubber 3/16" I.D. by 3/32" wall	X	X	X	X	X	
Pinch clamps strong enough for tight compression of rubber tubing above	X	X	X	X	X	
Pipettes, 10 ml, graduated, Mohr type, sterile, dispense 10 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipettes, 1 ml, graduated, Mohr type, sterile, dispense 24 per can per working space per day. (Resterilize daily to meet need).	X	X	X	X	X	
Pipette boxes, sterile, for 1 ml and 10 ml pipettes (sterilize above pipettes in these boxes)	X	X	X	X	X	
Cylinders, 100 ml graduated, sterile, (resterilize daily to meet need).	X	X	X	X	X	
Jars, to receive used pipettes	X	X	X	X	X	
Gas burner, Bunsen or similar laboratory type	X	X	X	X	X	X
Wax pencils, red, suitable for writing on glass	X	X	X	X	X	
Sponge in dilute iodine, to wipe down the desk tops	X	X	X	X	X	
Membrane filters (white, grid marked, sterile,and suitable pore size for microbiological analysis of water)	X	X	X	X	X	
Absorbent pads for nutrient, (47 mm in diameter), sterile, in units of 10 pads per package Not required if medium contains agar	X	X	X	X	X	
Petri dishes, disposable, plastic, 50 X 12 mm, sterile	X	X	X	X	X	
Waterbath incubator 44.5 ± 0.2°C				X		
Vegetable crispers, or cake boxes, plastic, with tight fitting covers, for membrane filter incubations	X	X	X		X	
Fluorescent lamp, with extension cord equipped with a simple lens of about 4X magnification	X	X	X	X	X	X
Ring stand, with clamps, utility type	X	X	X	X	X	

Table 1 EQUIPMENT, SUPPLIES AND MEDIA (Cont'd)

	Standard Tests		Nonstandard Tests			
Item	M-Endo Broth	L. E. S. Coliform	Delayed Coliform	Fecal Coliform	Fecal Streptococcus	Verified Test
M-FC Broth for fecal coliform, dehydrated medium in 25 X 95 mm flat bottomed screw-capped glass vials, 1.23 g per tube, sufficient for 30 ml of culture medium				X		
Rosolic acid, 1% solution, in 0.2N NaOH, in 25 X 150 mm flat bottomed screw-capped tubes, about 5 ml per tube, freshly prepared				X		
M-Enterococcus Agar, dehydrated medium in 25 X 150 mm screw-capped tubes, sufficient for 30 ml, 1.26 g per tube					X	
Dilution bottles, 6-oz, preferable boro-silicate glass, with screw-cap (or rubber stopper protected by paper), each containing 99 ml of sterile phosphate buffered distilled water	X	X	X	X	X	
Electric hot plate surface	X	X	X	X	X	
Beakers, 400 - 600 ml (for water-bath in preparation of membrane filter culture media)	X	X	X	X	X	
Crucible tongs, to be used at electric hot plates, for removal of hot tubes of culture media for boiling waterbath	X	X	X	X	X	

6 Deliver enough culture medium to saturate each absorbent pad,* using a sterile pipette.

Exact quantities cannot be stated because pads and culture containers vary. Sufficient medium should be applied so that when the culture container is tipped, a good-sized drop of culture medium freely drains out of the absorbent pad.

7 Organize supplies and equipment for convenient sample filtration. In training courses, laboratory instructors will suggest useful arrangements, eventually the individual will select a system of bench-top organization most suited to his own needs. The important point in any arrangement is to have all needed equipment and supplies conveniently at hand, in such a pattern as to minimize lost time in useless motions.

8 Lay a sterile membrane filter on the filter holder, grid-side up, centered over the porous part of the filter support plate.

Membrane filters are extremely delicate and easily damaged. For manipulation, the sterile forceps should always grasp the outer part of the filter disk, outside the part of the filter through which the sample passes.

9 Attach the funnel element to the base of the filtration unit.

To avoid damage to the membrane filter, locking forces should only be applied at the locking arrangement. The funnel element never should be turned or twisted while being seated and locked to the lower element of the filter holding unit. Filter holding units featuring a bayonet joint and locking ring to join the upper element to the lower element require special care on the part of the operator. The locking ring should be turned sufficiently to give a snug fit, but should not be tightened excessively.

10 Shake the sample thoroughly.

11 Measure sample into the funnel with vacuum turned off.

The primary objectives here are: 1) accurate measurement of sample; and 2) optimum distribution of colonies on the filter after incubation. To meet these objectives, methods of measurement and dispensation to the filtration assembly are varied with different sample filtration volumes.

a With samples greater than 20 ml, measure the sample with a sterile graduated cylinder and pour it into the funnel. It is important to rinse this graduate with sterile buffered distilled water to preclude the loss of excessive sample volume. This should be poured into the funnel.

b With samples of 10 ml to 20 ml, measure the sample with a sterile 10 ml or 20 ml pipette, and pipette on a dry membrane in the filtration assembly.

c With samples of 2 ml to 10 ml, pour about 20 ml of sterile dilution water into the filtration assembly, then measure the sample into the sterile buffered dilution water with a 10 ml sterile pipette.

d With samples of 0.5 to 2 ml, pour about 20 ml of sterile dilution water into the funnel assembly, then measure the sample into the sterile dilution water in the funnel with a 1 ml or a 2 ml pipette.

e If a sample of less than 0.5 ml is to be filtered, prepare appropriate dilutions in sterile dilution water, and proceed as applicable in item c or d above.

When dilutions of samples are needed, always make the filtrations as soon as possible after dilution of the sample, this never should exceed

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30 minutes. Always shake sample dilutions thoroughly before delivering measured volumes.

12 Turn on the vacuum.

Open the appropriate spring clamp or valve, and filter the sample.

After sample filtration a few droplets of sample usually remain adhered to the funnel walls. Unless these droplets are removed, the bacteria contained in them will be a source of contamination of later samples. (In laboratory practice the funnel unit is not routinely sterilized between successive filtrations of a series). The purpose of the funnel rinse is to flush all droplets of a sample from the funnel walls to the membrane filter. Extensive tests have shown that with proper rinsing technique, bacterial retention on the funnel walls is negligible.

13 Rinse the sample through the filter.

After all the sample has passed through the membrane filter, rinse down the sides of the funnel walls with at least 20 ml of sterile dilution water. Repeat the rinse twice after all the first rinse has passed through the filter. Cut off suction on the filtration assembly.

14 Remove the funnel element of the filter holding unit.

If a ring stand with split ring is used, hang the funnel element on the ring, otherwise, place the inverted funnel element on the inner surface of the wrapping material. This requires care in opening the sterilized package, but it is effective as a protection of the funnel ring from contamination.

15 Take the membrane filter from the filter holder and carefully place it, grid-side up on the medium.

Check that no air bubbles have been trapped between the membrane filter and the underlying absorbent pad or agar. Relay the membrane if necessary.

16 Place in incubator after finishing filtration series.

Invert the containers. The immediate atmosphere of the incubating membrane filter must be at or very near 100% relative humidity.

17 Count colonies which have appeared after incubating for the prescribed time.

A stereoscopic microscope magnifying 10-15 times and careful illumination give best counts.

For reporting results, the computation is:

bacteria/100 ml =

$$\frac{\text{No. colonies counted} \times 100}{\text{Sample volume filtered in ml}}$$

Example:

A total of 36 colonies grew after filtering a 10 ml sample. The number reported is:

$$\frac{36 \text{ colonies}}{10 \text{ ml}} \times 100 = 360 \text{ per } 100 \text{ ml}$$

II MF LABORATORY TESTS FOR COLIFORM GROUP

A Standard Coliform Test (Based on M-Endo Broth MF)

1 Culture medium

a M-Endo Broth MF Difco 0749-02 or the equivalent BBL M-Coliform Broth 01-494

Preparation of Culture Medium (M-Endo Broth) for Standard MF Coliform Test

Yeast extract	1.5	g
Casitone or equivalent	5.0	g
Thiopeptone or equivalent	5.0	g
Tryptose	10.0	g
Lactose	12.5	g
Sodium desoxycholate	0.1	g
Dipotassium phosphate	4.375	g
Monopotassium phosphate	1.375	g
Sodium chloride	5.0	g
Sodium lauryl sulfate	0.05	g
Basic fuchsin (bacteriological)	1.05	g
Sodium sulfite	2.1	g
Distilled water (containing 20.0 ml ethanol)	1000	ml

This medium is available in dehydrated form and it is recommended that the commercially available medium be used in preference to compounding the medium of its individual constituents.

To prepare the medium for use, suspend the dehydrated medium at the rate of 48 grams per liter of water containing ethyl alcohol at the rate of 20 ml per liter.

As a time-saving convenience, it is recommended that the laboratory worker preweigh the dehydrated medium in closed tubes for several days, or even weeks, at one operation.

With this system, a large number of increments of dehydrated medium (e.g., 1.44 grams), sufficient for some convenient (e.g., 30 ml) volume of finished culture medium are weighed and dispensed into screw-capped culture tubes, and stored until needed. Storage should preferably be in a darkened desiccator.

A supply of distilled water containing 20 ml stock ethanol per liter is maintained.

When the medium is to be used, it is reconstituted by adding 30 ml of the distilled water-ethanol mixture per tube of pre-weighed dehydrated culture medium.

b Medium is "sterilized" as directed in I, C.

c Finished medium can be retained up to 96 hours if kept in a cool, dark place. Many workers prefer to reconstitute fresh medium daily.

2 Filtration and incubation procedures are as given in I, D.

Special instructions:

a For counting, use the wide field binocular dissecting microscope, or simple lens. For illumination, use a light source perpendicular to the plane of the membrane filter. A small fluorescent lamp is ideal for the purpose.

b Coliform colonies have a "metallic" surface sheen under reflected light which may cover the entire colony, or it may appear only in the center. Non-coliform colonies range from colorless to pink, but do not have the characteristic sheen.

c Record the colony counts on the data sheet, and compute the coliform count per 100 ml of sample.

B Standard Coliform Tests (Based on L.E.S. Endo Agar)

The distinction of the L.E.S. count is a two hour enrichment incubation on LST broth. M-Endo L.E.S. medium is used as agar rather than the broth.

1 Preparation of culture medium (L.E.S. Endo Agar) for L.E.S. coliform test

a Formula from McCarthy, Delaney, and Grasso [2]

Bacto-Yeast Extract	1.2 g
Bacto-Casitone	3.7 g
Bacto-Thiopeptone	3.7 g
Bacto-Tryptose	7.5 g
Bacto-Lactose	9.4 g
Dipotassium phosphate	3.3 g
Monopotassium phosphate	1.0 g
Sodium chloride	3.7 g
Sodium desoxycholate	0.1 g
Sodium lauryl sulfate	0.05 g
Sodium sulfite	1.6 g
Bacto-Basic fuchsin	0.8 g
Agar	15 g
Distilled water (containing 20 ml ethyl alcohol)	1000 ml

b To rehydrate the medium, suspend 51 grams in the water-ethyl alcohol solution.

c Medium is "sterilized" as directed in I, C.

d Pour 4-6 ml of freshly prepared Agar into the smaller half of the container. Allow the medium to cool and solidify.

2 Procedures for filtration and incubation

a Lay out the culture dishes in a row or series of rows as usual. Place these with the upper (lid) or top side down.

b Place one sterile absorbent pad in the larger half of each container (lid). Use sterile forceps for all manipulations of the pads. (Agar occupies smaller half or bottom).

c Using a sterile pipette, deliver enough single strength lauryl sulfate tryptose broth to saturate the pad only. Excess interferes.

d Follow general procedures for filtering in I, D. Place filters on pad with lauryl sulfate tryptose broth.

e Upon completion of the filtrations, invert the culture containers and incubate at 35° C for 1 1/2 to 2 hours.

3 2-hour procedures

a Transfer the membrane filter from the enrichment pad in the upper half to the agar medium in the lower half of the container. Carefully roll the membrane onto the agar surface to avoid trapping air bubbles beneath the membrane.

b Removal of the used absorbent pad is optional.

c The container is inverted and incubated 22 hours $\pm$ 2 hours $\pm$ 0.5°C.

4 Counting procedures are as in I, D.

5 L.E.S. Endo Agar may be used as a single-stage medium (no enrichment step) in the same manner as M-Endo Broth, MF.

C Delayed Incubation Coliform Test

This technique is applicable in situations where there is an excessive delay between sample collection and plating. The procedure is unnecessary when the interval between sample collection and plating is within acceptable limits.

1 Preparation of culture media for delayed incubation coliform test

a Preservative media M-Endo Broth base

To 30 ml of M-Endo Broth MF prepared in accordance with directions in II, A, 1 of this outline, add 1.0 ml of a sterile 12% aqueous solution of sodium benzoate.

L.E.S. MF Holding Medium-Coliform: Dissolve 12.7 grams in 1 liter of distilled water. No heating is necessary. Final pH 7.1 ± 0.1. This medium contains sodium benzoate.

b Growth media

M-Endo Broth MF is used, prepared as described in II, A, 1 earlier in this outline. Alternately, L.E.S. Endo Medium may be used.

2 General filtration followed is in I, D.

Special procedures are:

a Transfer the membrane filter from the filtration apparatus to a pad saturated with benzoated M-Endo Broth.

b Close the culture dishes and hold in a container at ambient temperature. This may be mailed or transported to a central laboratory. The mailing or transporting tube should contain accurate transmittal data sheets which correspond to properly labeled dishes.

Transportation time, in the case of mailed containers, should not exceed three days to the time of reception by the testing laboratory.

c On receipt in the central laboratory, unpack mailing carton, and lay out the culture containers on the laboratory bench.

d Remove the tops from the culture containers. Using sterile forceps, remove each membrane and its absorbent pad to the other half of the culture container.

e With a sterile pipette or sterile absorbent pad, remove preservative medium from the culture container.

f Place a sterile absorbent pad in each culture container, and deliver enough freshly prepared M-Endo Broth to saturate each pad.

g Using sterile forceps, transfer the membrane to the new absorbent pad containing M-Endo Broth. Place the membrane carefully to avoid entrapment of air between the membrane and the underlying absorbent pad. Discard the absorbent pad containing preservative medium.

h After incubation of 20 ± 2 hours at 35° C, count colonies as in the above section A, 2.

i If L.E.S. Endo Agar is used, the steps beginning with (e) above are omitted; and the membrane filter is removed from the preservative medium and transferred to a fresh culture container with L.E.S. Endo Agar, incubated, and colonies counted in the usual way.

D Verified Membrane Filter Coliform Test

This procedure applies to identification of colonies growing on Endo-type media used for determination of total coliform counts. Isolates from these colonies are studied for gas production from lactose and typical coliform morphology. In effect, the procedure corresponds with the Completed Test stage of the multiple fermentation tube test for coliforms.

Procedure:

1 Select a membrane filter bearing several well-isolated coliform-type colonies.

2 Using sterile technique, pick all colonies in a selected area with the inoculation needle, making transfers into tubes of phenol red lactose broth (or lauryl sulfate tryptose lactose

broth). Using an appropriate data sheet record the interpretation of each colony, using, for instance, "C" for colonies having the typical color and sheen of coliforms, "NC" for colonies not conforming to coliform colony appearance on Endotype media.

3 Incubate the broth tubes at 35° C ± 0.5°C.

4 At 24 hours:

a Read and record the results from the lactose broth fermentation tubes. The following code is suggested:

Code	
O	No indication of acid or gas production, either with or without evidence of growth.
A	Evidence of acid but not gas (applies only when a pH indicator is included in the broth medium)
G	Growth with production of gas. If pH indicator is used, use symbol AG to show evidence of acid. Gas in any quantity is a positive test.

b Tubes not showing gas production are returned to the 35° C incubator.

c Gas-positive tubes are transferred as follows.

1) Prepare a streak inoculation on EMB agar for colony isolation, and using the same culture.

2) Inoculate a nutrient agar slant.

3) Incubate the EMB agar plates and slants at 35° C ± 0.5°C.

5 At 48 hours:

a Read and record results of lactose broth tubes which were negative at 24 hours and were returned for further incubation.

b Gas-positive cultures are subjected to further transfers as in 4c. Gas-negative cultures are discarded without further study; they are coliform- negative.

c Examine the cultures transferred to EMB agar plates and to nutrient agar slants, as follows:

1) Examine the EMB agar plate for evidence of purity of culture; if the culture represents more than one colony type, discard the nutrient agar culture and reisolate each of the representative colonial types on the EMB plate and resume as with 4c for each isolation. If purity of culture appears evident, continue with c (2) below.

2) Prepare a smear and Gram stain from each nutrient agar slant culture. The Gram stain should be made on a culture not more than 24 hours old. Examine under oil immersion for typical coliform morphology, and record results.

6 At 72 hours:

Perform procedures described in 5c above, and record results.

7 Coliform colonies are considered verified if the procedures demonstrate a pure culture of bacteria which are gram negative nonspore-forming rods and produce gas from lactose at 35° C within 48 hours.

E Fecal Coliform Count (Based on M-FC Broth Base)

The count depends upon growth on a special medium at 44.5 ± 0.2°C.

1 Preparation of Culture Medium (M-FC Broth Base) for Fecal Coliform Count

a Composition

Tryptose	10.0 g
Proteose Peptone No. 3	5.0 g
Yeast extract	3.0 g
Sodium chloride	5.0 g
Lactose	12.5 g
Bile salts No. 3	1.5 g
Rosolic acid* (Allied Chemical)	10.0 ml
Aniline blue (Allied Chemical)	0.1 g
Distilled water	1000 ml

b To prepare the medium dissolve 37.1 grams in a liter of distilled water which contains 10 ml of 1% rosolic acid (prepared in 0.2 N NaOH).

Fresh solutions of rosolic acid give best results. Discard solutions which have changed from dark red to orange.

c To sterilize, heat to boiling as directed in I, C.

d Prepared medium may be retained up to 4 days in the dark at 2-8° C.

2 Special supplies

Small water proof plastic sacks capable of being sealed against water with capacity of 3 to 6 culture containers.

3 Filtration procedures are as given in I, D.

4 Elevated temperature incubation

a Place fecal coliform count membranes at 44.5 ± 0.2°C as rapidly as possible.

Filter membranes for fecal coliform counts consecutively and immediately place them in their culture containers. Insert as many as six culture containers all oriented in the same way (i.e., all grid sides facing the same direction) into the sacks and seal. Tear off the perforated top, grasp the side wires, and twirl the sack to roll the open end inside the folds of sack. Then submerge the sacks with culture containers inverted beneath the surface of a 44.5 ± 0.2°C waterbath.

b Incubate for 22 ± 2 hours.

5 Counting procedures

Examine and count colonies as follows.

a Use a wide field binocular dissecting microscope with 5 - 10X magnification.

b Low angle lighting from the side is advantageous.

c Fecal coliform colonies are blue, generally 1-3 mm in diameter.

d Record the colony counts on the data sheet, and report the fecal coliform count per 100 ml of sample. (I, D, 17 illustrates method)

III TESTS FOR FECAL STREPTOCOCCAL GROUP-MEMBRANE FILTER METHOD

A 48 hour incubation period on a choice of two different media, giving high selectivity for fecal streptococci, are the distinctive features of the tests.

*Prepare 1% solution of rosolic acid in 0.2 N NaOH. This dye is practically insoluble in water.

A Test for Members of Fecal Streptococcal (Tentative, Standard Methods) M-Enterococcus Agar Medium

1 Preparation of the culture medium

a Formula (The Difco formula is shown, but equivalent constituents from other sources are equally acceptable).

Bacto tryptose	20.0 g
Bacto yeast extract	5.0 g
Bacto dextrose	2.0 g
Dipotassium phosphate	4.0 g
Sodium Azide	0.4 g
Bacto agar	10.0 g
2, 3, 5, Triphenyl tetrazolium chloride	0.1 g

b The medium is prepared by rehydration at the rate of 42 grams per 1000 ml of distilled water. It is recommended that the medium in dehydrated form be preweighed and dispensed into culture containers (about 25 × 150 mm) in quantities sufficient for preparation of 30 ml of culture medium (1.26 g per tube).

c Follow I, C, for "sterilizing" medium and dispense while hot into culture containers. Allow plates to harden before use.

2 List of apparatus, materials, as given in Table I.

3 Procedure, in general, as given in I.

Special instructions

a Incubate for 48 hours, inverted, with 100% relative humidity, after filtrations are completed. If the entire incubator does not have saturated humidity, acceptable conditions can be secured by placing the cultures in a tightly closed container with wet paper, towels, or other moist material.

b After incubation, remove the cultures from the incubator, and count all colonies under wide field binocular dissecting microscope with magnification set at 10X or 20X. Fecal streptococcus colonies are 0.5 - 2 mm in diameter, and flat to raised smooth, and vary from pale pink to dark red in color.

c Report enterococcus count per 100 ml of sample. This is conveniently computed:

No. fecal streptococci per 100 ml =

$$\frac{\text{No. fecal streptococcus colonies counted}}{\text{Sample filtration volume in ml}} \times 100$$

B Test for Members of Fecal Streptococcal Group based on KF-Agar

1 Preparation of the culture medium

a Formula: (The dehydrated formula of Bacto 0496 is shown, but equivalent constituents from other sources are acceptable). Formula is in grams per liter of reconstituted medium.

Bacto proteose peptone #3	10.0	g
Bacto yeast extract	10.0	g
Sodium chloride (reagent grade)	5.0	g
Sodium glycerphosphate	10.0	g
Maltose (CP)	20.0	g
Lactose (CP)	1.0	g
Sodium azide (Eastman)	0.4	g
Sodium carbonate (Na_2CO_3 reagent grade)	0.636	g
Brom cresol purple (water soluble)	0.015	g
Bacto agar	20.0	g

b Reagent

2, 3, 5-Triphenyl tetrazolium chloride reagent (TPTC)

This reagent is prepared by making a 1% aqueous solution of the above chemical passing it through a Seitz filter or membrane filter. It can

be kept in the refrigerator in a screw-capped tube until used.

c The dehydrated medium described above is prepared for laboratory use as follows:

Suspend 7.64 grams of the dehydrated medium in 100 ml of distilled water in a flask with an aluminum foil cover.

Place the flask in a boiling water-bath, melt the dehydrated medium, and leave in the boiling waterbath an addional 5 minutes.

Cool the medium to 50°-60° C, add 1.0 ml of the TPTC reagent, and mix.

For membrane filter studies, pour 5-8 ml in each 50 mm glass or plastic culture dish or enough to make a layer approximately 1/8" thick. Be sure to pour plates before agar cools and solidifies.

For plate counts, pour as for standard agar plate counts.

NOTE: Plastic dishes containing media may be stored in a dark, cool place up to 30 days without change in productivity of the medium, provided that no dehydration occurs. Plastic dishes may be incubated in an ordinary air incubator. Glass dishes must be incubated in an atmosphere with saturated humidity.

2 Apparatus, and materials as given in Table 1.

3 General procedure is as given in I.

Special instructions

a Incubate 48 hours, inverted with 100% relative humidity after filtration.

b After incubation, remove the cultures from the incubator, and count colonies under wide field binocular dissecting microscope, with magnification set at 10X or 20X. Fecal streptococcus colonies are pale pink to dark wine-color. In size they range from barely visible to approximately 2mm in diameter. Colorless colonies are not counted.

c Report fecal streptococcus count per 100 ml of sample. This is computed as follows:

No. fecal streptococci per 100 ml =

$$\frac{\text{No. fecal streptococcus colonies}}{\text{Sample filtration volume in ml}} \times 100$$

C Verification of Streptococcus Colonies

1 Verification of colony identification may be required in waters containing large numbers of Micrococcus organisms. This has been noted particularly with bathing waters, but the problem is by no means limited to such waters.

2 A verification procedure is described in "Standard Methods for the Examination of Water and Wastewater," 13th ed. (1971). The worker should use this reference for the step-by-step procedure.

IV PROCEDURES FOR USE OF MEMBRANE FILTER FIELD UNITS

A Culture Media

1 The standard coliform media used with laboratory tests are used.

2 To simplify field operations, it is suggested that the medium be sent to the field, preweighed, in vials or capped culture tubes. The medium then requires only the addition of a suitable volume of distilled water-ethanol prior to sterilization.

3 Sterilization procedures in the field are the same as for laboratory methods.

4 Laboratory preparation of the media, ready for use, would be permissible provided that the required limitations on time and conditions of storage are met.

B Operation of the Sabro Field Unit

1 Equipment and materials

Sabro field unit

Membrane filters

Absorbent pads for nutrient

Culture containers

M-Endo Broth MF or L.E.S. Endo Medium

Provision for heating water (optional)

Source of electricity

2 Procedure (Based on M-Endo Broth MF)

a Connect the electric cord to the power source and to Sabro field unit. After about 15 minutes check the temperature in the incubator drawer. The required temperature is 35°C (95°F). If the temperature is too low it can be increased by turning the thermostat adjustment screw counterclockwise. This screw is located at the front on the recessed divider between the two incubator drawers. To lower temperature, turn the adjustment screw clockwise.

b Review the supply of expendable materials to be used with the unit and secure replacements as needed (culture containers, medium, membrane filters, absorbent pads for nutrient, fuel, etc.).

c Sterilize the funnel unit by one of the following procedures.

1) Immerse the equipment 2 minutes in boiling water. The temperature should be at least 78°C (170°F).

2) Flame-sterilize membrane filter holder inside and both ends of funnel (suggested by manufacturer).

d Lay in a row all the culture containers to be used in the filtration series, and number the containers to correspond with numbers of the data sheet.

e Place one sterile absorbent pad in each culture container. Use sterile forceps for all manipulation of absorbent pads and filters.

f Using sterile pipette deliver enough culture medium to saturate each absorbent pad. The amount of culture medium required is approximately 2 ml, but cannot be precisely stated. Sufficient medium should be applied, that when the culture container is tipped, a good-sized drop of culture medium freely drains out of the absorbent pad.

g Using sterile forceps, place a membrane filter, grid-side up, on the MF receptacle of the funnel unit. Place the funnel portion over the membrane, and clamp the unit with the spring clamp provided with the portable kit.

h Pour the water sample into the funnel using a sterile pipette or graduate.

i Connect the tubing of the vacuum pump to the receptable on the base of the filter unit and draw the sample through the membrane. After the sample has passed through the membrane

filter, rinse down the sides of the funnel walls with at least 20 ml of sterile dilution water. Repeat the rinse twice after all the first rinse has passed through the filter.

j Disassemble the funnel unit and with sterile forceps transfer the membrane filter grid-side up to the appropriate culture container. The membrane should be "rolled on" the absorbent pad containing culture medium, to prevent entrapment of air between the pad and the membrane filter.

k Repeat steps g - j for additional filtrations of the same or different sampling volumes for the water being tested.

l After completion of filtration, place the culture container in an inverted position (with membrane position grid-side down) in the incubator drawers.

m After completion of the last filtration from any one sample, resterilize the funnel unit by one of the procedures described in instruction 2c.

n Allow the cultures to incubate 20 - 24 hours.

o Remove the cultures from the incubator and count coliform colonies.

C Operation of Millipore Water Testing Kit, Bacteriological

1 Supporting supplies and equipment are the same as for the laboratory procedures.

2 Set the incubator voltage selector switch to the voltage of the available supply, turn on the unit and adjust as necessary to establish operating incubator temperature at 35 $\pm$ 0.5°C.

3 Sterilize the funnel unit assembly by exposure to formaldehyde or by immersion in boiling water. If a laboratory autoclave is available, this is preferred.

Formaldehyde is produced by soaking an asbestos ring (in the funnel base) with methanol, igniting, and after a few seconds of burning, closing the unit by placing the stainless steel flask over the funnel and base. This results in incomplete combustion of the methanol, whereby formaldehyde is produced. Leave the unit closed for 15 minutes to allow adequate exposure to formaldehyde.

4 Filtration and incubation procedures correspond with laboratory methods.

5 The unit is supplied with a booklet containing detailed step-by-step operational procedures. The worker using the equipment should become completely versed in its contents and application.

D Counting of Colonies on Membrane Filters

1 Equipment and materials

Membrane filter cultures to be examined

Illumination source

Simple lens, 2X to 6X magnification

Hand tally (optional)

2 Procedure

a Remove the cultures from the incubator and arrange them in numerical sequence.

b Set up illumination source as that light will originate from an area perpendicular to the plane of membrane filters being examined. A small fluorescent lamp is ideal for the purpose. It is highly desirable that a simple lens be attached to the light source.

c Examine results. Count all coliform and noncoliform colonies. Coliform colonies have a "metallic" surface sheen under reflected light, which may cover the entire colony or may appear only on the center

Noncoliform colonies range from colorless to pink or red, but do not have the characteristic "metallic" sheen.

d Enter the colony counts in the data sheets.

e Enter the coliform count per 100 ml of sample for each membrane having a countable number of coliform colonies. Computation is as follows·

No. coliform per 100 ml =

$$\frac{\text{No. coliform colonies on MF}}{\text{No. milliliters sample filtered}} \times 100$$

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater. APHA, AWWA, WPCF. 12th Edition. 1965.

2 McCarthy, J.A., Delaney, J.E. and Grasso, R.J. Measuring Coliforms in Water. Water and Sewage Works. 1961: R-426-31. 1961.

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V OXYGEN IMPORTANCE AND DETERMINATIONS

The dissolved oxygen content of water is a major water quality criteria in relationship to the nature, variety, and activity of aquatic organisms in it and degree of stabilization of it.

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DISSOLVED OXYGEN DETERMINATION (DO) - I

Winkler Iodometric Titration and Azide Modification

I The Dissolved Oxygen determination is a very important water quality criteria for many reasons:

A Oxygen is an essential nutrient for all living organisms. Dissolved oxygen is essential for survival of aerobic organisms and permits facultative organisms to metabolize more effectively. Many desirable varieties of macro or micro organisms cannot survive at dissolved oxygen concentrations below certain minimum values. These values vary with the type of organisms, stage in their life history, activity, and other factors.

B Dissolved oxygen levels may be used as an indicator of pollution by oxygen demanding wastes. Low DO concentrations are likely to be associated with low quality waters.

C The presence of dissolved oxygen prevents or minimizes the onset of putrefactive decomposition and the production of objectionable amounts of malodorous sulfides, mercaptans, amines, etc.

D Dissolved oxygen is essential for terminal stabilization wastewaters. High DO concentrations are normally associated with good quality water.

E Dissolved oxygen changes with respect to time, depth or section of a water mass are useful to indicate the degree of stability or mixing characteristics of that situation.

F The BOD or other respirometric test methods for water quality are commonly based upon the difference among an initial and final DO determination for a given sample time interval and condition. These measurements are useful to indicate·

1 The rate of biochemical activity in terms of oxygen demand for a given sample and conditions.

2 The degree of acceptability (a bioassay technique) for biochemical stabilization of a given microbiota in response to food, inhibitory agents or test conditions.

3 The degree of instability of a water mass on the basis of test sample DO changes over an extended interval of time.

4 Permissible load variations in surface water or treatment units in terms of DO depletion versus time, concentration, or ratio of food to organism mass, solids, or volume ratios.

5 Oxygenation requirements necessary to meet the oxygen demand in treatment units or surface water situations.

G The DO test is the only chemical test included in all Water Quality Criteria, Federal, State, Regional or local.

II FACTORS AFFECTING THE DO CONCENTRATION IN WATER

A Physical Factors:

1 DO solubility in water for an air/water system is limited to about 9 mg DO/liter of water at 20°C. This amounts to about 0.0009% as compared to 21% by weight of oxygen in air.

2 Transfer of oxygen from air to water is limited by the interface area, the oxygen deficit, partial pressure, the conditions at the

interface area, mixing phenomena and other items.

Certain factors tend to confuse reoxygenation mechanisms of water aeration:

a The transfer of oxygen in air to dissolved molecular oxygen in water has two principal variables:

1) Area of the air-water interface.

2) Dispersion of the oxygen-saturated water at the interface into the body liquid.

The first depends upon the surface area of the air bubbles in the water or water drops in the air, the second depends upon the mixing energy in the liquid. If diffusors are placed in a line along the wall, dead spots may develop in the core. Different diffusor placement or mixing energy may improve oxygen transfer to the liquid two or threefold.

b Other variables in oxygen transfer include

3) Oxygen deficit in the liquid.

4) Oxygen content of the gas phase.

5) Time.

If the first four variables are favorable, the process of water oxygenation is rapid until the liquid approaches saturation. Much more energy and time are required to increase oxygen saturation from about 95 to 100% than to increase oxygen saturation from 0 to about 95%. For example· An oxygen-depleted sample often will pick up significant DO during DO testing, changes are unlikely with a sample containing equilibrium amounts of DO.

c The limited solubility of oxygen in water compared to the oxygen content of air does not require the interchange of a large mass of oxygen per unit volume of water to change DO saturation. DO increases from zero to 50% saturation are common in passage over a weir.

d Aeration of dirty water is practiced for cleanup. Aeration of clean water results in washing the air and transferring fine particulates and gaseous contaminants to the liquid.

e One liter of air at room temperature contains about 230 mg of oxygen. A 5 gal carboy of water with 2 liters of gas space above the liquid has ample oxygen supply for equilibration of DO after storage for 2 or 3 days or shaking for 30 sec.

f Aeration tends toward evaporative cooling. Oxygen content becomes higher than saturation values at the test temperature, thus contributing to high blanks.

3 Oxygen solubility varies with the temperature of the water. Solubility at 10° C is about two times that at 30° C. Temperature often contributes to DO variations much greater than anticipated by

solubility. A cold water often has much more DO than the solubility limits at laboratory temperature. Standing during warmup commonly results in a loss of DO due to oxygen diffusion from the supersaturated sample. Samples warmer than laboratory temperature may decrease in volume due to the contraction of liquid as temperature is lowered. The full bottle at higher temperature will be partially full after shrinkage with air entrance around the stopper to replace the void. Oxygen in the air may be transferred to raise the sample DO. For example, a volumetric flask filled to the 1000 ml mark at 30° C will show a water level about 1/2 inch below the mark when the water temperature is reduced to 20° C. BOD dilutions should be adjusted to 20° C + or - 1 1/2° before filling and testing.

4 Water density varies with temperature with maximum water density at 4° C. Colder or warmer waters tend to promote stratification of water that interferes with distribution of DO because the higher density waters tend to seek the lower levels.

5 Oxygen diffusion in a water mass is relatively slow, hence vertical and lateral mixing are essential to maintain relatively uniform oxygen concentrations in a water mass.

6 Increasing salt concentration decreases oxygen solubility slightly but has a larger effect upon density stratification in a water mass.

7 The partial pressure of the oxygen in the gas above the water interface controls the oxygen solubility limits in the water. For example, the equilibrium concentration of oxygen in water is about 9 mg DO/l under one atmospheric pressure of air, about 42 mg DO/liter in contact with pure oxygen and 0 mg DO/liter in contact with pure nitrogen (@ 20° C).

B Biological or Bio-Chemical Factors

1 Aquatic life requires oxygen for respiration to meet energy requirements for growth, reproduction, and motion. The net effect is to deplete oxygen resources in the water at a rate controlled by the type, activity, and mass of living materials present, the availability of food and favorability of conditions.

2 Algae, autotrophic bacteria, plants or other organisms capable of photosynthesis may use light energy to synthesize cell materials from mineralized nutrients with oxygen released in process.

a Photosynthesis occurs only under the influence of adequate light intensity.

b Respiration of alga is continuous.

c The dominant effect in terms of oxygen assets or liabilities of alga depends upon algal activity, numbers and light intensity. Gross algal productivity contributes to significant diurnal DO variations.

3 High rate deoxygenation commonly accompanies assimilation of readily available nutrients and conversion into cell mass or storage products. Deoxygenation due to cell mass respiration commonly occurs at some lower rate dependent upon the nature of the organisms present, the stage of decomposition and the degree of predation, lysis, mixing and regrowth. Relatively high

deoxygenation rates commonly are associated with significant growth or regrowth of organisms.

4 Micro-organisms tend to flocculate or agglomerate to form settleable masses particularly at limiting nutrient levels (after available nutrients have been assimilated or the number of organisms are large in proportion to available food).

a Resulting benthic deposits continue to respire as bed loads.

b Oxygen availability is limited because the deposit is physically removed from the source of surface oxygenation and algal activity usually is more favorable near the surface. Stratification is likely to limit oxygen transfer to the bed load vicinity.

c The bed load commonly is oxygen deficient and decomposes by anaerobic action.

d Anaerobic action commonly is characterized by a dominant hydrolytic or solubilizing action with relatively low rate growth of organisms.

e The net effect is to produce low molecular weight products from cell mass with a correspondingly large fraction of feedback of nutrients to the overlaying waters. These lysis products have the effect of a high rate or immediate oxygen demand upon mixture with oxygen containing waters.

f Turbulence favoring mixing of surface waters and benthic sediments commonly are associated with extremely rapid depletion of DO. Recurrent resuspension of thin benthic deposits may contribute to highly erratic DO patterns.

g Long term deposition areas commonly act like point sources of new pollution as a result of the feedback of nutrients from the deposit. Rate of reaction may be low for old materials but a low percentage of a large mass of unstable material may produce excessive oxygen demands.

C Tremendous DO variations are likely in a polluted water in reference to depth, cross section or time of day. More stabilized waters tend to show decreased DO variations although it is likely that natural deposits such as leaf mold will produce differences related to depth in stratified deep waters.

III ANALYTICAL METHOD BACKGROUND

The basic Winkler procedure (1888) has been modified many times to improve its workability in polluted waters. None of these modifications have been completely successful. The most useful modification was proposed by Alsterberg and consists of the addition of sodium azide to control nitrite interference during the iodometric titration. The Azide modification of the iodometric titration is recommended as official by the EPA-OWP Quality Control Committee for relatively clean waters.

A Reactions

1 The determination of DO involves a complex series of interactions that must be quantitative to provide a valid DO result. The number of sequential reactions also complicates interference control. The reactions will be presented first followed by discussion of the functional aspects.

$$MnSO_4 + 2\ KOH \rightarrow Mn(OH)_2 + K_2SO_4 \quad (a)$$

$$2\ Mn(OH)_2 + O_2 \rightarrow 2\ MnO(OH)_2 \quad (b)$$

$$MnO(OH)_2 + 2\ H_2SO_4 \rightarrow Mn(SO_4)_2 + 3H_2O \quad (c)$$

$$Mn(SO_4)_2 + 2\ KI \rightarrow MnSO_4 + K_2SO_4 + I_2 \quad (d)$$

$$I_2 + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\ NaI \quad (e)$$

2 Reaction sequence

The series of reactions involves five different operational steps in converting dissolved oxygen in the water into a form in which it can be measured.

a $O_2 \rightarrow MnO(OH)_2 \rightarrow Mn(SO_4)_2 \rightarrow$ $I_2 \rightarrow$ Thiosulfate (thio) or phenylarsine oxide (PAO) titration.

b All added reagents are in excess to improve contact possibilities and to force the reaction toward completion.

3 The first conversion, $O_2 \rightarrow MnO(OH)_2$ (reactions a, b) is an oxygen transfer operation where the dissolved oxygen in the water combines with manganous hydroxide to form an oxygenated manganic hydroxide.

a The manganous salt can react with oxygen only in a highly alkaline media.

b The manganous salt and alkali must be added separately with addition below the surface of the sample to minimize reaction with atmospheric oxygen via air bubbles or surface contact. Reaction with sample dissolved oxygen is intended to occur upon mixing of the reagents and sample after stoppering the full bottle (care should be used to allow entrained air bubbles to rise to the surface before adding reagents to prevent high results due to including entrained oxygen).

c Transfer of oxygen from the dissolved state to the precipitate form involves a two phase system of solution and precipitate requiring effective mixing for quantitative transfer. Normally a gross excess of reagents are used to limit mixing requirements. Mixing by rapid inversion 25 to 35 times will accomplish the purpose. Less energy is required by inversion 5 or 6 times, allowing the solids to settle half way and repeat the process. Reaction is rapid, contact is the principal problem in the two phase system.

d If the alkaline floc is white, no oxygen is present.

4 Acidification (reactions c and d) changes the oxygenated manganic hydroxide to manganic sulfate which in turn reacts with potassium iodide to form elemental iodine. Under acid conditions, oxygen cannot react directly with the excess manganous sulfate remaining in solution.

5 Iodine (reaction e) may be titrated with sodium thiosulfate standard solution to indicate the amount of dissolved oxygen originally present in the sample.

a The blue color of the starch-iodine complex commonly is used as an indicator. This blue color disappears when elemental iodine has been reacted with an equivalent amount of thiosulfate.

b Phenylarsine oxide solutions are more expensive to obtain but have better keeping qualities than thiosulfate solutions. Occasional use, field operations and situations where it is not feasible to calibrate thio solutions regularly, usually encourage use of purchased PAO reagents.

6 For practical purposes the DO determination scheme involves the following operations.

a Fill a 300 ml bottle* under conditions minimizing DO changes. This means that the sample bottle must be flushed with test solution to displace the air in the bottle with water characteristic of the tested sample*.

*DO test bottle volumes should be checked - discard those outside of the limits of 300 ml + or - 3 ml.

b To the filled bottle

1) Add $MnSO_4$ reagent (2 ml)

2) Add KOH, KI, NaN_3 reagent (2 ml)
Stopper, mix by inversion, allow to settle half way and repeat the operation. Highly saline test waters commonly settle very slowly at this stage and may not settle to the half way point in the time allotted.

c To the alkaline mix (settled about half way) add 2 ml of sulfuric acid, stopper and mix.

d Transfer the contents of the bottle to a 500 ml Erlenmeyer flask and titrate with 0.0375 Normal Thiosulfate*. Each ml of reagent used represents 1 mg of DO/liter of sample.

The same thing applies for other sample volumes when using an appropriate titrant normality such as·

1) For a 200 ml sample, use 0.025 N Thio

2) For a 100 ml sample, use 0.0125 N Thio

*EPA-OWP Method

7 The addition of the first two DO reagents, ($MnSO_4$ and the KOH, KI and NaN_3 solutions) displaces an equal quantity of the sample. This is not the case when acid is added because the clear liquid above the floc does not contain dissolved oxygen as all of it should be converted to the particulate $MnO(OH)_2$. Some error is introduced by this displacement of sample during dosage of the first two reagents. The error upon addition of 2 ml of each reagent to a 300 ml sample is $\frac{4}{300} \times 100$ or 1.33% loss in DO. This may be corrected by an appropriate factor or by adjustment of reagent normality. It is generally considered small in relation to other errors in sampling, manipulation and interference, hence this error may be recognized but not corrected.

8 Reagent preparation and procedural details can be found in reference 1.

IV The sequential reactions for the Chemical DO determination provides several situations where significant interference may occur in application on polluted water, such as

A Sampling errors may not be strictly designated as interference but have the same effect of changing sample DO. Inadequate flushing of the bottle contents or exposure to air may raise the DO of low oxygen samples or lower the DO of supersaturated samples.

B Entrained air may be trapped in a DO bottle by·

1 Rapid filling of vigorously mixed samples without allowing the entrained air to escape before closing the bottle and adding DO reagents.

2 Filling a bottle with low temperature water holding more DO than that in equilibrium after the samples warm to working temperature.

3 Aeration is likely to cool the sample permitting more DO to be introduced than can be held at the room or incubator temperatures.

4 Samples warmer than working incubator temperatures will be only partially full at equilibrium temperatures.

Addition of DO reagents results in reaction with dissolved or entrained oxygen. Results for DO are invalid if there is any evidence of gas bubbles in the sample bottle.

C The DO reagents respond to any oxidant or reductant in the sample capable of reacting within the time allotted. HOCl of H_2O_2 may raise the DO titration while H_2S, 4 SH^- may react with sample oxygen to lower the sample titration. The items mentioned react rapidly and raise or lower the DO result promptly. Other items such as Fe^{++} or $SO_3^=$ may or may not react completely within the time allotted for reaction. Many organic materials or complexes from benthic deposits may have an effect upon DO results that are difficult to predict. They may have one effect during the alkaline stage to release iodine from KI while favoring irreversible absorption of iodine during the acid stage. Degree of effect may increase with reaction time. It is generally inadvisable to use the iodometric titration on samples containing large amounts of organic contaminants or benthic residues. It would be expected that benthic residues would tend toward low results because of the reduced iron and sulfur content - they commonly favor high results due to other factors that react more rapidly, often giving the same effect as in uncontrolled nitrite interference during titration.

D Nitrite is present to some extent in natural waters or partially oxidized treatment plant samples. Nitrite is associated with a cyclic reaction during the acid stage of the DO determination that may lead to erroneous high results.

1 These reactions may be represented as follows·

$$2HNO_2 + 2\,HI \rightarrow I_2 + 4H_2O + N_2O_2 \quad (a)$$

$$2HNO_2 \leftarrow + 1/2O_2 + H_2O + N_2O_2 \quad (b)$$

These reactions are time, mixing and concentration dependent and can be minimized by rapid processing.

2 Sodium azide (NaN_3) reacts with nitrite under acid conditions to form a combination of $N_2 + N_2O$ which effectively blocks the cyclic reaction by converting the HNO_2 to noninterfering compounds of nitrogen.

3 Sodium azide added to fresh alkaline KI reagent is adequate to control interference up to about 20 mg of HNO_2 - N/liter of sample. The azide is unstable and gradually decomposes. If resuspended benthic sediments are not detectable in a sample showing a returning blue color, it is likely that the azide has decomposed in the alkaline KI azide reagent.

E Surfactants, color and Fe^{+++} may confuse endpoint detection if present in significant quantities.

F Polluted water commonly contains significant interferences such as C. It is advisable to use a membrane protected sensor of the electronic type for DO determinations in the presence of these types of interference.

G The order of reagent addition and prompt completion of the DO determination is critical. Stable waters may give valid DO results after extended delay of titration during the acidified stage. For unstable water, undue delay at any stage of processing accentuates interference problems.

ACKNOWLEDGMENTS

This outline contains significant materials from previous outlines by J. W. Mandia. Review and comments by C. R. Hirth and R. L. Booth are greatly appreciated.

REFERENCE

1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH, July 1971.

This outline was prepared by F. J. Ludzack, Chemist National Training Center, MDS, OWP, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR DISSOLVED OXYGEN

Winkler-Azide Procedure

I INTRODUCTION

The azide modification is used for most sewage. effluents, and streams which contain nitrate nitrogen and not more than 1 mg of ferrous iron/l. If 1 ml 40% KF solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100-200 mg/l ferric iron.

The "Methods for Chemical Analysis of Water & Wastes, 1971," published by the Analytical Quality Control Laboratory of the EPA, recommends the Winkler-Azide method using the full bottle technique.

II REAGENTS

A Manganous Sulfate Solution

Dissolve 480 g $MnSO_4 \cdot 4H_2O$ in distilled water and dilute to 1 liter.

B Alkali-Iodide-Azide Reagent

Dissolve 500 g sodium hydroxide and 150 g potassium iodide in distilled water and dilute to 1 liter. To this solution add 10 g sodium azide. NaN_3 dissolved in 40 ml distilled water.

C Sulfuric Acid, Conc.

The strength of this acid is 36 N.

D Starch Solution

Prepare an emulsion of 10 g of soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 ml per liter of chloroform and storage in a 10° C refrigerator.

E Sodium Thiosulfate Stock Solution 0.75 N

Dissolve 186.15 g $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 ml chloroform.

F Sodium Thiosulfate Standard Titrant 0.0375N

Dilute 50.0 ml of stock solution to 1 liter. Preserve by adding 5 ml of chloroform. Standardize with potassium biiodate.

III PROCEDURE

A Addition of Reagents

1 Manganous sulfate and alkali-iodide-azide

To a full sample bottle (300 ml ± 3 ml BOD incubation), add 2 ml manganous sulfate solution and 2 ml alkaline-iodide reagent with the tip of each pipette below the surface of the sample.

2 Replace the stopper, rinse under running water and mix by inverting 4-5 times.

3 Allow the precipitate to settle until at least 100 ml of clear supernate have been produced. Repeat the inverting and settling.

4 Add 2 ml conc. sulfuric acid with the tip of the pipette above the surface of the sample.

5 Stopper the bottle, rinse under running water and mix to dissolve the precipitate.

6 Pour contents of bottle into a wide mouth 500 ml Erlenmeyer flask.

B Titration and Calculation

1 Titrate with 0.0375 N thiosulfate to a pale straw color, add 2 ml starch solution indicator and continue titrating to the disappearance of the blue color.

2. Calculation

$$\frac{\text{mls titrant} \times 0.0375 \times 8000}{300} = \text{mg/l DO}$$

$$\text{mls titrant} \times 0.0125 \times 80 = \text{mg/l DO}$$

$$\text{mls titrant} \times 1 = \text{mg/l DO}$$

$$\text{mls titrant} = \text{mg/l DO}$$

REFERENCE

Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, Ohio, July 1971.

This outline was prepared by J. W. Mandia, Chemist, formerly with National Training Center, and revised by C. R. Feldmann, Chemist, National Training Center, MDS, OWP, EPA, Cincinnati, OH 45268.

DISSOLVED OXYGEN DETERMINATION - II
ELECTRONIC MEASUREMENTS

I INTRODUCTION

A Electronic measurement of DO is attractive for several reasons·

1 Electronic methods are more readily adaptable for automated analysis, continuous recording, remote sensing or portability.

2 Application of electronic methods with membrane protection of sensors affords a high degree of interference control.

3 Versatility of the electronic system permits design for a particular measurement, situation or use.

4 Many more determinations per man-hour are possible with a minor expenditure of time for calibration.

B Electronic methods of analysis impose certain restrictions upon the analyst to insure that the response does, in fact, indicate the item sought.

1 The ease of reading the indicator tends to produce a false sense of security. Frequent and careful calibrations are essential to establish workability of the apparatus and validity of its response.

2 The use of electronic devices requires a greater degree of competence on the part of the analyst Understanding of the behavior of oxygen must be supplemented by an understanding of the particular instrument and its behavior during use.

C Definitions

1 Electrochemistry - a branch of chemistry dealing with relationships between electrical and chemical changes.

2 Electronic measurements or electrometric procedures - procedures using the measurement of potential differences as an indicator of reactions taking place at an electrode or plate.

3 Reduction - any process in which one or more electrons are added to an atom or an ion, such as $O_2 + 2e \rightarrow 2O^-$! The oxygen has been reduced.

4 Oxidation - any process in which one or more electrons are removed from an atom or an ion, such as $Zn^o - 2e \rightarrow Zn^{+2}$. The zinc has been oxidized.

5 Oxidation - reduction reactions - in a strictly chemical reaction, reduction cannot occur unless an equivalent amount of some oxidizable substance has been oxidized. For example:

$$2H_2 + O_2 \rightleftarrows 2H_2O$$
$$2H_2 - 4e \rightleftarrows 4H^{+1} \quad \text{hydrogen oxidized}$$
$$O_2 + 4e \rightleftarrows 2O^{-2} \quad \text{oxygen reduced}$$

Chemical reduction of oxygen may also be accomplished by electrons supplied to a noble metal electrode by a battery or other energizer.

6 Anode - an electrode at which oxidation of some reactable substance occurs.

7 Cathode - an electrode at which reduction of some reactable substance occurs. For example in I.C.3, the reduction of oxygen occurs at the cathode.

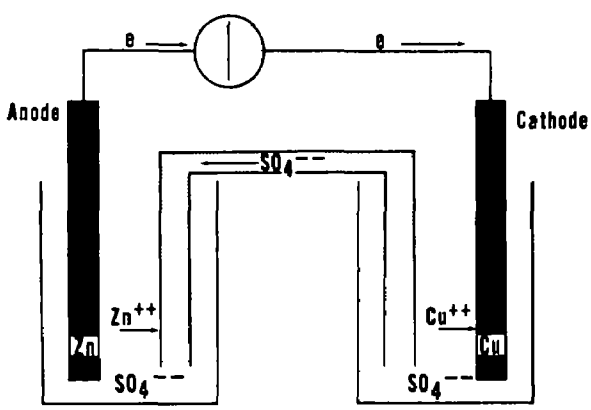
8 Electrochemical reaction - a reaction involving simultaneous conversion of chemical energy into electrical energy or the reverse. These conversions are

equivalent in terms of chemical and electrical energy and generally are reversible

9 Electrolyte a solution, gel, or mixture capable of conducting electrical energy and serving as a reacting media for chemical changes. The electrolyte commonly contains an appropriate concentration of selected mobile ions to promote the desired reactions

10 Electrochemical cell - a device consisting of an electrolyte in which 2 electrodes are immersed and connected via an external metallic conductor The electrodes may be in separate compartments connected by tube containing electrolyte to complete the internal circuit

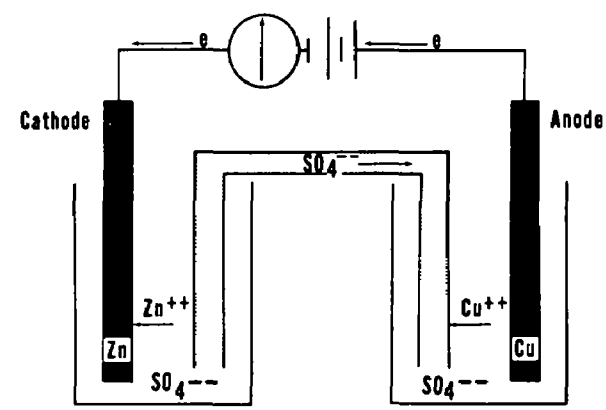
a Galvanic (or voltaic) cell - an electrochemical cell operated in such a way as to produce electrical energy from a chemical change, such as a battery (See Figure 1).



GALVANIC CELL

Figure 1

b Polarographic (electrolytic) cell - an electrochemical cell operated in such a way as to produce a chemical change from electrical energy (See Figure 2).



POLAROGRAPHIC CELL

Figure 2

D As indicated in I. C. 10 the sign of an electrode may change as a result of the operating mode. The conversion by the reactant of primary interest at a given electrode therefore designates terminology for that electrode and operating mode In electronic oxygen analyzers, the electrode at which oxygen reduction occurs is designated the cathode.

E Each cell type has characteristic advantages and limitations. Both may be used effectively.

1 The galvanic cell depends upon measurement of electrical energy produced as a result of oxygen

reduction. If the oxygen content of the sample is negligible, the measured current is very low and indicator driving force is negligible, therefore response time is longer

2 The polarographic cell uses a standing current to provide energy for oxygen reduction. The indicator response depends upon a change in the standing current as a result of electrons released during oxygen reduction. Indicator response time therefore is not dependent upon oxygen concentration.

3 Choice may depend upon availability, habit, accessories, or the situation. In each case it is necessary to use care and judgment both in selection and use for the objectives desired.

II ELECTRONIC MEASUREMENT OF DO

A Reduction of oxygen takes place in two steps as shown in the following equations:

1 $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

2 $H_2O_2 + 2e^- \rightarrow 2OH^-$

Both equations require electron input to activate reduction of oxygen. The first reaction is more important for electronic DO measurement because it occurs at a potential (voltage) which is below that required to activate reduction of most interfering components (0.3 to 0.8 volts relative to the saturated calomel electrode - SCE). Interferences that may be reduced at or below that required for oxygen usually are present at lower concentrations in water or may be minimized by the use of a selective membrane or other means. When reduction occurs, a definite quantity of electrical energy is produced that is proportional to the quantity of reductant entering the reaction. Resulting current measurements thus are more specific for oxygen reduction.

B Most electronic measurements of oxygen are based upon one of two techniques for evaluating oxygen reduction in line with equation II A.1. Both require activating energy, both produce a current proportional to the quantity of reacting reductant. The techniques differ in the means of supplying the activating potential; one employs a source of outside energy, the other uses spontaneous energy produced by the electrode pair.

1 The polarographic oxygen sensor relies upon an outside source of potential to activate oxygen reduction Electron gain by oxygen changes the reference voltage.

a Traditionally, the dropping mercury electrode (DME) has been used for polarographic measurements. Good results have been obtained for DO using the DME but the difficulty of maintaining a constant mercury drop rate, temperature control, and freedom from turbulence makes it impractical for field use.

b Solid electrodes are attractive because greater surface area improves sensitivity. Poisoning of the solid surface electrodes is a recurrent problem. The use of selective membranes over noble metal electrodes has minimized but not eliminated electrode contamination. Feasibility has been improved sufficiently to make this type popular for regular use.

2 Galvanic oxygen electrodes consist of a decomposable anode and a noble metal cathode in a suitable electrolyte to produce activating energy for oxygen reduction (an air cell or battery). Lead is commonly used as the anode because its decomposition potential favors spontaneous reduction of oxygen. The process is continuous as long as lead and oxygen are in contact in the electrolyte and the electrical energy released at the cathode may be dissipated by an outside circuit. The anode may be conserved by limiting oxygen availability. Interrupting the outside circuit may produce erratic behavior for a time after reconnection. The resulting

current produced by oxygen reduction may be converted to oxygen concentration by use of a sensitivity coefficient obtained during calibration. Provision of a pulsed or interrupted signal makes it possible to amplify or control the signal and adjust it for direct reading in terms of oxygen concentration or to compensate for temperature effects.

III ELECTRONIC DO ANALYZER APPLICATION FACTORS

A Polarographic or galvanic DO instruments operate as a result of oxygen partial pressure at the sensor surface to produce a signal characteristic of oxygen reduced at the cathode of some electrode pair. This signal is conveyed to an indicating device with or without modification for sensitivity and temperature or other influences depending upon the instrument capabilities and intended use.

1 Many approaches and refinements have been used to improve workability, applicability, validity, stability and control of variables. Developments are continuing. It is possible to produce a device capable of meeting any reasonable situation, but situations differ.

2 Most commercial DO instruments are designed for use under specified conditions. Some are more versatile than others. Benefits are commonly reflected in the price. It is essential to determine the requirements of the measurement situation and objectives for use. Evaluation of a given instrument in terms of sensitivity, response time, portability, stability, service characteristics, degree of automation, and consistency are used for judgment on a cost/benefit basis to select the most acceptable unit

B Variables Affecting Electronic DO Measurement

1 Temperature affects the solubility of oxygen, the magnitude of the resulting signal and the permeability of the protective membrane. A curve of oxygen solubility in water versus increasing temperature may be concave downward while a similar curve of sensor response versus temperature is concave upward. Increasing temperature decreases oxygen solubility and increases probe sensitivity and membrane permeability. Thermistor actuated compensation of probe response based upon a linear relationship or average of oxygen solubility and electrode sensitivity is not precisely correct as the maximum spread in curvature occurs at about 17° C with lower deviations from linearity above or below that temperature. If the instrument is calibrated at a temperature within + or - 5° C of working temperature, the compensated readout is likely to be within 2% of the real value. Depending upon probe geometry, the laboratory sensor may require 4 to 6% correction of signal per ° C change in liquid temperature.

2 Increasing pressure tends to increase electrode response by compression and contact effects upon the electrolyte, dissolved gases and electrode surfaces. As long as entrained gases are not contained in the electrolyte or under the membrane, these effects are negligible.

Inclusion of entrained gases results in erratic response that increases with depth of immersion.

3 Electrode sensitivity changes occur as a result of the nature and concentration of contaminants at the electrode surfaces and possible physical chemical or electronic side reactions produced. These may take the form of a physical barrier, internal short, high residual current, or chemical changes in the metal surface. The membrane is intended to allow dissolved gas penetration but to exclude passage of ions or particulates. Apparently some ions or materials producing extraneous ions within the electrode vicinity are able to pass in limited amounts which

become significant in time. Dissolved gases include 1) oxygen, 2) nitrogen, 3) carbon dioxide, 4) hydrogen sulfide, and certain others. Item 4 is likely to be a major problem. Item 3 may produce deposits in alkaline media, most electrolytes are alkaline or tend to become so in line with reaction II.A.1. The usable life of the sensor varies with the type of electrode system, surface area, amount of electrolyte and type, membrane characteristics, nature of the samples to which the system is exposed and the length of exposure. For example, galvanic electrodes used in activated sludge units showed that the time between cleanup was 4 to 6 months for electrodes used for intermittent daily checks of effluent DO, continuous use in the mixed liquor required electrode cleanup in 2 to 4 weeks. Each electrometric cell configuration and operating mode has its own response characteristics. Some are more stable than others. It is necessary to check calibration frequency required under conditions of use as none of them will maintain uniform response indefinitely. Calibration before and after daily use is advisable.

4 Electrolytes may consist of solutions or gels of ionizable materials such as acids, alkalies or salts. Bicarbonates, KCl and KI are frequently used. The electrolyte is the transfer and reaction media, hence, it necessarily becomes contaminated before damage to the electrode surface may occur. Electrolyte concentration, nature, amount and quality affect response time, sensitivity, stability, and specificity of the sensor system. Generally a small quantity of electrolyte gives a shorter response time and higher sensitivity but also may be affected to a greater extent by a given quantity of contaminating substances.

5 Membranes may consist of teflon, polyethylene, rubber, and certain other polymeric films. Thickness may vary from 0.5 to 3 mils (inches × 1/1000). A thinner membrane will decrease response time and increase sensitivity but is less selective and may be ruptured more easily. The choice of material and its uniformity affects response time, selectivity and durability. The area of the membrane and its permeability are directly related to the quantity of transported materials that may produce a signal. The permeability of the membrane material is related to temperature and to residues accummulated on the membrane surface or interior. A cloudy membrane usually indicates deposition and more or less loss of signal.

6 Test media characteristics control the interval of usable life between cleaning and rejuvenation for any type of electrode. More frequent cleanup is essential in low quality waters than for high quality waters. Reduced sulfur compounds are among the more troublesome contaminants. Salinity affects the partial pressure of oxygen at any given temperature. This effect is small compared to most other variables but is significant if salinity changes by more than 500 mg/l.

7 Agitation of the sample in the vicinity of the electrode is important because DO is reduced at the cathode. Under quiescent conditions a gradient in dissolved oxygen content would be established on the sample side of the membrane as well as on the electrode side, resulting in atypical response. The sample should be agitated sufficiently to deliver a representative portion of the main body of the liquid to the outer face of the membrane. It is commonly observed that no agitation will result in a very low or negigible response after a short period of time. Increasing agitation will cause the response to rise gradually until some minimum liquid velocity is reached that will not cause a further increase in response with increased mixing energy. It is important to check mixing velocity to reach a stable high signal that is independent of a reasonable change in sample mixing. Excessive

mixing may create a vortex and expose the sensing surface to air rather than sample liquid. This should be avoided. A linear liquid velocity of about 1 ft/sec at the sensing surface is usually adequate.

8 DO sensor response represents a potential or current signal in the milli-volt or milli-amp range in a high resistance system. A high quality electronic instrument is essential to maintain a usable signal-to-noise ratio. Some of the more common difficulties include.

a Variable line voltage or low batteries in amplifier power circuits.

b Substandard or unsteady amplifier or resistor components.

c Undependable contacts or junctions in the sensor, connecting cables, or instrument control circuits.

d Inadequately shielded electronic components.

e Excessive exposure to moisture, fumes or chemicals in the wrong places lead to stray currents, internal shorts or other malfunction.

C Desirable Features in a Portable DO Analyzer

1 The unit should include steady state performance electronic and indicating components in a convenient but sturdy package that is small enough to carry.

2 There should be provisions for addition of special accessories such as bottle or field sensors, agitators, recorders, line extensions, if needed for specific requirements. Such additions should be readily attachable and detachable and maintain good working characteristics.

3 The instrument should include a sensitivity adjustment which upon calibration will provide for direct reading in terms of mg of DO/liter.

4 Temperature compensation and temperature readout should be incorporated.

5 Plug in contacts should be positive, sturdy, readily cleanable and situated to minimize contamination. Water seals should be provided where necessary.

6 The sensor should be suitably designed for the purpose intended in terms of sensitivity, response, stability, and protection during use. It should be easy to clean, and reassemble for use with a minimum loss of service time.

7 Switches, connecting plugs, and contacts preferably should be located on or in the instrument box rather than at the "wet" end of the line near the sensor. Connecting cables should be multiple strand to minimize separate lines. Calibration controls should be convenient but designed so that it is not likely that they will be inadvertently shifted during use.

8 Agitator accessories for bottle use impose special problems because they should be small, self contained, and readily detachable but sturdy enough to give positive agitation and electrical continuity in a wet zone.

9 Major load batteries should be rechargeable or readily replaceable. Line operation should be feasible wherever possible

10 Service and replacement parts availability are a primary consideration. Drawings, parts identification and trouble shooting memos should be incorporated with applicable operating instructions in the instrument manual in an informative organized form.

D Sensor and Instrument Calibration

The instrument box is likely to have some form of check to verify electronics, battery or other power supply conditions for use. The sensor commonly is not included in this check. A known reference

sample used with the instrument in an operating mode is the best available method to compensate for sensor variables under use conditions. It is advisable to calibrate before and after daily use under test conditions. Severe conditions, changes in conditions, or possible damage call for calibrations during the use period. The readout scale is likely to be labeled - calibration is the basis for this label.

The following procedure is recommended·

1 Turn the instrument on and allow it to reach a stable condition. Perform the recommended instrument check as outlined in the operating manual.

2 The instrument check usually includes an electronic zero correction. Check each instrument against the readout scale with the sensor immersed in an agitated solution of sodium sulfite containing sufficient cobalt chloride to catalyze the reaction of sulfite and oxygen. The indicator should stabilize on the zero reading. If it does not, it may be the result of residual or stray currents, internal shorting in the electrode, or membrane rupture. Minor adjustments may be made using the indicator rather than the electronic controls. Serious imbalance requires electrode reconditioning if the electronic check is O.K. Sulfite must be carefully rinsed from the sensor until the readout stabilizes to prevent carry over to the next sample.

3 Fill two DO bottles with replicate samples of clarified water similar to that to be tested. This water should not contain significant test interferences.

4 Determine the DO in one by the azide modification of the iodometric titration.

5 Insert a magnetic stirrer in the other bottle or use a probe agitator. Start agitation after insertion of the sensor assembly and note the point of stabilization.

a Adjust the instrument calibration control if necessary to compare with the titrated DO.

b If sensitivity adjustment is not possible, note the instrument stabilization point and designate it as ua. A sensitivity coefficient, ϕ is equal to $\frac{ua}{DO}$ where DO is the titrated value for the sample on which ua was obtained. An unknown DO then becomes $DO = \frac{ua}{\phi}$. This factor is applicable as long as the sensitivity does not change.

6 Objectives of the test program and the type of instrument influence calibration requirements. Precise work may require calibration at 3 points in the DO range of interest instead of at zero and high range DO. One calibration point frequently may be adequate.

Calibration of a DO sensor in air is a quick test for possible changes in sensor response. The difference in oxygen content of air and of water is too large for air calibration to be satisfactory for precise calibration for use in water.

IV This section reviews characteristics of several sample laboratory instruments. Mention of a specific instrument does not imply FWQA endorsement or recommendation. No attempt has been made to include all the available instruments, those described are used to indicate the approach used at one stage of development which may or may not represent the current available model.

A The electrode described by Carrit and Kanwisher (1) is illustrated in Figure 3. This electrode was an early example of those using a membrane. The anode was a silver - silver oxide reference cell with a platinum disc cathode (1-3 cm diameter). The salt bridge consisted of N/2 KCl and

KOH. The polyethylene membrane was held in place by a retaining ring. An applied current was used in a polarographic mode. Temperature effects were relatively large. Thermistor correction was studied but not integrated with early models.

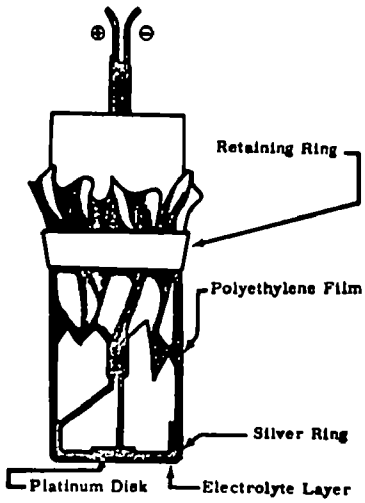


Figure 3

B The Beckman oxygen electrode is another illustration of a polarographic DO sensor (Figure 4). It consists of a gold cathode, a silver anode, an electrolytic gel containing KCl, covered by a teflon membrane. The instrument has a temperature readout and compensating thermistor, a source polarizing current, amplifier with signal adjustment and a readout DO scale with recorder contacts.

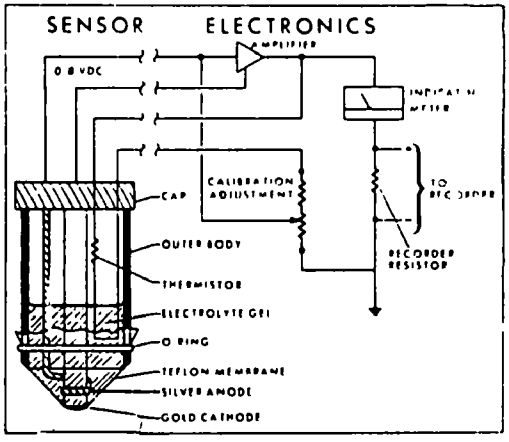


Figure 4. THE BECKMAN OXYGEN SENSOR

C The YSI Model 51 (3) is illustrated in Figure 5. This is another form of polarographic DO analyzer. The cell consists of a silver anode coil, a gold ring cathode and a KCl electrolyte with a teflon membrane The instrument has a sensitivity adjustment, temperature and DO readout. The model 51 A has temperature compensation via manual preset dial. A field probe and bottle probe are available

YSI Model 51 DO Sensor

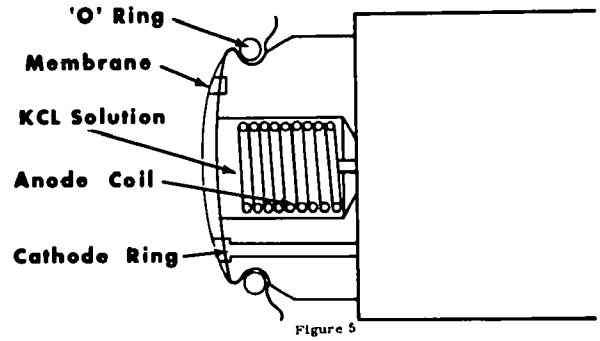


Figure 5

D The Model 54 YSI DO analyzer (4) is based upon the same electrode configuration but modified to include automatic temperature compensation, DO readout, and recorder jacks. A motorized agitator bottle probe is available for the Model 54 (Figure 6)

YSI Model 54 Agitator Probe

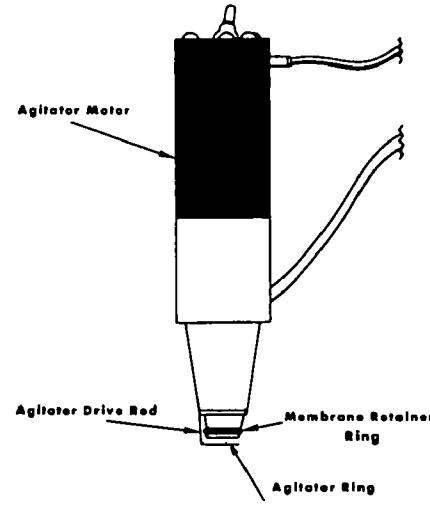


Figure 6

E The Galvanic Cell Oxygen Analyzer (7, 8) employs an indicator for proportional DO signal but does not include thermistor compensation or signal adjustment. Temperature readout is provided. The sensor includes a lead anode ring, and a silver cathode with KOH electrolyte (4 molar) covered by a membrane film (Figure 7).

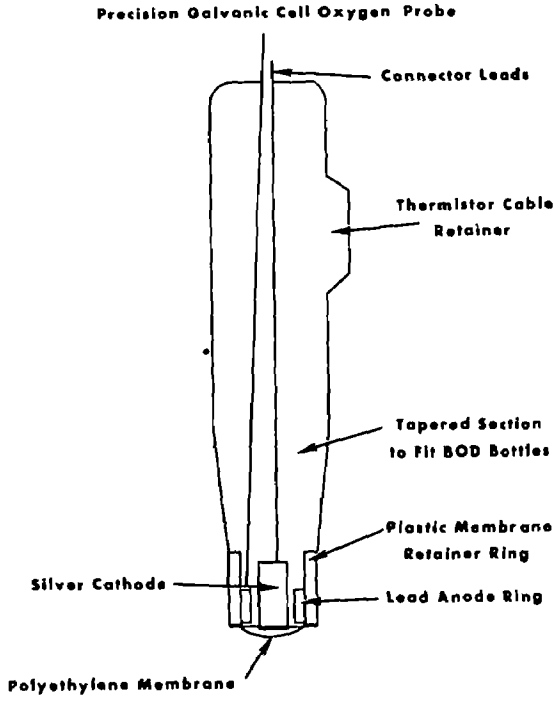


Figure 7

F The Weston and Stack Model 300 DO Analyzer (8) has a galvanic type sensor with a pulsed current amplifier adjustment to provide for signal and temperature compensation. DO and temperature readout is provided. The main power supply is a rechargeable battery. The sensor (Figure 8) consists of a lead anode coil recessed in the electrolyte cavity (50% KI) with a platinum cathode in the tip. The sensor is covered with a teflon membrane. Membrane retention by rubber band or by a plastic retention ring may be used for the bottle agitator or depth sampler respectively. The thermistor and agitator are mounted in a sleeve that also provides protection for the membrane.

G The EIL Model 15 A sensor is illustrated in Figure 9. This is a galvanic cell with thermistor activated temperature compensation and readout. Signal adjustment is provided. The illustration shows an expanded scheme of the electrode which when assembled compresses into a sensor approximating 5/8 inch diameter and 4 inch length exclusive of the enlargement at the upper end. The anode consists of compressed lead shot in a replaceable capsule (later models used fine lead wire coils), a perforated silver cathode sleeve around the lead is covered by a membrane film. The electrolyte is saturated potassium bicarbonate. The large area of lead surface, silver and membrane provides a current response of 200 to 300 microamperes in oxygen saturated water at 20° C for periods of up to 100 days use (8). The larger electrode displacement favors a scheme described by Eden (9) for successive DO readings for BOD purposes.

V Table 1 summarizes major characteristics of the sample DO analyzers described in Section IV. It must be noted that an ingenious analyst may adapt any one of these for special purposes on a do-it-yourself program. The sample instruments are mainly designed for laboratory or portable field use. Those designed for field monitoring purposes may include similar designs or alternate designs generally employing larger anode, cathode, and electrolyte capacity to approach better response stability with some sacrifice in response time and sensitivity. The electronic controls, recording, telemetering, and accessory apparatus generally are semi-permanent installations of a complex nature.

ACKNOWLEDGMENTS

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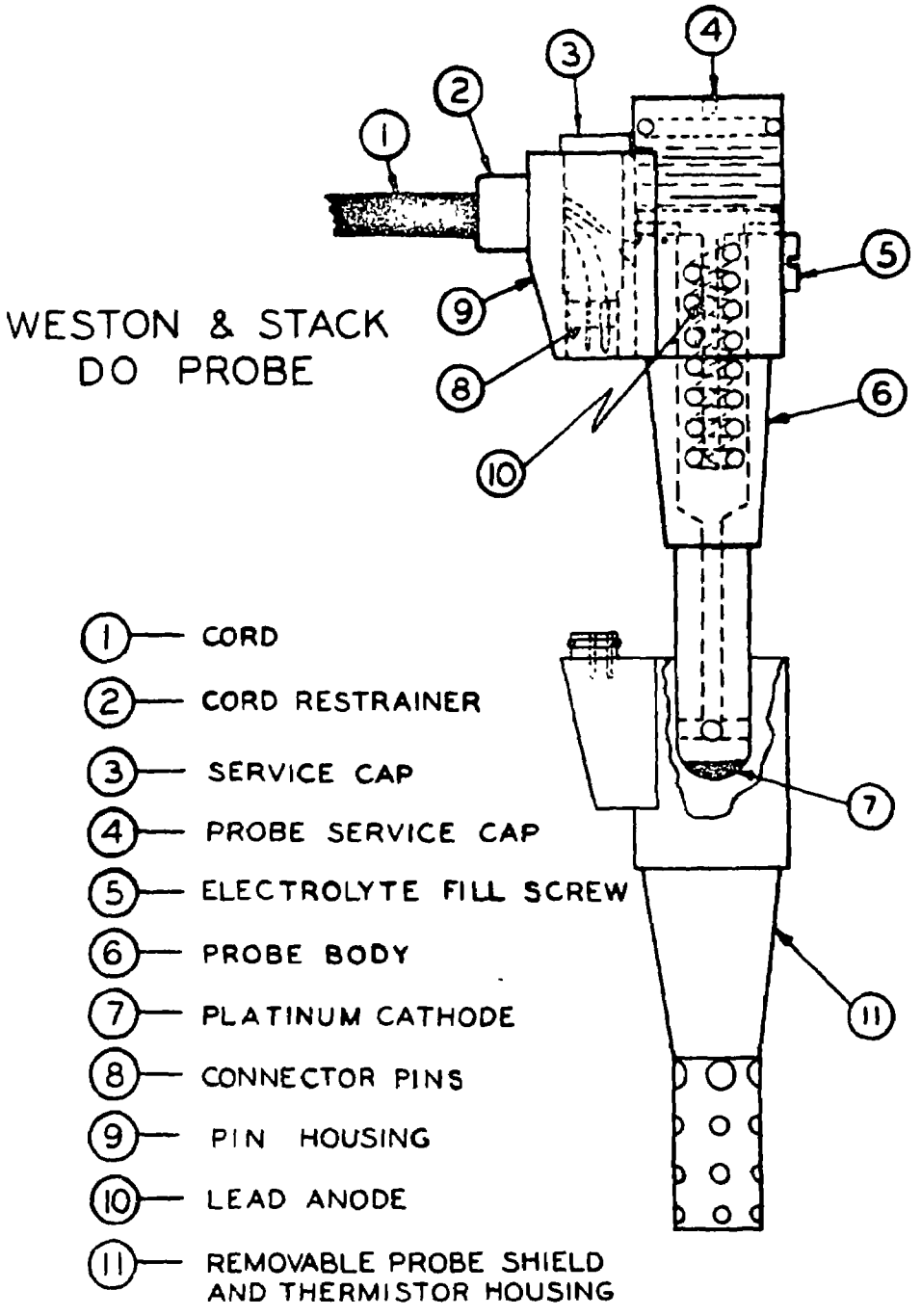
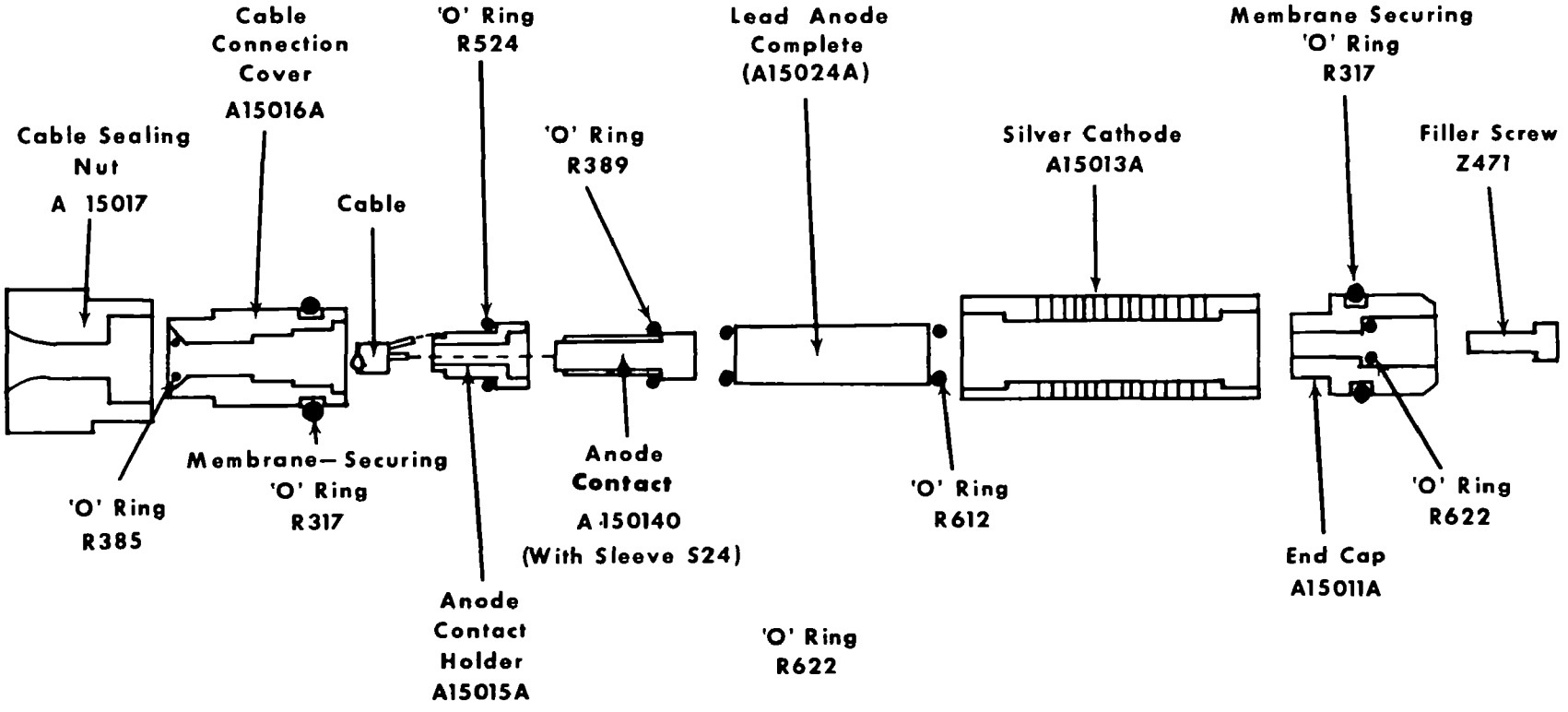

WESTON & STACK
DO PROBE
1 CORD
2 CORD RESTRAINER
3 SERVICE CAP
4 PROBE SERVICE CAP
5 ELECTROLYTE FILL SCREW
6 PROBE BODY
7 PLATINUM CATHODE
8 CONNECTOR PINS
9 PIN HOUSING
10 LEAD ANODE
11 REMOVABLE PROBE SHIELD AND THERMISTOR HOUSING

Figure 8

Model A15A ELECTRODE COMPONENT PARTS



Note: Red wire of cable connects to Anode Contact Holder

Black wire of cable connects to Anode Contact

Membrane not shown E. I. L. part number T22

Figure 9

TABLE 1

CHARACTERISTICS OF VARIOUS LABORATORY DO INSTRUMENTS

	Anode	Cathode	Elec	Type	Membr	DO Sig. Adj	Temp. Comp / Temp. Rdg.	Accessories for which designed
Carrit & Kanwisher	silver-silver ox. ring	Pt disc	KC1 KOH N/2	pol	polyeth	no	no	Recording temp. & signal adj. self assembled
Beckman	Aq ring	Au disc	KCL gel	pol	teflon	yes	yes / yes	recording
Yellow Springs 51	Ag coil	Au ring	KC1 soln sat.	pol	teflon	yes	no* / yes	field and bottle probe
Yellow Springs 54	"	"	"	"	"	yes	yes / yes	recording field bottle & agitator probes
Precision Sci	Pb ring	silver disc	KOH 4N	galv	polyeth	no	no / yes	
Weston & Stack 300	Pb coil	Pt disc	KI 40%	galv	teflon	yes	yes / yes	agit. probe depth sampler
EIL	Pb	Ag	$KHCO_3$	galv	teflon	yes	yes / yes	recording
Delta 75	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes / no	field bottle & agitator probe
Delta 85	Lead	Silver disc	KOH 1N	galv	teflon	yes	yes / yes	field bottle & agitator probe

*Pol - Polarographic (or amperimetric)
Galv - Galvanic (or voltametric)

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This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB MDS, Environmental Protection Agency, OWP, Cincinnati, OH 45268 and Nate Malof, Chemist National Field Investigations Center, Environmental Protection Agency, WPO, Cincinnati, OH.

BIOCHEMICAL OXYGEN DEMAND TEST PROCEDURES

I OXYGEN DEMAND OF POLLUTED WATERS

Established practice includes common use of the BOD test as a tool for estimation of the bio-oxidizable fraction of surface waters or wastewaters discharged to them. Any index including a quantity per unit time such as the BOD_5 is a rate expression. The ultimate demand is more important than any one point on the progression. The results of a bottle test with minimum seeding and quiescent storage are not likely to be as high as those on the same influent in a mixing situation and abundant seed of secondary treatment or receiving waters. The BOD_5 is "a" fraction of total oxygen requirements.

A The particular technique used for BOD commonly is specified by State agencies and/or supervisors. They are required to interpret the results as obtained by laboratory testing. It is essential that the tester and the interpreters have a common understanding of what was done and how. It is highly advisable to maintain a given routine until all concerned agree upon a change.

1 Each particular routine has many undefinable factors. The particular routine is not as important as the consistency and capability with which the result was obtained.

2 This outline and Standard Methods[1] discusses several valid approaches for obtaining BOD results. Selection of "method" is not intended in this outline or in the EPA Methods Manual[2].

B The common 5-day incubation period for BOD testing is a result of tradition and cost. Initial lags are likely to be over and some unknown fraction of the total oxidizable mass has been satisfied after 5 days

C A series of observations over a period of time makes it possible to estimate the total oxidizable mass and the fraction oxidized or remaining to be oxidized at any given time The problem is to define the shape of the deoxygenation pattern and its limits A fair estimate of the shape of the deoxygenation pattern is available by observations at 1, 2, or 3 days, 7 days and 14 days. Increased observations are desirable for more valid estimates of curve shape, rate of oxidation and total oxidizable mass or ultimate BOD

D Increasing impoundment of surface waters and concurrent increases in complexity and stability of wastewater components emphasize the importance of long-term observation of BOD. The 5-day observation includes most of the readily oxidizable materials but a very small fraction of the stable components that are the main factors in impoundment behavior

II DIRECT METHOD

A With relatively clean surface waters, the BOD may be determined by incubation of the undiluted sample for the prescribed time interval. This method is applicable only to those waters whose BOD is less than 8 mg/1 and assumes the sample contains suitable organisms and accessory nutrients for optimum biological stabilization.

B Treated effluents, polluted surface waters, household and industrial wastewaters commonly require dilution to provide the excess oxygen required for the oxygen demand determination General guidelines for dilution requirements for a given BOD range in terms of the percent of sample in BOD dilution water are

For a 5-day BOD of
5-20 mg/1, use 25 to 100% sample

For a BOD of
20-100 mg/1, use 5 to 25% sample

For a BOD of
100-500 mg/1, use 1 to 5% sample

For a BOD of
500-5000 mg/1, use 0 1 to 1 0% sample

III PROCEDURES

A Cylinder Dilution Technique

1 Using an assumed or estimated BOD value as a guide, calculate the factors for a range of dilutions to cover the desired depletions. Those dilutions ranging from a depletion of 2 mg/l and a residual of 1 mg/l are most reliable At least three dilutions in duplicate should be used for an unknown sample.

2 Into a one-liter graduate cylinder (or larger container if necessary) measure accurately the required amount of mixed sample to give one liter of diluted waste Fill to the one liter mark with dilution water. Carefully mix The initial DO by calculation includes IDOD (VIII) a determined initial does not. Both are essential to estimate significance of IDOD. Entrapment of air bubbles during manipulation must be avoided.

3 Siphon the mixture from the cylinder into three 300 ml glass stoppered bottles, filling the bottles to overflowing

4 Determine the DO concentration on one of the bottles by the appropriate Winkler modification and record as "Initial DO".

5 Incubate the two remaining bottles at 20°C in complete darkness The incubated bottles should be water-sealed by immersion in a tray or by using a special water-seal bottle.

6 After 5 days of incubation, or other desired interval, determine the DO on the bottles. Average the DO concentration of the duplicates and report as "Final DO".

B Direct Dilution Technique

1 It may be more convenient to make the dilution directly in sample bottles of known capacity. A measured volume of sample may be added (as indicated in A-1) and the bottle filled with dilution water to make the desired sample concentration for incubation In this case, the sample must be precisely measured, the bottle carefully filled, but not overfilled, and the bottle volumes comparable and known. Precision is likely to be poorer than for cylinder dilution.

2 Continue the procedure as in A-4, 5, and 6

C Seeded Cylinder Dilution Technique

1 Many wastewaters may be partially or completely sterile as a result of chlorination, effects of other toxic chemicals, heat, unfavorable pH or other factors detrimental to biological activity Validity of the BOD result depends upon the presence of organisms capable of prompt and effective biodegradation and favorable conditions during the particular test Correction of the cause resulting in sterilization must be corrected by adjustment, dilution, etc., prior to reinoculation to achieve meaningful BOD data Receiving water, biologically treated effluents, and soil suspensions are a good source of organisms likely to be adapted for stabilization of wastewaters. Untreated wastewaters provide numerous organisms but are likely to contain nutrients contributing to excessive seed corrections and may require appreciable time for adaptation before test waste oxidation becomes significant

2 The amount of added inoculant must be determined by trial. The concentration added should initiate biochemical activity promptly but should not exert enough oxygen demand to unduly reduce the oxygen available for sample requirements.

3 Estimate the sample concentration desired in accordance with A-1 and C-2 and add the sample aliquot to the dilution cylinder

4 Add approximately half of the required amount of dilution water to the sample and mix. This is necessary to assure that the concentrated waste does not exert a toxic effect on the seed organisms

5 Measure a suitable aliquot of seed into the bottle or cylinder and fill with dilution water Mix the combined sample, seed and dilution water without excessive air entrainment

6 Continue as in III-A steps 4, 5, and 6

IV INTERPRETATION OF RESULTS

Standard Methods[1] includes a calculation section that is valid and concise. Preceding it are details of reagent preparation and

procedures for the test. These will not be reprinted here. This section considers certain items that may cause concern about the validity of results unless they are carefully considered and controlled.

A The initial DO of the BOD test obviously should be high. The method of attaining a high DO can trap the analyst.

1 Aeration of dilution water is the most commonly considered treatment. This technique does produce a high DO but it is a treacherous ally.

a Dirty air passing through clean dilution water can produce clean air and dirty water. This is a simple air-washing operation. Filtering the entering air stream may remove brickbats and 2 x 4's, but filters tend to pass organic gases, fine aerosols, and particulates.

b A stream of air passing through water tends to cool the water by evaporation 1 to 3°C below ambient temperature. The cooled liquid picks up more DO than it can hold at ambient temperature. The physical loss of oxygen may produce an erroneously high depletion value for a determined initial DO, or a low depletion on a calculated initial DO. Erroneous blanks are a particular concern. The dilution water temperature/DO shift is critical.

2 Raising DO by allowing the sample to equilibrate in a cotton-plugged bottle for 2 or 3 days permits oxygenation with minimum air volume contact.

3 Shaking a partially filled bottle for a few seconds also oxygenates with minimum opportunity of gas washing contamination, supersaturation, or temperature changes.

B Seeding always is a precarious procedure but a very necessary one at times. Often the application of seed corrections is a "_____ if you do, _____ if you don't" situation. Hopefully, seed corrections are small because each individual biological situation is a "universe" of its own.

1 Unstable seeding materials such as fresh wastewater have "seed" organisms characteristic of their origin and history Saprophytes resulting in surface water stabilization may be a small fraction of the population. Reactable oxygen-demanding components produce excessive demands upon test oxygen resources.

2 A seed containing viable organisms at a lower energy state because of limited nutritional availability theoretically is the best available seed source. An organism population grown under similar conditions should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. The population should not be stored too long because organism redistribution and die-out become limiting. This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular material under consideration.

3 Seed sources and amounts can only be evaluated by trial. Different seed sources and locations require checkout to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

C Chlorination and BOD results fundamentally are incompatible. Chlorination objectives include disinfection as the number one goal. Chlorine is notoriously non-specific in organism effects. Chlorine acts like an oxidant in the DO determination. Test organisms are less suitable for activity than they were before chlorination. Nutrients may be less available after chlorination. Certainly the conditions are less suitable for biological response after chlorination. Dechlorination is feasible with respect to the oxidizing power of

chlorine, but many organic chlorine compounds that do not show strong oxidizing action still have toxic effects on biologic response.

Numbers are obtainable after dechlorination and reseeding. The meaning of these numbers is obscure. At least two states (New York and New Jersey) specify BOD's before chlorination only.

V PRECISION OF THE BOD TEST

A The DO test precision often has been used to suggest precision of the BOD result

DO precision is a relatively minor and controllable factor contributing to BOD results. Other factors such as organism suitability, members, adaptation and conditional variables are much more difficult to control or to evaluate.

B The Analytical Reference Service report on Water Oxygen Demand, July, 1960 (Sample type VII) included the results of seeded samples of glucose-glutamic acid BOD results from 34 agencies on 2, 3, 5 and 7 day incubations.

The relative geometric standard deviation (average) was 19% on 2% sample and 24% on 1% sample concentrations. Rate coefficients ranged from 0 10 to 0.27 with a median of 0.16 from 21 different laboratories that participated in rate studies

VI ALTERNATE BOD TECHNIQUES

Reaeration methods are becoming increasingly popular in order to approach more nearly the actual waste concentration in the receiving water It is common to obtain "sliding" BOD results related to the concentration of waste in a series of dilutions of the same sample. This may result from greater possibilities for toxic effects at higher concentrations, or to a different selection of organisms and change in oxidation characteristics at low concentrations of sample The most reliable estimate of stream behavior is likely to be from that dilution closest to the wastewater dilution in the receiving water.

A Reaeration can be accomplished by the usual series techniques by dumping all of the remaining sealed bottles into a common container when the residual DO reaches about 1.0 mg/1 After reoxygenation, the remaining bottles are refilled and a new initial DO determined. Subsequent dissolved oxygen depletions are added incrementally as a summation of the total oxygen depletion from the start of the test If necessary, the reaeration technique may be performed several times but at a sacrifice of double DO determinations for each day on which reaeration occurs

B Special methods of reaeration have evolved to minimize the extra manipulation for reaeration of individual sample dilutions.

1 Elmore Method

A relatively large volume of the sample is stored in an unsealed bottle. Small bottles are withdrawn in sets of 5 or more, sealed, incubated, and the DO determined at appropriate intervals When the DO concentration in the smaller bottles reaches 1.0 mg/1, a new set is withdrawn from the large unsealed bottle, after reoxygenation if necessary

2 Orford Method

The deoxygenation is carried out in a large sealed jug from which samples for DO are withdrawn at appropriate intervals. To maintain the waste level and a sufficient DO in the jug, additional waste is added from a second open container See diagram

C Excess oxygen may be provided by oxygenation with commercial oxygen instead of with air to increase the initial oxygen content for incubation while limiting the number of dilutions or reaeration steps. When oxygen is used in place of air the oxygen saturation in water at 20°C is about 40 mg/1 instead of 9 mg/1 Limited results are available hence the analyst must verify his technique The DO tends to decrease as soon as the bottle is opened hence, about 35 mg/1 of oxygen content is the top of the practical working concentration There has been no evidence that the biota is inhibited by the higher oxygen content with respect to BOD progression

D Reaeration or Oxygenation Advantages and Limitations

1 Reaeration expands the range of BOD results obtainable directly at field concentrations, but is not advisable for applications when the sample BOD exceeds 50 mg/1

2 Dilution water problems are eliminated, to the extent that the stream sample may be tested without dilution.

3 Incubator storage space becomes a real problem for multiple sample routine.

VII Dissolved oxygen electrodes, polarographic and others, are feasible for use in BOD determinations, often making it possible to make an estimate of DO or BOD when sample interference prevents a valid Winkler DO determination

Electronic probe DO makes it possible to determine many successive DO's at different time intervals on the same bottle with negligible sample loss. Reaeration or extended time series, therefore, are more feasible.

Another outline in this series describes response of reaerated BOD_5 with electronic DO probes.

A It is the responsibility of the analyst to evaluate

1 Applicability of the specified technique and sample.

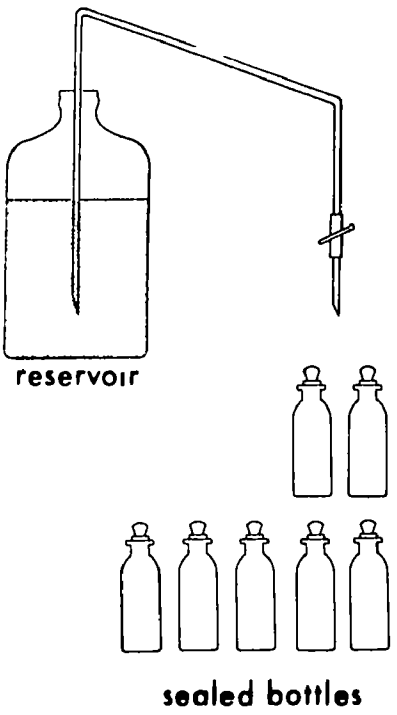
2 To determine requirements for mixing and possible thermal effects while mixing in terms of instrument response and biochemical reaction.

3 To evaluate long-term calibration or standardization and their effects upon precision and accuracy of the BOD result.

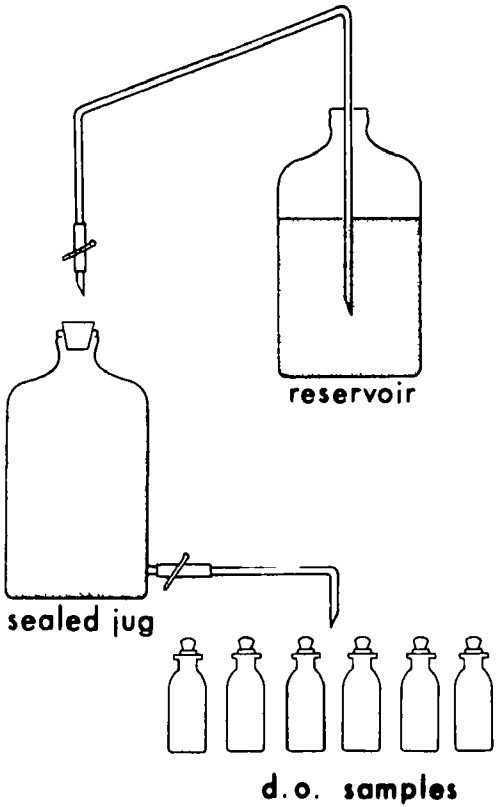
VIII IMMEDIATE DISSOLVED OXYGEN DEMAND (IDOD)

Immediate dissolved oxygen demand includes dissolved oxygen utilization requirements of substances such as those in I-A-3 An arbitrary period of 15 min. has been selected to distinguish IDOD from BOD.

REAERATION METHODS FOR B.O.D. DETERMINATION



ELMORE METHOD



ORFORD METHOD

A The IDOD is an apparent response as indicated by a specified technique Since DO titration is based upon iodine titration, any factor that causes I_2 response different from that produced by the reaction of KI and molecular oxygen confuses the IDOD determination.

B IDOD Determination

1 The IDOD determination includes the determination of DO on a sample and dilution water separately. A waste likely to have a significant IDOD is unlikely to show a DO.

2 According to mixing theory, it should be possible to calculate the DO of any definite mixture of the sample and dilution water from the DO of component parts and their proportion. (See IV. E. 1).

3 The same relative proportions of sample and dilution water should be mixed without air entrainment and the DO determined after 15 minutes.

4 Any difference between the calculated initial DO as obtained in VIII-B-2, and the determined DO in VIII-B-3 may be designated as IDOD.

5 Sample aeration, DO interference, and other factors confuse results for IDOD.

C Sample Calculation of IDOD

1 Sample DO checked and shown to be 0. 0 mg/ 1

Dilution water DO found to be 8. 2 mg/ 1

Assume a mixture of 9 parts of dilution water and 1 part (V/ V) of sample

Calculated DO =

$1 \times 0 = 0$

$9 \times 8.2 = 73.8$

10 parts of the mixture contain 73. 8/ 10 or 7. 4 mg DO/ 1. Note that mixing has reduced the DO concentration because the original amount is present in a larger package.

2 The mixture described in C-3 was held for 15 minutes and the DO determined was 4. 3 mg/ 1

$$IDOD = DO_{calc} - DO_{detm} \times \frac{100}{\% \text{ sample used}}$$

$$= 7.4 - 4\ 3 \times 10$$

$$= 31 \text{ mg IDOD/ 1}$$

Bibliography

1 Standard Methods, APHA-AWWA-WPCF 13th Ed. 1971.

2 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL. 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

BIOCHEMICAL OXYGEN DEMAND TEST
DILUTION TECHNIQUE

I OBJECTIVES: to prepare a sample dilution for series observation of oxygen depletion versus time for the estimation of BOD_t, rate and ultimate demand. The azide modification of the Winkler iodometric titration of dissolved oxygen will be used. An electronic DO analyzer will be used to check the titrimetric DO.

II PROCEDURE:

A Each group will prepare 1 dilution of either effluent or influent at an assigned concentration to enable filling 8 standard BOD bottles. One of these will be used for electronic DO monitoring by the lab instructor, the other seven are for daily student determinations. Identify each bottle with group number, I for influent or E for effluent and the assigned concentration in %.

B Calculate the volume of sample to be used to make up a total diluted volume of 3500 ml at the assigned concentration. Then dilute the sample as follows:

1 Add distilled water to the dilution bottle to make up about 1/2 of the total amount of distilled water to be used. Add 1 ml each of the four mineral supplements. (These solutions are prepared at 4 times the concentration of Standard Methods mineral supplement reagents so the added volume is lower). Mix the supplemented dilution water thoroughly.

2 Carefully mix the bottle of sample to be used to a uniform suspension. If large chunks are evident, homogenize. Rapidly pour out, with mixing, into a beaker, the approximate amount of sample required and return to lab station. Rapidly measure out, with mixing, the required sample aliquot to make the 3500 ml sample dilution.

Mixing, time, and precision are important. Your sample may be non-representative to the extent of carelessness in mixing, allowing settling while adjusting measurement or measurement errors.

Add the measured quantity to the dilution bottle.

3 Carefully add distilled water to make up 3500 ml of diluted sample.

4 Stopper and shake the dilution bottle to mix the sample and to ensure that you have an acceptable initial DO. This step will add DO or reduce supersaturation. About 15 seconds of mixing is usually adequate.

C Filling bottles is simplified if you place the dilution bottle on the center pedestal, insert the siphon and line up your identified bottles with stoppers removed. Have a container larger than the bottles available to accommodate the overflow while filling them. Otherwise, move the apparatus near a sink. Fill the siphon tube and waste some of the diluted sample to rinse this tube. Be sure that the siphon tube is used in a manner so that it is always full of the diluted sample. Agitate the diluted sample regularly to avoid undue collection of solids on the bottom.

1 Place first bottle to be filled in the overflow container or hold over sink. Insert the siphon delivery tip all of the way down into the bottom of the test bottle. Tilt the bottle to cover the delivery tip with water promptly. Start the siphon flow slowly to decrease turbulence until the delivery tip is well-covered. Fill to overflowing. Slowly raise the delivery tip while overflowing the sample. Stop the flow with the bottle completely full. Insert the bottle stopper and turn it slightly to seat it. Fill the remaining bottles in the same manner.

2 Each bottle will have the same DO and sample mix as the next if you have followed a consistent procedure, mixed carefully, and have not changed technique enroute.

3 One of the eight bottles is to be retained for initial titration of DO by your group. Another bottle is to be turned in to the lab instructor for use in performance checks on all class dilutions. The remainder are to be placed into trays for 20° cincubation. Be certain that all bottles are properly identified.

D Dissolved Oxygen Determination

1 You are encouraged to check the DO of your sample on the electronic analyzer. A calibrated unit will be available with assistance. The DO analyzer will not result in more than 1/2 ml loss of sample on probe insertion. You will be able to add DO reagents and continue the Winkler titrimetric DO on the same bottle.

2 An initial DO value (DO_o) using the Winkler method must be determined today On succeeding days, the DO_t will be determined on the incubated samples for estimation of BOD progression. (If reaeration is required, the technique will be demonstrated). All data is to be recorded on the sheet provided in this manual.

The Winkler-Azide procedure as presented in outline 43 is to be used for these DO determinations.

3 A blank value for each stock bottle of distilled water will be determined by the staff.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, WPO, EPA, Cincinnati, OH 45268

BOD DATA SHEET

Group No._____ Initial Date_____

Buret Readings	DO_o	Buret Readings	DO_t	ΔDO	Sf/ID	BOD

Buret readings - initial and final

DO_o - initial DO in mg/liter

DO_t = DO at indicated incubation time t

ΔDO= DO change (DO_o - DO_t), a depletion

Sf = the sample content as a decimal fraction in the dilution

ID = sample identification (influent or effluent)

BOD= $\frac{DO_o - DO_t}{Sf}$ (according to Standard Methods, 13th edition, 1971)

BOD DETERMINATION - REAERATED BOTTLE PROBE TECHNIQUE

I INTRODUCTION

A Customary dilution technique has certain limitations with respect to the BOD determination such as a prior commitment on sample dilutions to be used, number of bottles to be included to permit Winkler DO determinations to be made throughout a predetermined test interval, (with sample destruction for each DO test), possibility for anomalous effects due to dilution water, dilution, or inconsistent response between test bottles.

B DO probe technique offers the advantages of nondestructive DO testing, possibilities of adjusting routine according to sample behavior during test, and retaining the sample with minor losses for long term observation (if desired) and for supplementary tests at the end of the BOD test interval.

Note: Use of the probe results in a loss of about 1/2 ml of each 300 ml sample. Use dilution water with a glass rod or bead to bring the sample volume back to 300 ml for further testing.

C This test was performed in triplicate on the same sample at 100% concentration with reaeration as needed to illustrate results obtainable.

II PROCEDURE

A Sample

Final effluent (catch sample at 1100 hours) of the Advanced Waste Treatment Research Activated Sludge (Unit B) Sanitary Engineering Center Experimental Wing (September 3, 1970). One hundred percent concentration.

B DO Probe Calibration

The probe was calibrated daily in air saturated tap water versus the azide modification of the iodometric titration. A replicate test bottle of the titrated sample was retained for initial probe adjustment, if necessary, and to recheck sensor response at the end of the use period. Zero sensor response in cobalt treated sulfite solution was checked at three day intervals. Electronic response and battery condition were checked daily.

C Reaeration

1 When the DO test indicated 1.0 mg DO/liter or appeared likely to reach that point prior to the next test reading the test bottles were reaerated and a new initial DO was obtained.

2 An adapter was inserted into the neck of the sample bottle indicating low DO and an empty bottle inserted on the opposite end. The combination was shaken vigorously, while in an inverted position to allow the transfer of sample to the empty bottle, immediately after transfer the combination was reinverted, shaken as before and sample returned to the original bottle.

3 The sample was allowed to stand for at least five minutes to allow entrained air to rise (generally formed a froth ring). The froth ring was raised into the neck of the bottle by displacement with about 1/2 ml of dilution water. It usually was necessary to tilt the bottle slightly and roll it to sweep fine air bubbles off the shoulder of the bottle and into the neck. After careful reinsertion of the DO sensor these bubbles were displaced by tipping the assembly and discarding contents of the BOD bottle lip containing the frothy residue.

4 It is recognized that surface active materials and possibly other components would concentrate in the discarded froth. It would be possible to limit this effect

by allowing a longer time for the froth to break--say 15 to 30 minutes. This was not attempted for this demonstration.

D No attempt was made to determine DO at any regular time interval. Observation time is recorded for each test on a 24-hour clock basis with zero time at midnight.

E Results are recorded in tabular form including the date (Column 1), time in hours (2), sample temperature (3), hours of incubation (4), and the triplicate sample data. For each of the samples the observed DO, the change since the last observation (ΔDO) and the summation of all the DO depletions observed during the incubated interval are recorded as Σ ΔDO. A bold face line in the tabulated data in the DO column indicates DO before reaeration, the number below the line indicates DO after reaeration to be used as a new initial for the next observation.

F Near the end of the fifth day of incubation (116 hours) one of the triplicates (no. 3) was not reaerated to check the effect of complete depletion of oxygen. It was reaerated on the following day and thereafter.

G The results plotted in graphic form are presented following the BOD tabular data.

H The results of a similar respiration test on mixed liquor from the same activated sludge units are tabulated to show the effects of different types of feed on DO depletion (respiratory activity). In this case, time is in minutes, DO is in mg/l and ΔDO/minute also is in mg/l. The first test in Table 2 represented 100 ml of return sludge plus plant effluent to fill the bottle. Approximately one percent nutrient agar solution was added by syringe (1.0 ml) to produce the response noted in the second series, one ml of five percent mercuric chloride produced the effects in series 3. A new replicate sample (100 ml sludge) and plant influent to fill the bottle produced the results in series 4.

III SUMMARY

A Table 1 shows that it is possible to obtain consistent results using a DO probe plus reaeration for BOD technique. The major requirement is to carefully calibrate the DO instrument and sensor on a daily basis. It is not recommended to reaerate as many times as found necessary here. It could have been diluted at any time since the sample was available. Had the BOD response at five days been substantially complete only two reaerations would have been necessary. However, since the sample was working into second stage BOD it proceeded to react accordingly. The oxygen demand of 15 mg/liter at five days increased to 30 mg/liter in seven days and reached 45 mg/liter in ten days. The sample that was allowed to deplete on the fifth day slowly recovered but by the twelfth day or thereafter equaled or exceeded the BOD of the samples with residual DO at all times. This technique requires much less incubator space, time and manipulation with an added advantage of retaining the sample for adjustment or extended observation. The results do not depend upon a preconceived guestimate which may or may not fit the situation.

B Table 2 indicates the possibility for estimating feed acceptability in an activated sludge treatment plant. On many occasions an inquiry from an industrial plant, a different type of inflow or perhaps a new plant requires a decision regarding effects at the treatment plant. A respiration test by probe technique offers an opportunity to rate the situation within ten minutes after probe calibration. A test of sludge and effluent oxygen demand gives an estimate sludge respiratory activity now. When the same sludge is subjected to a new feed of an acceptable nature respiratory activity rises as in series 2. If the feed happened to be highly toxic as in series 3 (mercuric chloride) respiratory activity stops--

obviously a dead or severely shocked condition. A replicate sludge (Series 4) with the regular plant influent shows a prompt increase in activity Oxygen requirements, permissible load ratio, acceptable feed to sludge ratios (return sludge adjustment) all may be estimated on the basis of DO tests related to overall plant performance.

C The graphic of BOD versus time includes the COD--an estimate of first stage oxygen demand and the TKN for the composite effluent sample. If the total Kjeldahl nitrogen (TKN) is multiplied by its oxygen equivalent (4.57) then the COD plus TKN times 4.57 gives an estimate of ultimate oxygen demand (138 mg/l). The twenty day BOD was about 55 X 100/138 or forty percent of this value. The effluent sample obtained at 1100 hours is unlikely to contain a significant portion of the high load period considering the primary, aerator and secondary clarifier detention. Also the COD and TKN may have included materials that were chemically oxidized but gave a poor response to biological oxidation The BOD gives the rapid respiratory oxygen demand. COD plus the oxygen equivalent of N (on the same sample) gives a quick estimate of potential ultimate demand.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

TABLE 1
BOD Tabulated Results - Reaerated Sample, Probe DO

9/3/70 100% Sample

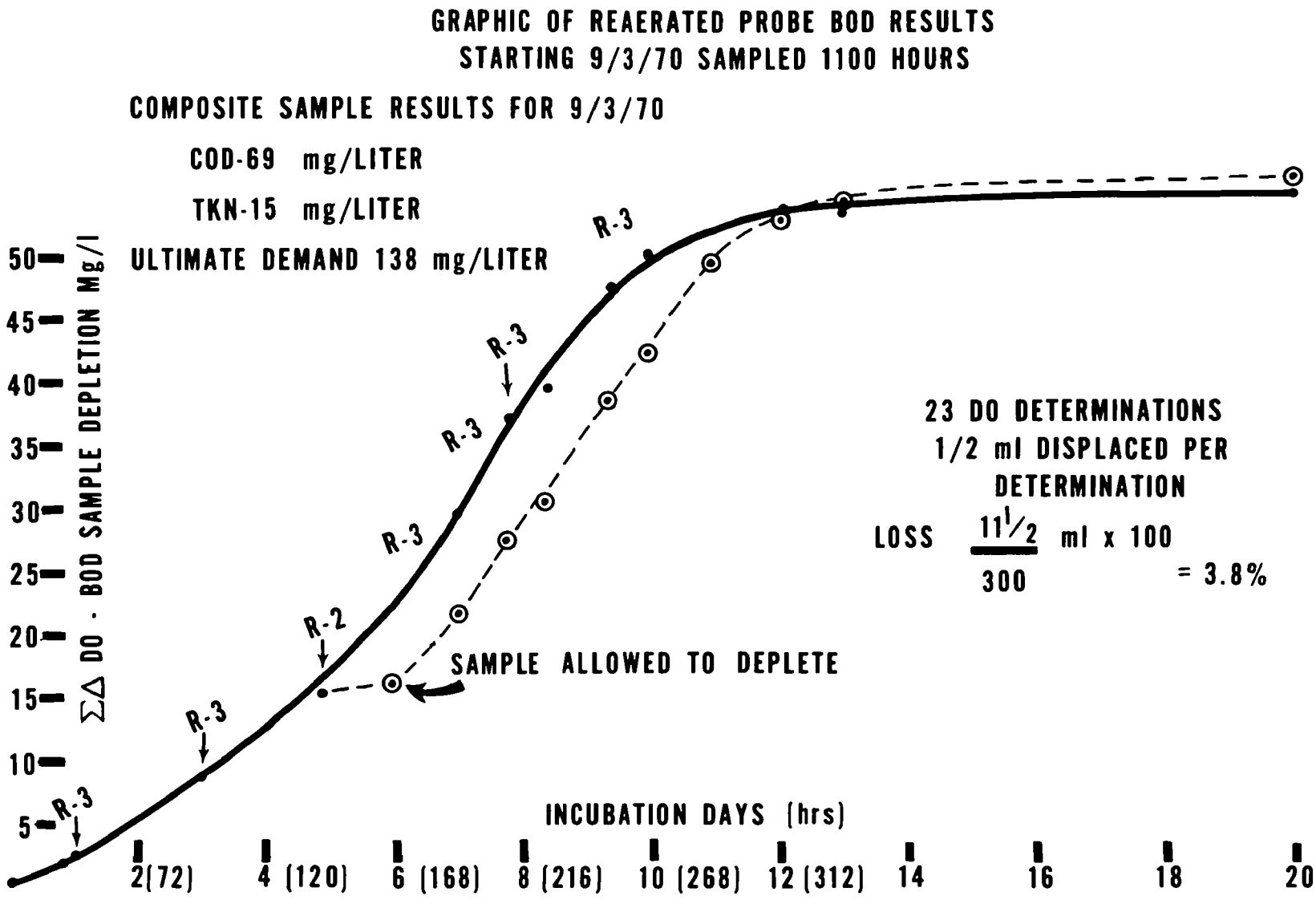
Date	Time	T°C	Hrs. Incub	Sample 1 DO	Sample 1 ΔDO	Sample 1 ΣΔDO	Sample 2 DO	Sample 2 ΔDO	Sample 2 ΣΔDO	Sample 3 DO	Sample 3 ΔDO	Sample 3 ΣΔDO
9/3/70	1340	24.0	0	6.7			6.7			6.8		
9/3/70	1355	24.0	0.25	6 7	-		6.7	-		6 8	-	
9/3/70	1620	22.0	2.6	6 5	0.2	0.2	6.5	0 2	0.2	6.6	0.2	0.2
9/4/70	1010	20 2	20.5	4.9	1.6	1.8	4.7	1 8	2 0	4.8	1.8	2.0
				8.2			8.3			7.9		
9/4/70	1600	20.6	26 3	7.4	0.8	2.6	7.5	0.8	2 8	6 9	1.0	3.0
9/6/70	1340	19.5	72	1.0	6.4	9.0	1 5	6 0	8.8	1.1	5 8	8.8
				8.1			8 1			7 4		
9/8/70	0940	19.8	116	0.6	7.5	16.5	0 5	7.6	16 4	0.4	7.0	15 8
				8.6			7 6					
9/9/70	1235	20.0	143	1 1	7 5	24 0	1.1	6.5	22 9	0 0	0.4	16.2
				8.2			8 2			7.6		
9/10/70	1200	20 0	166	1 0	7.2	31.2	1.2	7 0	29.9	2 0	5.6	21.8
				8.2			8.6			8 0		
9/11/70	0800	20.0	186	1 2	7.0	38 2	1 1	7.5	37 4	2.0	6 0	27 8
				8.4			8 3			8.5		
9/11/70	1400	20.0	192	5.0	3.4	41.6	5.3	3 0	40.4	5.7	2.8	30.6
				8.1			8 4			8.6		
9/12/70	2035	20.0	222	0 5	7.6	49 2	0.6	7 8	48 2	0.4	8.2	38.8
				8.2			8.2			8 2		
9/13/70	1130	20 0	238	6.3	1.9	51 1	3.8	4.4	52.6	4 6	3 6	42.4
				8 3			8.4			8.3		
9/14/70	1200	20.0	262	7.0	1.3	52.4	6 9	1.5	53 1	0.9	7 4	49 8
										8.3		
9/15/70	1340	19.6	288	5 7	1 3	53 7	6 2	0.7	53 8	4.5	3.8	53 6
										8.2		
9/16/70	1200	19.3	310	5.1	0.6	54.3	5.8	0.4	54.2	7 2	1.0	54.6
*9/20/70	1240	21.6	480	3.8	1.3	55 6	4.6	1.2	55 4	4.9	2.3	56 9

* Incubator power off

TABLE 2

ACTIVATED SLUDGE RESPIRATION DATA DO PROBE TECHNIQUE

Time Min	Temp	DO	ΔDO	Sample
			Series 1	
0	24°	7.2		100 ml Return Sludge & Effluent
1		6.6	0.6	
2		6.2	0.4	
3		5 6	0 6	
4	24.5	5 1	0 5	
0		4.7	Series 2	+ 1 ml Nutrient agar
1		3 8	0.9	
2		2.7	1 1	
3		1.7	1.0	
0	24 8	4.7	Series 3	+ 1 ml $HgCl_2$ Soln
1		4 8		
2		4.7	Nil	
3		4.7		
0	24.2	5 9	Series 4	100 ml Sludge + Influent
1		4 6	1.3	
2		3 3	1.3	
3		2.0	1.3	


GRAPHIC OF REAERATED PROBE BOD RESULTS
STARTING 9/3/70 SAMPLED 1100 HOURS
COMPOSITE SAMPLE RESULTS FOR 9/3/70
COD-69 mg/LITER
TKN-15 mg/LITER
ULTIMATE DEMAND 138 mg/LITER
ΣΔ DO - BOD SAMPLE DEPLETION Mg/l
50
45
40
35
30
25
20
15
10
5
R-3
R-2
SAMPLE ALLOWED TO DEPLETE
23 DO DETERMINATIONS
1/2 ml DISPLACED PER
DETERMINATION
LOSS 11 1/2 / 300 ml x 100 = 3.8%
INCUBATION DAYS (hrs)
2(72)
4 (120)
6 (168)
8 (216)
10 (268)
12 (312)
14
16
18
20

EFFECT OF SOME VARIABLES ON THE BOD TEST

I TIME

A The common equation $y_t = L(1 - 10^{-kt})$ for BOD relationship indicates time as a variable. The rate coefficient (k_1) indicates that a specific percentage of material initially present (oxygen) will be used during a given time unit. Each successive unit of time has less reactant present initially than the preceding interval, hence a definite precentage decrease results in successively smaller amounts of reactant use per unit of lapsed time. Increasing k_1 results in a larger percentage oxygen use per unit of time and also increases the change in reactant mass among successive time intervals.

B Adney's work for the British Royal Commission cited 5 days passage time from source to the ocean as maximum for English streams. The 8th Report (1909) largely established BOD philosophy including the 5-day interval. At 5 days, initial lags generally have terminated and a substantial fraction of the long-term oxygen demand has been exerted. If only one time interval can be used, 7 days permits better scheduling. Any one time interval is "a" fraction of the total oxygen requirement, this is a poor reference point if we do not know how it arrived. For example, the percentage of oxidizable material stabilized in terms of oxygen use at various rate factors are.

k_1 ($\log_{10}$)	% oxidized in 5 days	K_1 ($\log_e$)
0.05	42%	0.11
0.10	67%	0.23
0.15	84%	0.34
0.25	94%	0.57
0.50	99+%	1.15

This range ($K_1 = 2.3\ k_1$) is commonly encountered in wastewater stabilization with the higher rates characteristic of fresh oxidizable material that is readily converted. The lower coefficients are characteristic of cell mass at later stages of oxidation and of low-rate reactants in general

C The oxygen utilization at specified intervals of time are required to estimate k_1, and L, the estimate of oxygen use at infinite time. It is common to observe results at equal intervals of time but this is not essential as long as the time intervals are accurately known. The initial time periods are critical as an error of a few hours in time represents a relatively large change in reactant mass in a system at maximum instability. Unequal time periods can be plotted to define the curve from which any given intervals can be selected as desired.

D Increasing impoundment of surface water provides more time for stabilization of relatively inert soluble or suspended pollutants and for organism adaptation to the situation or pollutants. Long term BOD's are essential to indicate changes in the pattern of oxygen demand vs. time. It may be expected that one or more plateaus will be evident in the BOD curve followed by a temporary rise in rate during second stage oxidation or thereafter. Anaerobiosis may cause a rise in rate coefficient after aerobic conditions are re-established. Eventually k_1 stabilizes at very low values.

1 Rate coefficients tend to be difficult to interpret during long term BOD's because of progressive changes and other factors.

a The relative error of the DO test may be a large fraction of the incremental DO change during low rate periods.

b Cell mass may agglomerate under quiescent test conditions and decrease nutrient availability.

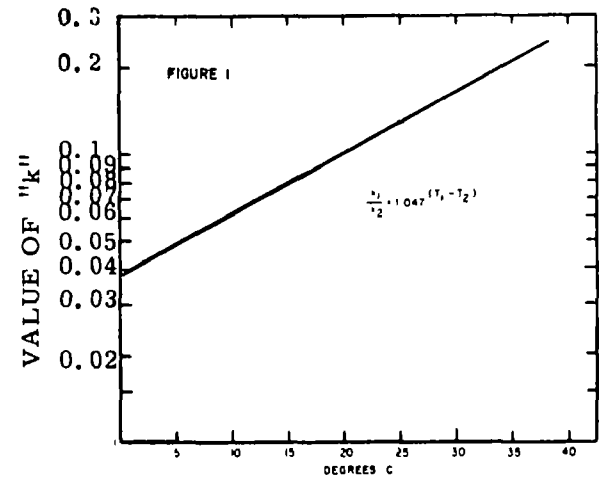
c It is not likely that recycled nutrients under aerobic test conditions will have as much effect as recycle from anaerobic benthic deposits in a stream.

2 The BOD result tends to underestimate deoxygenation relative to surface water behavior because of interchanges, turbulence, biota, and boundary effects. Reseeding does not occur in a sealed bottle but reseeding is inevitable in a stream or treatment unit.

II TEMPERATURE

A Effect on Oxidation Rate

Temperature is one of the important controlling factors in any biological system. In the BOD reaction, changes in temperature produce acceleration or depression of the rate of oxidation. Figure 1 shows the changes in the value of k at temperatures from 0 - 25°C on a common wastewater.



VARIATION IN "k" WITH CHANGE IN TEMPERATURE

B Test Temperature

In the BOD test procedure an arbitrary temperature is usually selected for convenience even though a wide temperature range exists under natural conditions.

Incubation of the test containers at 20°C for the whole period is now accepted practice in the U.S., 18.5°C is preferred in England. Camp (ASCE, SA5 91:1, Oct. 65) recommended light and dark bottle immersion in the stream.

C Temperature Correction

When it is necessary to calculate the rate of oxidation at a temperature other than 20°, the following relationship may be used

$$\frac{k_1}{k_2} = \theta^{(T_1 - T_2)}$$

where

k_1 = rate coefficient at temperature T_1

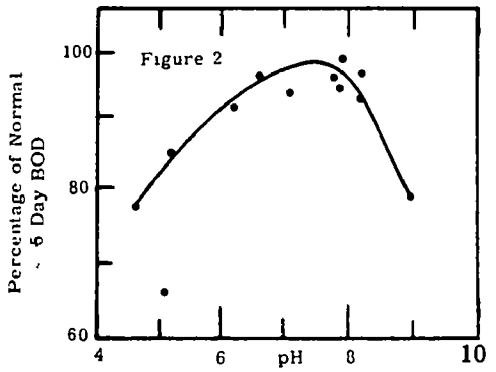
k_2 = rate at coefficient at temperature T_2

θ = temperature coefficient, for which Streeter and Phelps obtained the value 1.047. θ changes with temperature, it appears to be higher in the range of 5-15°C than in the range of 30 to 40°C. The value given refers to 15-30°.

The cited temperature coefficient appears reasonable for household wastes. It may not apply for other wastes where developing or seed organisms may not tolerate temperature changes as readily. A given temperature coefficient should be checked for applicability under specified conditions.

III pH

A The organisms involved in biochemical conversions apparently have an optimum response near a pH of 7.0 providing other environmental factors are favorable, a pH range of about 6.5 to 8.3 apparently is acceptable (Figure 2). Reactivity is likely to be significantly lower on both sides of the acceptable pH range but microbial adaptation may extend the limits appreciably. For example, trickling filters have operated with better than 50% treatment efficiency at pH 3 and 10 after adaptation.



B Adjustment of Concentrated Samples

When wastes are more acid than pH 6.5 or more alkaline than pH 8.3, adjustment to pH 7.2 is advisable before reliable BOD values can be obtained.

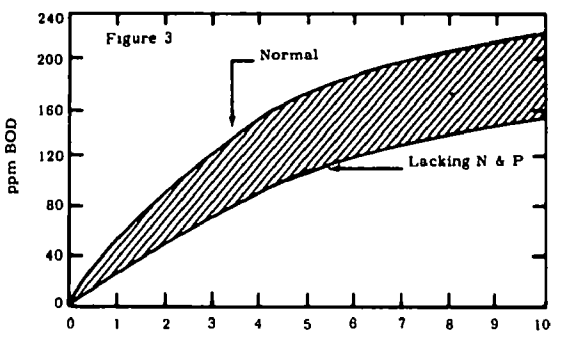
C Dilution Samples

Standard dilution water is buffered at pH 7.2. Sample-dilution water mixtures should be checked to make sure that the sample buffer capacity does not exceed the capacity of the dilution water for pH adjustment.

IV ESSENTIAL MINERAL NUTRIENTS

A Importance

In 1932 Butterfield reported on the role of certain minerals in the biochemical oxidation of sewage and concluded that deficient minerals often upset metabolic response. In addition, he found that inadequate nitrogen and/or phosphorus was a common cause of low BOD results in industrial wastewaters. (Figure 3)



Effect of Mineral Nutrients on BOD

B Standard Methods Dilution Water

The dilution water specified for the BOD test approximates USGS estimates for an average U.S. mineral content of surface water except for added phosphate buffer. It is assumed to provide essential mineral nutrients for most wastewaters but cannot be expected to meet requirements for grossly deficient wastewater nutrients both mineral and organic. Ruchhoft (S.W.J. 13 669, 1941) summarized committee action leading to the present dilution water.

C Other Dilution Considerations

There is a trend toward the use of receiving water, storage-stabilized if necessary, to evaluate waste behavior. It is advisable to minimize dilution and consider the nutrient level likely in the receiving water as most valid. Any change in the environment, such as dilution, upsets the microbial balance and requires adaptive changes.

V MICROBIOLOGICAL POPULATION

A Need for Complex Flora and Fauna

Butterfield, Purdy, and Theriault (Pub. Health Rep. 393, 1931) demonstrated that an isolated species of organisms was not as effective in biological stabilization as a variety of species. Figure 4 summarizes some of their data. Bhatta and Gaudy (ASCE, SA3, 91·63, June 1965) reinvestigated this factor. Many studies have emphasized the need for a mixed biota in the BOD test. It appears that bacteria are capable of varied activities, but all species are not capable of synthesizing all required nutrients. Certain bacterial species may be capable of producing enzymes, amino acids, or growth factors needed for their use and by other species for optimum performance. It has been shown that oxygen demand becomes minimal when some limit of bacterial population has been reached. Predation prevents such an approach to maximum numbers and maintains a continuing bacterial growth and recycle of nutrients among a mixed population. The net effect is a symbiotic relation among mixed organisms tending to enhance the rate of stabilization or utilization of oxygen as in the BOD test.

B Organism Adaptation

1 Early investigations in relation to the BOD test considered domestic wastewaters primarily. The saprophytic organisms involved in stabilization either were present in adequate numbers or quickly multiplied to attain effective populations.

2 The period of adjustment required to shift enzyme production needed to utilize an energy source different from that previously utilized or to shift population variety from that favored by one food to that favored by another food is considered an adaptation period. Dilution, temperature, oxygen tension, pH, nutrient type, inhibitory substances, light and other changes all are common inducements for microbial adaptation. Mutation of organisms may be encountered during adaptation but usually is not a factor.

3 The developments in industry and technology have resulted in discharge of new and more varied wastewater constituents. Microorganisms may adapt themselves to the use of a new substance as an energy source providing the energy and environment are favorable. The receiving stream usually shows development of an adapted microbiota for a new or different discharge constituent within hours, days or weeks after fairly regular discharge. The time for adaptation depends on the nature of the constituent, available energy, tolerance of the organisms, and environmental conditions.

C Seeding

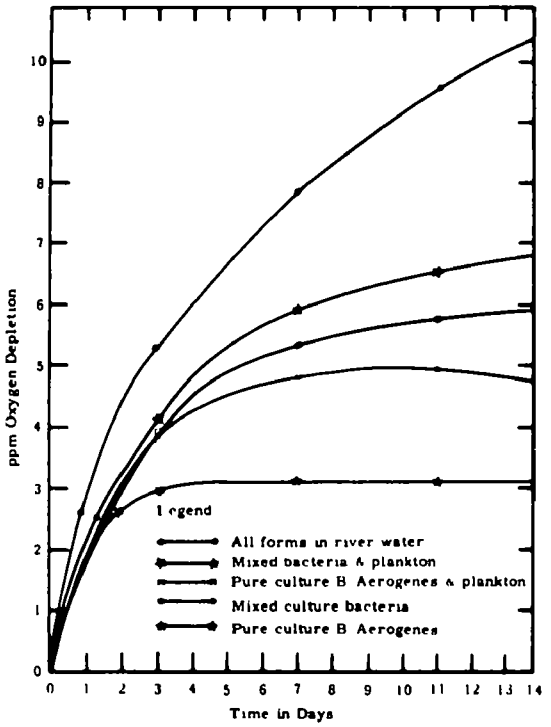
The amount of seed and its selection must be determined experimentally. The most effective inoculant would be that which would produce the maximum BOD response with minimum lag period and negligible seed demand. This would mean some maximum population adapted to feed and conditions at a minimum equilibrium energy nutrient supply.

1 Figure 5 indicates corrected BOD progression on a synthetic feed with river water and stale sewage inoculants at several concentrations. The river water resulted in higher BOD with negligible lag and seed correction. The seed correction at 20% concentration of inoculant was less than 0.3 mg. DO/l at 5 days. It would be possible to use this river water as a diluent without excessive oxygen loss to produce more valid BOD progression for that receiving water. The lower wastewater inoculant concentration resulted in a definite BOD lag. Higher wastewater concentrations produced comparable BOD progression earlier but resulted in high seed corrections and lowered availability of dissolved oxygen for the sample.

2 A good secondary treated effluent produced results similar to river water inoculation with higher seed corrections per increment of applied inoculant. Soil suspensions also are very effective sources of seed organisms with minor seed corrections if they are reasonably stabilized surface soils.

3 It appears that the BOD progression most nearly indicating receiving water oxidation would be one based upon receiving water dilution or inoculated with organisms from it.

4 A new or unusual wastewater may require adapted organisms not present in sufficient numbers in the receiving water. Development of an adapted seed from soil suspensions, plant effluents or receiving water may be necessary to evaluate oxidation potential in a plant or receiving water at some future time. Enrichment culture technique is beneficial where small concentrations of the test wastewater are applied regularly with increases in wastewater concentrations as BOD or respiration activity indicates increasing tolerance and oxidation of the test waste. Both time and concentration limits are useful to characterize the wastewater and its acceptability for biological stabilization.



Effect of Biological Forms on Oxygen Depletion
Figure 4

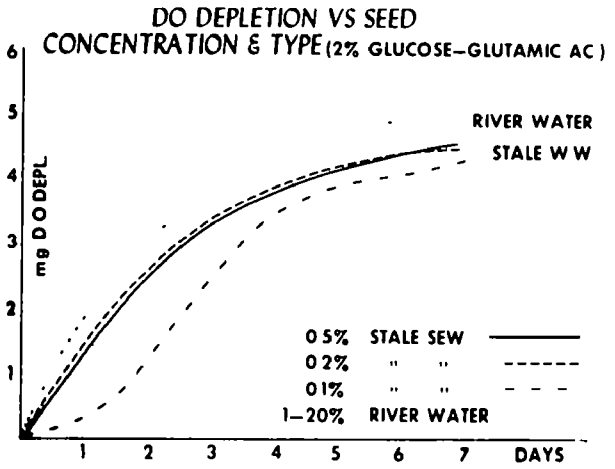


Figure 5

5 It must be recognized that BOD progressions are most likely to err on the low side. A meaningful BOD test should seek the highest consistent oxygen demand feasible for sample and conditions.

D Algae

When large numbers of algae are present in surface waters, they produce significant changes in the oxygen content. Under the influence of sunlight excess oxygen is produced while a net deficit occurs in the dark. The result is a wide variation in surface water DO depending on sample time.

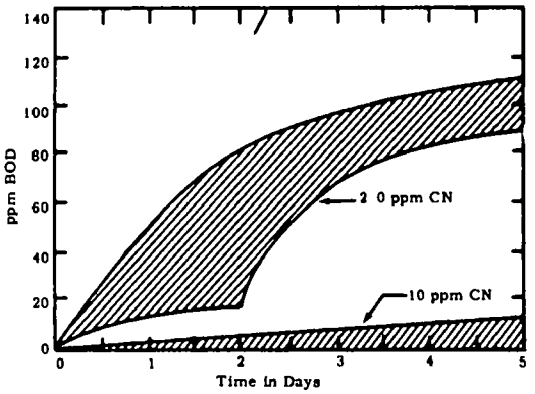
When stream samples containing algae are incubated in the laboratory the algae survive for a time, then die because of the lack of light. Short-term BOD determinations may show the influence of oxygen production by the algae. When the algae die, they release the stored organic load for recycle and increase the BOD. Therefore, samples incubated in the dark may not be representative of the deoxygenation process in the stream, since the benefits of photosynthesis are lacking. Conversely, samples incubated in the light, under conditions of continual photosynthesis, will yield low BOD values.

The influence of algae on BOD is one of the most difficult variables to evaluate. More research is needed to develop satisfactory methods for the accurate determination of BOD in the presence of large numbers of algae. Light and dark bottle incubations suggest the magnitude of effects.

IV TOXICITY

A Effect

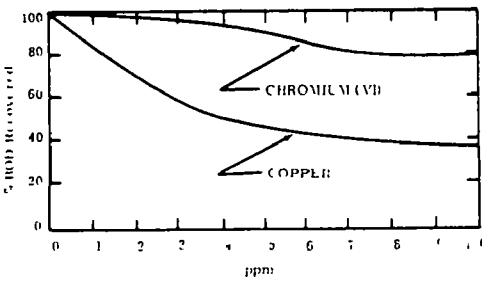
Since satisfaction of the BOD is accomplished through the action of microorganisms, the presence of toxic substances will result in depression of the oxidation rate. In many cases, toxicity will produce a lag period, until tolerant organism activity becomes significant. Figure 6 shows the effect of cyanide on the BOD curve. A prominent lag period is exhibited in the 2 ppm curve, while at 10 ppm the lag extends beyond the fifth day. An activated sludge may be adapted to work effectively in degradation of 60 mg CN/l.



Effect of Cyanide on BOD of Domestic Sewage
(2% Sewage in Formula C Dilution Water)

Figure 6

Heavy metals have similar effects depending on history and environment. The effects of copper and chromium are illustrated in Figure 7.



EFFECT OF HEAVY METALS ON BOD

Figure 7

B Detection

In laboratory determinations of BOD the absence of toxic substances including chlorine must be established before the results can be accepted as valid. Comparison of BOD values for several dilutions of the waste will indicate the presence or absence of toxicity. In Table 1 the calculated BOD for the dilutions show higher values in the more dilute concentrations. It is apparent that toxicity was present and that the toxic effect was diluted out at a waste concentration of 2% or less.

Table I

Waste conc.	Depletion	5 day BOD
10%	3.51	35
5%	4.53	91
2%	2.80	140
1%	1.52	152
0.5%	0.74	148

VII NITRIFICATION

A Mechanism

The oxidation process, as exemplified by the equation

$$y = L\,(1-10^{-kt})$$

presumably involves the oxidation of carbonaceous matter or 1st stage oxygen demand.

$$C\ H\ O \xrightarrow{O_2} CO_2 + H_2O$$

The rate coefficient is normally high, giving nearly complete oxidation in a few days. When nitrogenous material is present its oxidation can be shown as

$$NH_3 \xrightarrow{O_2} NO_2^- \xrightarrow{O_2} NO_3^-$$

Nitrogen oxidation may be delayed for several days during BOD tests unless suitable micro-biota are initially available. Under some circumstances these two oxidations can proceed simultaneously and the resultant BOD curve will be a composite of the two reactions.

$$y_t = \left[L_c(1-10^{-k_c t}) + L_n\ (1-10^{-k_n t}) \right]$$

where y_t = the simultaneous BOD of the carbonaceous and nitrogenous phases or 1st and 2nd stage demands.

k_c and k_n = the rate coefficients applicable to the carbonaceous and nitrogenous materials respectively.

L_c and L_n = the ultimate oxygen demands characteristic of the two phases respectively. This is the general formula for any system characterized by two simultaneous reactions.

Principal conditions governing simultaneous carbon and nitrogen oxidation:

1 Presence of an effective nitrifying culture at the beginning of the test interval (nitrifiers grow relatively slowly).

2 Maintenance of adequate DO, believed to be a minimum of 0.5 to 1.0 mg/l, for nitrifier activity.

3 Available nitrogen - in excess of that required for synthesis. This is believed to require a minimum of about 7 mg/l to support active nitrification on a continuous basis.

4 Nitrifiers appear to be more sensitive to toxicity than most saprophytic organisms, hence are likely to be inhibited more readily. This is particularly evident during nitrite to nitrate conversion.

B It may require 5 to 10 days to establish nitrification if the population was not nitrifying initially. This is the basis for the sequential carbonaceous and nitrogenous oxidation of sewage oxidation.

1 Effects on the BOD curve indicate a typical pattern such as in Figure 8. The influence of nitrification in the production of a secondary rise in the BOD curve is so well known that any secondary rise may be erroneously attributed to nitrification whether or not nitrification was involved. Actually, a secondary rise in the curve may be due to any oxidation system assuming dominance after the initial oxidation system has been completed.

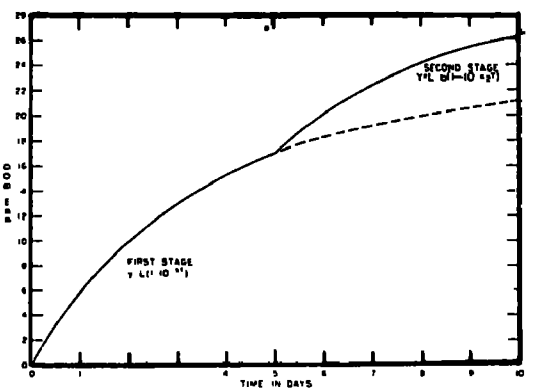
2 The nitrification phenomena occurs simultaneously in many streams, treated effluents or partially stabilized samples. The designation of a secondary BOD rise to nitrification should be based on analysis, not curve shape.

C The extent of nitrification is conclusively shown only by periodic analysis of ammonia, organic, nitrite and nitrate nitrogen. The conversion of ammonia and organic nitrogen to oxidized nitrogen is a definite indication of nitrification.

D Nitrification Inhibition

Plant efficiencies from a BOD standpoint can be erroneous because nitrification generally is not established during the usual incubation of influent samples but may be a major factor in effluent incubations. It requires about 2 times the oxygen to convert NH_3 -N to NO_3 -N as to convert C to CO_2 hence this is a major fraction of stream oxygen use. Most secondary treated effluents are characterized by a larger fraction of carbon than nitrogen removal which accentuates the problem.

Pasteurization of samples, methylene blue, chromium, and acid treatment followed by neutralization have been used to inhibit nitrification for estimation of carbonaceous BOD only. Any inhibition of nitrification also produces a change in the sample or its behavior and may partially inhibit carbonaceous oxidation. Nitrification is a factor in stream self-purification and treatment. It does not appear realistic to alter it for convenience. The most realistic approach to carbonaceous oxidation is the measurement of CO_2 or COD.



EFFECT OF NITRIFICATION ON B O D

Figure 8

VIII EFFECT OF DILUTION

When a series of dilutions are made on a BOD sample usually the results vary to the extent that only an approximate BOD value is obtained.

Table 2

INTERPRETATION OF BOD DATA

Sample conc.	DO	Depletion	BOD
Initial	8 2	-	-
Final			
1%	5 5	2 7	270
2%	3 3	4 9	245
4%	0 0	Complete	-

A For example, in Table 2, 1%, 2% and 4% concentrations of sample were used. The 4% concentration became anaerobic before the end of 5 days. The 5-day BOD of the 1% concentration was 270 and that of the 2% concentration was 245.

B Statistically one value is more reliable than the other.

Dilution	DO depletion
1%	5.5 mg/l
2%	3.3 mg/l
Difference	2.2 mg/l

The difference in depletion between 1 and 2% dilutions is 2.2 mg/l. This difference may be attributed to an additional 1% of sample added to the original 1%. If the difference is multiplied by the dilution factor of 100 to obtain the BOD, the result is 220 mg/l.

1 We now have three estimates of the BOD on a one percent concentration basis from the two dilutions:

a the actual 1% depletion gives 270
b 2%/2 depletion gives 245
c (2%- 1%) depletion gives 220

Statistically the probabilities of being nearer the actual value goes with the nearest two of three. The 4% value of 8.2 depletion/4 as a minimum possible BOD 1% concentration gives a BOD of at least 200.

2 There is the possibility that higher concentrations may reflect significant toxicity while lower concentrations tend to reflect a greater proportion of dilution water. The toxicity problem does not appear to be significant since the 4% sample concentration indicated a BOD of at least 200. The higher BOD at 1% sample concentration may be due to a contaminated dilution water or to the fact that a similar number of seed organisms had less food and utilized certain fractions that they had passed by when they had more choice with the 2% sample concentration. Data is insufficient to resolve this one.

3 Incubations having a depletion of at least 2 mg DO/liter and a residual of at least 1 mg DO/liter are indicated to be most valid[1]. Both the 1 and 2% concentrations fit this requirement in Table 2. An average error of + or -0.1 ml on the DO titration would have a smaller relative error upon the 2% depletion.

4 We have a reasonable presumption that the sample BOD of about 230 was a good estimate. We do not have an unequivocal basis for so stating. Possible variations in results with different dilutions of a given sample are subject to many uncertainties in the test routine.

If some cause is known - such as a titration eror, the inclusion of extraneous substances producing high or low response, or a definite procedural error that rules out a valid estimate of the sample BOD- that result should be labeled as a lost cause or unreported. Otherwise, report what was obtained to the best of your ability with the provision of uncertainty for uncontrollables.

ACKNOWLEDGMENT:

Certain portions of this outline contain training material from prior outlines by D. G. Ballinger and J. W. Mandia.

REFERENCE:

Standard Methods, APHA-AWWA-WPCF, 13th edition, 1971.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, WPQ, EPA, Cincinnati, OH 45268.

CHEMICAL OXYGEN DEMAND AND COD/BOD RELATIONSHIPS

I DEFINITION

A The Chemical Oxygen Demand (COD) is an estimate of the proportion of the sample matter susceptible to oxidation by a strong chemical oxidant. The current edition of Standard Methods,[2] specifies organic material which is generally the situation but not necessarily applicable.

B A variety of terms have been and are used for the test described here as COD·

1 Oxygen absorbed (OA) primarily in British practice.

2 Oxygen consumed (OC) preferred by some, but unpopular.

3 Chemical oxygen demand (COD) current preference.

4 Complete oxygen demand (COD) misnomer.

5 Dichromate oxygen demand (DOC) earlier distinction of the current preference for COD by dichromate or a specified analysis such as Standard Methods.

6 Others have been and are being used. Since 1960, terms have been generally agreed upon within most professional groups as indicated in I-A and B-3 and the explanation in B-5.

C The concept of the COD is almost as old as the BOD. Many oxidants and variations in procedure have been proposed, but none have been completely satisfactory.

1 Ceric sulfate has been investigated, but in general it is not a strong oxidant.

2 Potassium permanganate was one of the earliest oxidants proposed and until recently appeared in Standard Methods (9th ed.) as a standard procedure. It is currently used in British practice as a 4-hr. test at room temperature.

a The results obtained with permanganate were dependent upon concentration of reagent, time of oxidation, temperature, etc., so that results were not reproducible.

3 Potassium iodate or iodic acid is an excellent oxidant but methods employing this reaction are time-consuming and require a very close control.

4 A number of investigators have used potassium dichromate under a variety of conditions. The method proposed by Moore at SEC is the basis of the standard procedure.[1, 2] Statistical comparisons with other methods are described.[3]

5 Effective determination of elemental carbon in wastewater was sought by Buswell as a water quality criteria.

a Van Slyke[4] described a carbon determination based on anhydrous samples and mixed oxidizing agents including sulfuric, chromic, iodic and phosphoric acids to obtain a yield comparable to the theoretical on a wide spectrum of components.

b Van Hall, et al.,[5] used a heated combustion tube with infrared detection to determine carbon quickly and effectively by wet sample injection.

6 Current development shows a trend to instrumental methods automating

conventional procedures or to seek elemental or more specific group determination.

II RELATIONSHIP OF THE COD TEST WITH OTHER OXIDATION CRITERIA IS INDICATED IN TABLE 1.

A Table 1

Test	Test Temp. °C	Reaction time	Oxidation system	Variables
BOD	20	days	Biol. prod. Enz. Oxidn.	Compound, environment, biota, time, numbers. Metabolic acceptability, etc.
COD	145	2 hrs.	50% H_2SO_4 $K_2Cr_2O_7$ May be catalyzed	Susceptibility of the test sample to the specified oxidation
IDOD	20	15'	Diss. oxyg.	Includes materials rapidly oxidized by direct action, Fe^{+2}, SH.
Van Slyke Carbon detn.	400+	1 hr.	H_3PO_4 HIO_3 H_2SO_4 $K_2Cr_2O_7$ Anhydrous	Excellent approach to theoretical oxidation for most compounds (N-nil)
Carbon by combustion +IR	950	minutes	Oxygen atm. catalyzed	Comparable to theoretical for carbon only.
Chlorine Demand	20	20 min.	HOCl soln.	Good NH_3 oxidn. Variable for other compounds.

B From Table 1 it is apparent that oxidation is the only common item of this series of separate tests.

1 Any relationships among COD & BOD or any other tests are fortuitous because the conditions of test tend to give results indicating the susceptibility of a given sample to oxidation under specified conditions that are different for each test.

2 If the sample is primarily composed of compounds that are oxidized by both procedures (BOD and COD) a relationship may be established.

a The COD procedure may be substituted (with proper qualifications) for BOD or the COD may be used as an indication of the dilution required for setting up BOD analysis.

b If the sample is characterized by a predominance of material that can be chemically, but not biochemically oxidized, the COD will be greater than the BOD. Textile wastes, paper mill wastes, and other wastes containing high concentrations of cellulose have a high COD, low BOD.

c If the situation in item b is reversed the BOD will be higher than the COD. Distillery wastes or refinery wastes may have a high BOD, low COD, unless catalyzed by silver sulfate.

d Any relationship established as in 2a will change in response to sample history and environment. The BOD tends to decrease more rapidly than the COD. Biological cell mass or detritus produced by biological action has a low BOD but a relatively high COD. The COD/BOD ratio tends to increase with time, treatment, or conditions favoring stabilization.

III ADVANTAGES AND LIMITATIONS OF THE COD TEST[2] AS RELATED TO BOD

A Advantages

1 Time, manipulation, and equipment costs are lower for the COD test.

2 COD oxidation conditions are effective for a wider spectrum of chemical compounds.

3 COD test conditions can be standardized more readily to give more precise results.

4 COD results are available while the waste is in the plant, not several days later, hence, plant control is facilitated.

5 COD results are useful to indicate downstream damage potential in the form of sludge deposition.

6 The COD result plus the oxygen equivalent for ammonia and organic nitrogen is a good estimate of the ultimate BOD for many municipal wastewaters

B Limitations

1 Results are not applicable for estimating BOD except as a result of experimental evidence by both methods on a given sample type.

2 Certain compounds are not susceptible to oxidation under COD conditions or are too volatile to remain in the oxidation flask long enough to be oxidized.

Ammonia, aromatic hydrocarbons, saturated hydrocarbons, pyridine, and toluene are examples of materials with a low analytical response in the COD test.

3 Dichromate in hot 50% sulfuric acid requires close control to maintain safety during manipulation.

4 Oxidation of chloride to chlorine is not closely related to BOD but may affect COD results.

5 It is not advisable to expect precise COD results on saline water.

IV BACKGROUND OF THE STANDARD METHODS COD PROCEDURE

A The COD procedure[1] considered dichromate oxidation in 33 and 50 percent sulfuric acid. Results indicated preference of the 50 percent acid concentration for oxidation of sample components. This is the basis for the present standard procedure.

B Muers[6] suggested addition of silver sulfate to catalyze oxidation of certain low molecular weight aliphatic acids and alcohols. The catalyst also improves oxidation of most other organic components to some extent but does not make the COD test universally applicable for all chemical pollutants.

C The unmodified COD test result (A) includes oxidation of chloride to chlorine. Each mg of chloride will have a COD equivalent of 0.23 mg Chlorides must be determined in the sample and the COD result corrected accordingly.

1 For example, if a sample shows 300 mg of COD per liter and 200 mg Cl^- per liter the corrected COD result will be 300 -(200 x 0.23)or 300 - 46 = 254 mg COD/l on a chloride corrected basis.

2 Silver sulfate addition as a catalyst tends to cause partial precipitation of silver chloride even in the hot acid solution. Chloride corrections are questionable unless the chloride is oxidized before addition of silver sulfate, i.e., reflux for 15 minutes for chloride oxidation, add Ag_2SO_4, and continue the reflux or use of $HgSO_4$(D).

D Dobbs and Williams[7] proposed prior complexation of chlorides with $HgSO_4$ to prevent chloride oxidation during the test. A ratio of about 10 of Hg^{++} to 1 of Cl^- (wt. basis) appears essential. The Cl^- must be complexed in acid solution before addition of dichromate and silver sulfate.

1 For unexplained reasons the $HgSO_4$ complexation does not completely prevent chloride oxidation in the presence of high chloride concentrations

2 Factors have been developed to provide some estimate of error in the result due to incomplete control of chloride behavior. These tend to vary with the sample and technique employed.

E It is not likely that COD results will be precise for samples containing high chlorides Sea water contains 18000 to 21000 mgCl^-/l normally. Equivalent chloride correction for COD exceeds 4000 mg/l. The error in chloride determination may give negative COD results upon application of the correction.

Incomplete control of chloride oxidation with $HgSO_4$ may give equally confusing results.

$HgSO_4$ appears to give precise results for COD when chlorides do not exceed about 2000 mg/l. Interference increases with increasing chlorides at higher levels.

F The 12th edition of Standard Methods reduced the amount of sample and reagents to 40% of amounts utilized in previous editions. There has been no change in the relative proportions in the test This step was taken to reduce the cost of providing expensive mercury and silver sulfates required. Results are comparable as long as the proportions are identical Smaller aliquots of sample and reagents require more care during manipulation to promote precision.

G The EPA Methods for COD

1 For routine level COD (samples having an organic carbon concentration greater than 15 mg/liter and a chloride concentration less than 2000 mg/liter), the EPA specifies the procedures found in Standard Methods [2] and in ASTM[8].

2 For low level COD (samples with less than 15 mg/liter organic carbon and chloride concentration less than 2000 mg/liter), EPA provides an analytical procedure [9]. The difference from the routine procedure primarily involves a greater sample volume and more dilute solutions of dichromate and ferrous ammonium sulfate.

3 For saline samples (chloride level exceeds 1000 mg/liter and COD is greater than 250 mg/liter), EPA provides an analytical procedure[9] involving preparation of a standard curve of COD versus mg/liter chloride to correct the calculations. Volumes and concentrations for the sample and reagents are adjusted for this type of determination.

V The precision of the unmodified COD result shows a standard deviation of ± 4% of the mean [3] on low chloride samples. Silver sulfate modified COD results are likely to show a standard deviation about twice that without catalysis, due to questionable chloride behavior. The determination of chloride frequently shows a coefficient of variation (s/x) of 10 to 15%, hence high chloride samples result in COD precision controlled more by chloride behavior than organic oxidation.

VI REMARKS PERTINENT TO EFFECTIVE COD DETERMINATIONS INCLUDE·

A Sample size and COD limits for 0.25 N reagents are approximately as given. For 0.025 N reagents multiply COD by 0.1. Use the weak reagents for COD's in the range of 5-50 mg/l, (low level).

Sample Size	mg COD/l
20 ml	2000
10 ml	4000
5 ml	8000

B Most organic materials oxidize relatively rapidly under COD test conditions. A significant fraction of oxidation occurs during the heating upon addition of acid. The color change of dichromate after acid addition indicates the approximate fraction of dichromate remaining. If the mixed sample color changes from yellow to green after acid addition the sample was too large. Discard without reflux and repeat with a smaller aliquot until the color after mixing does not go beyond a brownish hue. The dichromate color change is less rapid with sample components that are slowly oxidized under COD reaction conditions.

C Chloride concentrations should be known for all test samples and results interpreted accordingly.

D Special precautions advisable for the regular COD procedure and essential when using 0.025 N reagents include:

1 Keep the apparatus assembled when not in use.

2 Plug the condenser breather tube with glass wool to minimize dust entrance.

3 Wipe the upper part of the flask and lower part of the condenser with a wet towel before disassembly to minimize sample contamination.

4 Steam out the condenser after use for high concentration samples and periodically for regular samples. Use the regular blank reagent mix and heat, without use of condenser water, to clean the apparatus of residual oxidizable components

5 Distilled water and sulfuric acid must be of very high quality to maintain low blanks on the refluxed samples for the 0.025 N oxidant

ACKNOWLEDGEMENT:

Certain portions of this outline contain training material from prior outlines by R. C. Kroner, R. J. Lishka, and J. W Mandia.

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See Next Page.

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LABORATORY PROCEDURE FOR ROUTINE LEVEL CHEMICAL OXYGEN DEMAND

I REAGENTS

A Standard Potassium Dichromate (0.250 N):

Dissolve 12.259 g of primary standard grade $K_2Cr_2O_7$, which had been dried at 103° C for two hours, in distilled water and dilute to one liter.

B Ferrous Ammonium Sulfate (0.1N):

Dissolve 39 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water.
Carefully add 20 ml of concentrated H_2SO_4.
Cool and dilute to one liter.

C Ferroin Indicator:

Dissolve 1.485 g 1, 10-phenanthroline monohydrate and 0.695 g $FeSO_4 \cdot 7H_2O$ in water and dilute to 100 ml. This indicator may be purchased already prepared.

D Concentrated Sulfuric Acid (36 N):

E Mercuric Sulfate: Analytical Grade

F Silver Sulfate: Analytical Grade

G Concentrated Sulfuric Acid - Silver Sulfate:

Dissolve 22 g of silver sulfate in a 9 lb bottle of concentrated sulfuric acid. (1-2 days required for dissolution)

II EQUIPMENT PREPARATION

Before use, the Erlenmeyer flask (500 ml, 24/40 standard taper joint) and reflux condenser (Friedrichs, 24/40 standard taper joint) should be steamed out to remove trace organic contaminants. Add 10 ml of 0.250 N $K_2Cr_2O_7$, 50 ml of distilled water, and several bumping stones to the flask. Carefully add 20 ml of concentrated H_2SO_4 and mix thoroughly. Connect the flask and condenser, but do not turn on the water to the condenser. Boil the mixture so that steam emerges from the top of the condenser for several minutes. Cool the mixture, carefully discard the acid, and rinse the condenser and flask with distilled water. In order to prevent contamination from air-borne particles, the top of the condenser should be lightly plugged with glass wool during storage and use.

III STANDARDIZATION OF FERROUS AMMONIUM SULFATE

A Dilute 10.0 ml of the standard potassium dichromate to about 100 ml with distilled water.

B Add 30 ml of concentrated H_2SO_4 and allow to cool.

C Add 2-3 drops of ferroin indicator and titrate to a reddish-brown end point with the ferrous ammonium sulfate. Calculate the normality, N, of the ferrous ammonium sulfate.

D Calculation

N of $Fe(NH_4)_2(SO_4)_2$ =

$$\frac{\text{ml } K_2Cr_2O_7 \times \text{N of } K_2Cr_2O_7}{\text{ml } Fe(NH_4)_2(SO_4)_2}$$

IV PROCEDURE

A Place 0.4 g $HgSO_4$ in a 500 ml 24/40 standard taper Erlenmeyer flask. Add 20 ml of sample, or an aliquot diluted to 20 ml with distilled water.

B Add 2 ml of concentrated H_2SO_4 and swirl to dissolve the $HgSO_4$.

C Add 10.0 ml of the 0.250 N $K_2Cr_2O_7$ and mix.

D Carefully add 28 ml of the sulfuric acid - silver sulfate reagent.

E Add several pumice granules or glass beads to prevent bumping, and then swirl the mixture to insure complete mixing.

F Reflux the mixture for two hours.

G Allow the solution to cool, wash down the condenser with distilled water, and bring the volume of liquid to about 100 ml with water.

H Add 2-3 drops of the ferroin indicator and titrate the solution to a reddish-brown end point with the ferrous ammonium sulfate.

I A blank consisting of 20 ml of distilled water and containing all reagents is refluxed and titrated in the same manner as the sample.

J Calculation

$$\text{mg COD/l} = \frac{(A-B)\ N \times 8 \times 1000}{\text{ml of sample}}$$

COD = chemical oxygen demand

A = ml $Fe(NH_4)_2(SO_4)_2$ used for blank

B = ml $Fe(NH_4)_2(SO_4)_2$ used for sample

N = N of $Fe(NH_4)_2(SO_4)_2$

8 = equivalent weight of oxygen

ACKNOWLEDGEMENT:

Portions of this outline were taken from an outline prepared by R. J. Liska.

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This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

TOTAL CARBON ANALYSIS

I INTRODUCTION

A History of Carbon Analyses

In the wake of a rapid population growth, and the increasing heavy use of our natural waterways, the nation, and indeed the world, is presented with the acute problem of increased pollutional loads on streams, rivers and other receiving bodies. This has resulted in a growing awareness of the need to prevent the pollution of streams, rivers, lakes and even the oceans. Along with this awareness has developed a desire for a more rapid and precise method of detecting and measuring pollution due to organic materials.

B The Methods

In the past, two general approaches have been used in evaluating the degree of organic water pollution.

1 The determination of the amount of oxygen or other oxidants required to react with organic impurities.

2 The determination of the amount of total carbon present in these impurities.

C Oxygen Demand Analyses

The first approach is represented by conventional laboratory tests for determining Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). One of the principal disadvantages of these tests is that they are limited primarily to historical significance, that is, they tell what a treatment plant had been doing, since they require anywhere from two hours to five days to complete. Since up to now no faster method has been available, traditional BOD and COD determinations have become accepted standards of measure in water pollution control work even though they are essentially ineffective for process control purposes.

Until the introduction of the Carbonaceous Analyzer, all methods taking the second approach, the total carbon method of evaluating water quality, also proved too slow.

II THE ANALYSIS OF CARBON

A Pollution Indicator

Now the carbonaceous analyzer provides a means to determine the total carbon content of a dilute water sample in approximately two minutes. With proper sample preparation to remove inorganic carbonates, the instrument determines the total organic carbon content in the sample.

B Relationship of Carbon Analysis to BOD and COD

This quantity varies with the structure from 27 percent for oxalic acid through 40 percent for glucose to 75 percent for methane. The ratio of COD to mg carbon also varies widely from 0.67 for oxalic acid through 2.67 for glucose to 5.33 for methane. Representative secondary sewage effluents have given a ratio of COD to carbon content of between 2.5 and 3.5 with the general average being 3.0.

The BOD, COD and carbon contents of these and some other representative compounds are summarized in the following table.

Sample	5-Day BOD-mg/mg	COD-mg/mg	% Carbon
Stearic Acid - $C_{18}H_{36}O_2$	786	2.91	76
Glucose - $C_6H_{12}O_6$	.73	1.07	40
Oxalic Acid - $C_2H_2O_4$	14	.18	27
Benzoic Acid - $C_7H_6O_2$	1.38	1 97	69
Phenol - C_6H_6O	05 to 2 1 depending upon concentration	2.36	77
Potassium Acid Phthalate $KHC_8H_4O_4$	.95	1 15	47
Salicylic Acid - $C_7H_6O_3$	1.25	1 60	61
Secondary Effluent, Clarified	13*	75*	21*
" " "	23*	67*	12*
" " "	4*	36*	7*

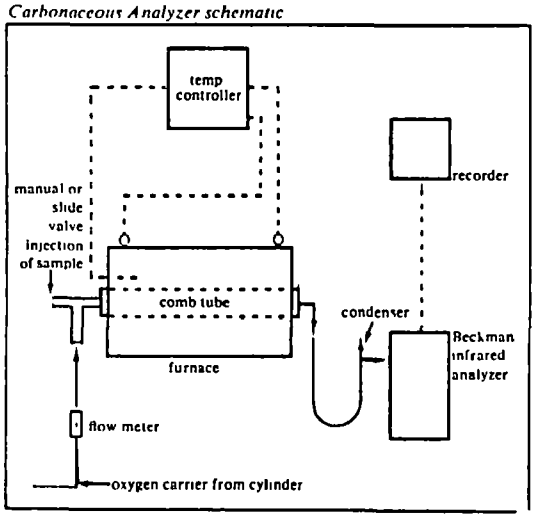
*In units of mg/l

III THE CARBON ANALYZER

A Principle of Operation

Basically the carbonaceous analyzer consists of three sections - a sampling and oxidizing system, a Beckman Model 315 Infrared Analyzer, and a strip-chart recorder

Carbonaceous Analyzer schematic



A micro sample (20-40 μl) of the water to be analyzed is injected into a catalytic combustion tube which is enclosed by an electric furnace thermostated at 950°C. The water is vaporized and the carbonaceous material is oxidized to carbon dioxide (CO_2) and steam in a carrier stream of pure oxygen. The oxygen flow carries the steam and CO_2 out of the furnace where the steam is condensed and the condensate removed The CO_2, oxygen and remaining water vapor enter an infrared analyzer sensitized to provide a measure of CO_2 The output of the infrared analyzer is recorded on a strip chart, after which, the curve produced can be evaluated by comparing peak height with a calibration curve based upon standard solutions. Results are obtained directly in milligrams of carbon per liter

B Application

Results[1] show that the method is applicable for most, if not all, water-soluble organic compounds -- including those that contain sulfur, nitrogen, and volatiles.

Nonvolatile organic substances can be differentiated from volatiles, such as carbon dioxide or light hydrocarbons by

determination of carbon both before and after the sample solution has been blown with an inert gas.

C Sample Preparation

The Carbonaceous Analyzer is often referred to as a total carbon analyzer because it provides a measure of all the carbonaceous material in a sample, both organic and inorganic. However, if a measure of organic carbon alone is desired, the inorganic carbon content of the sample can be removed during sample preparation

1 Removal of inorganic carbon

The simplest procedure for removing inorganic carbon from the sample is one of acidifying and blowing. A few drops of HCl per 100 ml of sample will normally reduce pH to 2 or less, releasing all the inorganic carbon as CO_2. Five minutes of blowing with a gas free of CO_2 sweeps out the CO_2 formed by the inorganic carbon. Only the organic carbon remains in the sample and may be analyzed without the inorganic interference

2 Volatile carbonaceous material

Volatile carbonaceous material that may be lost by blowing is accounted for by using a dual channel carbon analyzer. Beckman's new analyzer has the previously detailed high temperature (950°C) furnace plus a low temperature (150°C) one. Using quartz chips wetted with phosphoric acid, the low temperature channel senses only the CO_2 (freed by the acid) in the original sample. The remaining organics and water are retained in the condenser connected to this low temperature furnace. None of the organics are oxidized by the 150°C furnace.

By injecting a sample into the low temperature furnace, a peak representing the inorganic carbon is obtained on the strip chart. Injecting a non-acidified sample into the high temperature furnace yields a peak representing the total carbon. The difference between the values determined for the two peaks is the total organic carbon.

3 Dilute samples

If the sample is dilute (less than 100 mg/liter carbon) and is a true solution (no suspended particles) no further preparation is required.

4 Samples containing solids

If the sample contains solids and/or fibers which are to be included in the determination, these must be reduced in size so that they will be able to pass through the needle which has an opening of 170 microns (needles having larger openings may be obtained if necessary). In most cases, mixing the sample in a Waring Blender will reduce the particle size sufficiently for sampling.

IV PROCEDURE FOR ANALYSIS

A Interferences

Water vapor resulting from vaporization of the sample, causes a slight interference in the method Most of the water is trapped out by the air condenser positioned immediately after the combustion furnace. However, a portion of the water vapor passes through the system into the infrared detector and appears on the strip chart as carbon. The water blank also appears on the standard calibration curve, and is therefore removed from the final calculation In tests of solutions containing the following anions: NO_3^-, Cl^-, SO_4^{-2}, PO_4^{-3}, no interference was encountered with concentrations up to one percent.

B Precision and Accuracy

The recovery of carbon from standard solutions is 98.5 - 100.0 percent The minimum detectable concentration using the prescribed operating instructions is 1 mg/l carbon. Generally, the data are reproducible to $\pm$ 1 mg/l with a standard deviation of 0.7 mg/l at the 100 mg/l level

V APPLICATIONS

Several of the many research and industrial applications of the Carbonaceous Analyzer are listed below

A Determine the efficiency of various waste-water renovation processes, both in the laboratory and in the field.

B Compare a plant's waste outlet with its water inlet to determine the degree of contamination contributed

C Monitoring a waste stream to check for product loss.

D Follow the rate of utilization of organic nutrients by micro-organisms

E To detect organic impurities in inorganic compounds.

VI ADVANTAGES OF CARBON ANALYZER

A Speed

The Carbonaceous Analyzer's most important advantage is its speed of analysis One analysis can be performed in 2-3 minutes. This speed of analysis brings about another advantage, economy of operation Working with dilute samples, one man can run ten to twenty carbon determinations per hour. This is probably more than the number of COD or BOD tests that can even be started, much less completed, in the same period of time.

B Total Carbon

Another advantage is that the measure of carbon is a total one. The oxidizing system of the analyzer brings about complete oxidation of any form of carbon No compound has been found to which the method is inapplicable.

VII CONCLUSIONS

The Carbonaceous Analyzer provides a rapid and precise measurement of organic carbon in both liquid and air samples. It should be found useful for many research and industrial applications, a few of which have been mentioned.

Because of its rapidity it may be found more useful than the more time-consuming BOD and COD measurements for monitoring industrial waste streams or waste treatment processes.

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This outline was prepared by Robert T. Williams, Chief, Analytical Applications Laboratory, AWTL, NERC, Cincinnati, OH 45268 and revised by Charles J. Moench, Jr., Waste Identification and Analysis, AWTL, NERC, Cincinnati, OH 45268.

VI NUTRIENTS AND RELATED DETERMINATIONS

Chemical indications of water quality other than oxygen demand determinations are considered in this section. These criteria have an influence upon nutritional aspects of the aquatic environment of a favorable or unfavorable connotation depending upon concentration, balance, and desired uses.

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ACIDITY, ALKALINITY, pH AND BUFFERS

I DEFINITIONS OF ACIDS AND BASES

A Arrhenius Theory of Acids and Bases (Developed about 1887)

1 Acid: A substance which produces, in aqueous solution, a hydrogen ion (proton), H^+.

2 Base· A substance which produces, in aqueous solution, a hydroxide ion, OH^-.

3 The Arrhenius theory was confined to the use of water as a solvent.

B Bronsted and Lowry Theory of Acids and Bases (Developed about 1923)

1 Acid A substance which donates, in chemical reaction, a hydrogen ion (proton).

2 Base· A substance which accepts, in chemical reaction, a hydrogen ion (proton).

3 Bronsted and Lowry had expanded the acid-base concept into non-aqueous media, i.e., the solvent could, but did not have to be, water.

C Further development of acid-base theory dealing with electron pair donation or acceptance is unimportant.

II DEFINITIONS OF ACIDITY, ALKALINITY AND NEUTRALITY

A Acidity

A condition in which there is a preponderance of acid materials present in the water.

B Alkalinity

A condition in which there is a preponderance of alkaline (or basic) materials present in the water.

C Neutrality

1 It is possible to have present in the water chemically equivalent amounts of acids and bases. The water would then be described as being neutral; i.e., there is a preponderance of neither acid nor basic materials. The occurrence of such a condition would be rare.

2 The term "neutralization" refers to the combining of chemically equivalent amounts of acids and bases. The two products of neutralization are a salt and water.

$$HCl + NaOH = NaCl + H_2O$$

Hydrochloric acid	Sodium Hydroxide	Sodium Chloride (a salt)

D The key word in the above definitions is "preponderance." It is possible to have a condition of acidity while there are basic materials present in the water, as well as conversely.

III HOW ARE DEGREES OF ACIDITY AND ALKALINITY EXPRESSED?

The pH scale is used to express various degrees of acidity and alkalinity. Values can range from 0 to 14. These two extremes are of theoretical interest and would never be encountered in a natural water or in a waste water. pH readings from 0 to just under 7 indicate an acidic condition, from just over 7 to 14, an alkaline condition. Neutrality exists if the pH value is exactly 7. pH paper, or a pH meter, provides the most convenient method of obtaining pH readings. Some common liquids and their pH values are listed in Table 1.

TABLE 1. pH Values of Common Liquids	
Household lye	13.7
Bleach	12.7
Ammonia	11.3
Milk of magnesia	10.2
Borax	9.2
Baking soda	8.3
Sea water	8.0
Blood	7.3
Distilled water	7.0
Milk	6.8
Corn	6.2
Boric acid	5.0
Orange juice	4.2
Vinegar	2.8
Lemon juice	2.2
Battery acid	0.2

5.9 - 8.4 is the common pH range for most natural waters.

IV HARD AND SOFT WATERS

In addition to being acidic or basic, water can also be described as being hard or soft.

A Hard water contains large amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Hard water is objectionable because it forms insoluble compounds with ordinary soap.

B Soft water contains small amounts of calcium, magnesium, strontium, manganese and iron ions, relative to the amount of sodium and potassium ions present. Soft water does not form insoluble compounds with ordinary soap.

V TITRATIONS

A The conversion of pH readings into such quantities as milligrams (mg) of acidity, alkalinity, or hardness, is not easily carried out. These values are more easily obtained by means of a titration.

B In a titration, an accurately measured volume of sample (of unknown strength) is combined with an accurately measured volume of standard solution (of known strength) in the presence of a suitable indicator.

C The strength (called normality) of the sample is then found using the following expression:

milliliters (ml) of sample × normality (N) of sample = ml of standard solution × N of standard solution.

Three of the four quantities are known, and

N of sample = ml of standard solution × N of standard solution/ml of sample.

D In modified form, and a more specific application of the above equation, alkalinity is calculated in the following manner (12th ed. Standard Methods).

mg of alkalinity as mg $CaCO_3$/liter (1) = ml of standard H_2SO_4 × N of standard H_2SO_4 × 50 × 1000/ml sample.

VI INDICATORS

The term "suitable indicator" was used above. At the end of a titration, the pH of the solution will not necessarily be 7. It may be above or below 7. A suitable indicator, therefore, is one which undergoes its characteristic color change at the appropriate pH. Below are a few examples of indicators and the pH range in which they undergo their characteristic color changes. In some cases, mixed indicators may be used in order to obtain a sharper and more definite color change.

Indicator	Operational pH Range
Methyl Yellow	2.8 - 4.0
Methyl Orange	3.1 - 4.4
Methyl Red	4.4 - 6.2
Cresol Purple	7.4 - 9.0
Phenolphthalein	8.0 - 9.6
Alizarine Yellow	10.0 - 12.0

TABLE 2. pH Range of Indicators

VII BUFFERS

A A buffer is a combination of substances which, when dissolved in water, resists a pH change in the water, as might be caused by the addition of acid or alkali. Listed below are a few chemicals which, when combined in the proper proportions, will tend to maintain the pH in the indicated range.

Chemicals	pH Range
Acetic Acid + Sodium Acetate	3.7 - 5.6
Sodium Dihydrogen Phosphate + Disodium Hydrogen Phosphate	5.8 - 8.0
Boric Acid + Borax	6.8 - 9.2
Borax + Sodium Hydroxide	9.2 - 11.0

TABLE 3. pH Range of Buffers

B A buffer functions by supplying ions which will react with hydrogen ions (acid "spill"), or with hydroxide ions (alkali "spill").

C In many instances, the buffer is composed of a weak acid and a salt of the weak acid; e.g., acetic acid and sodium acetate.

1 In water, acetic acid ionizes or "breaks down" into hydrogen ions and acetate ions.

$$HC_2H_3O_2 = H^+ + C_2H_3O_2^-$$

(acetic acid) (hydrogen ion) (proton) (acetate ion)

This ionization occurs to only a slight extent, however, most of the acetic acid remains in the form of $HC_2H_3O_2$; only a small amount of hydrogen and acetate ions is formed.

2 Thus, acetic acid is said to be a weak acid.

3 In the case of other acids, ionization into the component ions occurs to a large degree, and the term strong acid is applied; e.g., hydrochloric acid.

$$HCl = H^+ + Cl^-$$

(hydrochloric acid) (hydrogen ion) (proton) (chloride ion)

4 The terms "strong" and "weak" are also applied to bases. In water solutions, those which break down into their component ions to a large extent are termed "strong", and those which do not are "weak". Sodium hydroxide is a relatively strong base, while ammonium hydroxide is realtively weak.

5 Sodium acetate (a salt of acetic acid) dissociates or "breaks down" into sodium ions and acetate ions when placed in water.

$$NaC_2H_3O_2 = Na^+ + C_2H_3O_2^-$$

(sodium acetate) (sodium ion) (acetate ion)

This dissociation occurs to a large extent, and practically all of the sodium acetate is in the form of sodium ions and acetate ions.

D It would be difficult and expensive to prepare large quantities of buffers for use in a treatment plant. However, certain naturally occurring buffers may be available (carbon dioxide is an example). It dissolves in water to form the species indicated below.

$$CO_2 + H_2O = H_2CO_3$$

(carbon dioxide) (Water) (carbonic acid)

$$H_2CO_3 = H^+ + HCO_3^-$$

(hydrogen ion) (proton) (hydrogen carbonate ion) (bicarbonate)

The hydrogen ions react with hydroxide ions which might appear in the water as the result of an alkali "spill".

$$H^+ + OH^- = H_2O$$

(in the buffer) (hydroxide ion "spilled")

The hydrogen carbonate ions react with hydrogen ions which might appear in the water as the result of an acid "spill".

$$H^+ + HCO_3^- = H_2CO_3$$

(hydrogen ion) (proton) "spilled" (in the buffer)

This buffering action will be in effect as long as there is carbonic acid present.

E Buffering action is not identical with a process in which acid wastes are "neutralized" with alkali wastes, or conversely. The desired effect is achieved in both cases, however (i.e., the pH is maintained within a desired range.)

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ALKALINITY AND RELATIONSHIPS AMONG THE VARIOUS TYPES OF ALKALINITIES

I PRELIMINARY

The property of water referred to as alkalinity is usually caused by the presence of hydroxyl, carbonate and bicarbonate ions. To a lesser extent, borates, phosphates and silicates contribute but are generally present in negligible amounts.

The concentration and ratio of the OH^-, CO_3^{--} and HCO_3^- ions may be measured by titrating a sample to certain specified pH's or end points which are detected either by use of a pH meter or by color indicators. Phenolphthalein is used for visual detection of the first end point, (approximately pH 8) which indicates the neutralization of NaOH and conversion of CO_3^{--} to HCO_3^-. A number of indicators (methyl orange, methyl purple, brom cresol green, etc.) are used for detection of the second end point (pH 3-5) which indicates the complete conversion of HCO_3^- to H_2O and CO_2. The final end point is determined by the amount of CO_3^{--} and HCO_3^- originally present in the sample. If the end points are determined electrometrically they are taken as the mid-point of the greatest rate of pH change per unit volume of titrant.

II RELATIONSHIPS BETWEEN HYDROXIDE, CARBONATE, AND BICARBONATE ALKALINITIES

The results obtained from phenolphthalein and total alkalinity measurements offer a means of classification of the principal forms of alkalinity, if certain assumptions are made. It must first be assumed that interferences are absent and that bicarbonate and hydroxide do not exist in the same solution. According to the system presented in Standard Methods, 12th Edition:

A Hydroxide alkalinity is present if the phenolphthalein alkalinity is more than one-half the total alkalinity.

B Carbonate alkalinity is present if the phenolphthalein alkalinity is not zero but is less than the total alkalinity.

C Bicarbonate alkalinity is present if the phenolphthalein alkalinity is less than one-half the total alkalinity.

Table 1. Relationships Between Phenolphthalein Alkalinity, Total Alkalinity, Carbonate Alkalinity, Bicarbonate Alkalinity and Hydroxide Alkalinity

Lecture Notes	Result of Titration	OH^- Alkalinity as $CaCO_3$	CO_3^{--} Alkalinity as $CaCO_3$	HCO_3^- Alkalinity as $CaCO_3$
Case 1	P = T	T	O	O
Case 2	P = ½T	O	2P	O
Case 3	P = O	O	O	T
Case 4	P > ½T	2P-T	2(T-P)	O
Case 5	P < ½T	O	2P	T-2P

P = Phenolphthalein Alkalinity T = Total Alkalinity

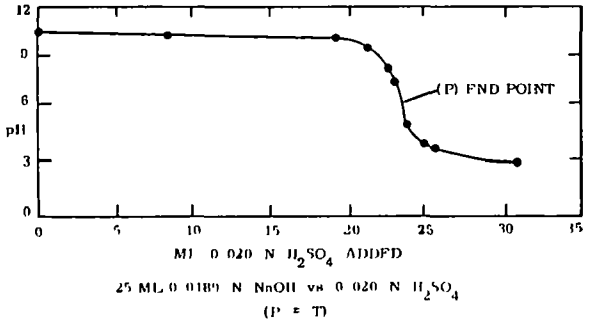
Table 2. Stoichiometric Volumes of Solutions of Different Normalities

Standard Solution	H_2SO_4	NaOH	Na_2CO_3	$NaHCO_3$
Normality	0.0200	0.0189	0.0199	0.0125
Equivalent Volumes, ml	10.0	10.6	9.9	16.0
	9.4	10.0	9.5	15.1
	10.0	10.5	10.0	15.9
	6.3	6.6	6.3	10.0

III CASE EXAMPLES

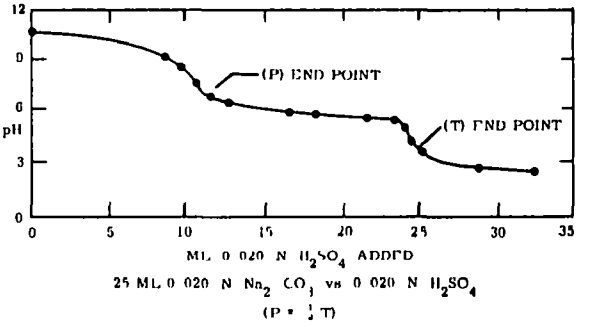
The relationships involved in Table 1 may best be explained by reference to the following graphs. These were prepared by titrating volumes of standard solutions of sodium hydroxide, sodium carbonate, and sodium bicarbonate with standard sulfuric acid. The stoichiometric volumes of the various solutions are summarized in Table 2 for convenience in the interpretation of the charts.

A CASE 1 - Where phenolphthalein alkalinity = total alkalinity



The sharp break occurs at the point where all of the NaOH has been exactly neutralized by the acid. The pH and concentration of the end products (Na_2SO_4 and H_2O) determine the pH at the equivalence point between NaOH and H_2SO_4, in this case, approximately 7.0.

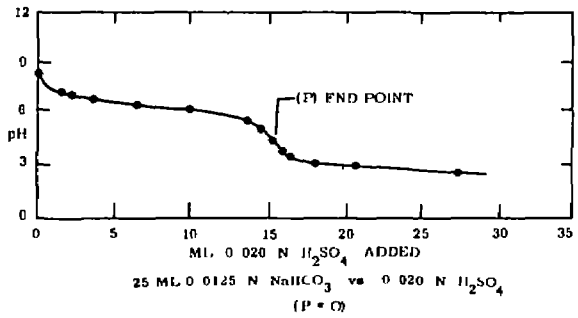
B CASE 2 - Where phenolphthalein alkalinity = one-half the total alkalinity



The titration proceeds in 2 stages wherein all of the CO_3^{--} is converted, first to HCO_3^- and finally to H_2CO_3. The first end point occurs at approximately pH 8, and at exactly half the volume of acid used for the total titration. The end point which occurs at approximately pH 4 represents the total alkalinity and requires exactly twice the volume of acid used for the first end point.

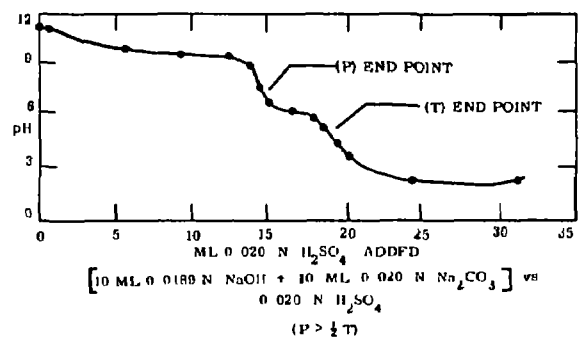
If either HCO_3^- or OH^- ions had been present the titration volumes for the curves would not have been of equal magnitude.

C CASE 3 - Where phenolphthalein alkalinity = 0



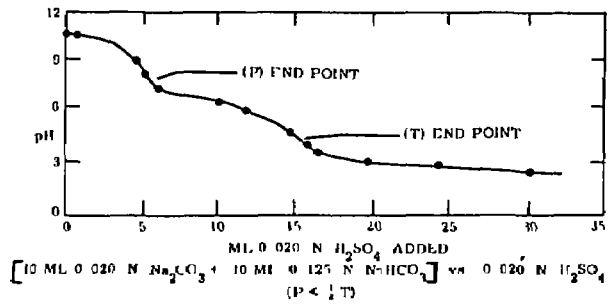
T The reaction proceeds in one stage with the initial pH at approximately 8.5 and final pH at 4.0. In this case the phenolphthalein alkalinity is zero and since no conversion of CO_3^{--} to HCO_3^- is noted the total alkalinity can only be due to the HCO_3^- ion.

D CASE 4 - Where phenolphthalein alkalinity is greater than one-half the total alkalinity



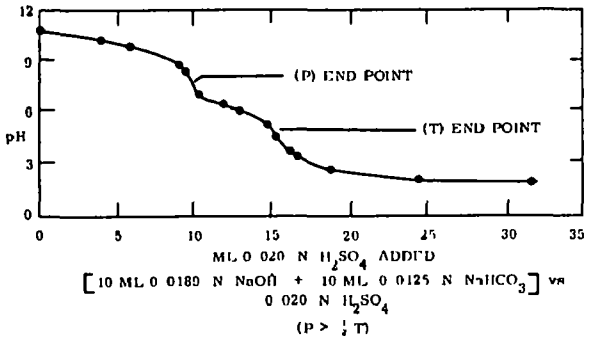
The volume of acid required for the first end point (phenolphthalein alkalinity) is due to the OH^- neutralization and conversion of the CO_3^{--} to HCO_3^-. The second end point represents the complete conversion of HCO_3^- to H_2CO_3. Referring to Case 2 where the volume of acid was similar for each end point, it is apparent that a base responding to phenolphthalein but which is not CO_3^{--} must be present. Since it was originally assumed that OH^- and HCO_3^- do not exist in the same solution we must conclude that the total alkalinity is due to OH^- and CO_3^{--}.

E CASE 5 - Where phenolphthalein alkalinity is less than one half of the total alkalinity



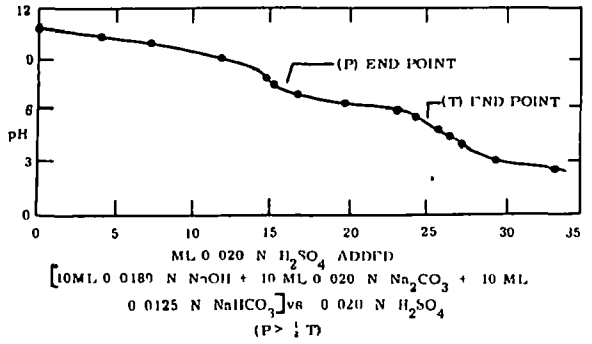
If, in the reaction $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$, the NaOH exists in excess quantity, the final sample contains NaOH and Na_2CO_3, (Case 4) in which the volume of acid required for the phenolphthalein end point is greater than one half the total. In this case, however, the situation is reversed, wherein the volume of acid required for the HCO_3^- end point is greater than one half the total. Referring again to the reaction $NaOH + NaHCO_3 \rightarrow Na_2CO_3 + H_2O$, if $NaHCO_3$ is in excess the end products must consist of Na_2CO_3 and $NaHCO_3$ and OH^- must be absent. The end points consist, therefore, of $Na_2CO_3 \rightarrow NaHCO_3$ (phenolphthalein end point) and $NaHCO_3 \rightarrow H_2CO_3$.

F CASE 6 - Where phenolphthalein alkalinity is greater than one-half total alkalinity.



Following the original assumption that OH^- and HCO_3^- are not compatible, with HCO_3^- being converted to CO_3^{--} we have a condition similar to Case 4 (P > ½ T).

G CASE 7 - Where phenolphthalein alkalinity is greater than one-half total alkalinity.



The first end point occurs at the stoichiometric sum of the equivalent volumes as follows:

$$(9.4 - 6.3) + \frac{(10 + 12.6)}{2}$$

where (9.4 - 6.3) = volume of N acid required for excess OH^- after OH^- + HCO_3^- reaction,

and $\frac{(10 + 12.6)}{2}$ = volume of acid required for conversion of CO_3^{--} to HCO_3^-. The second end point occurs at $\frac{(10 + 12.6)}{2}$ ml, the volume of HCO_3^- which is converted to H_2CO_3. This then becomes the same as Case 4.

IV COMPARISON OF ANALYTICAL METHODS FOR ALKALINITY (According to the Analytical Reference Service Report JAWWA Vol. 55, No. 5, 1963)

May 1963 DETERMINATION METHODS

TABLE 2—*Statistical Summary (contd.)*

Method	Year	No of Values Reported	Concn Added mg/l	Concn Determined mg/l			50% Range mg/l	Standard Deviation mg/l
				Mean	Low	High		
Alkalinity								
Methyl orange	1956	38	19	20 0	11 23	30 0	± 2	5 049
	1958	19	17	19 5	16	24	± 1	2 483
	1961	27	42 5	43 7	38	50	± 1.5	3 250
Electrometric	1956	14	19	19 7	19 0	21 0	± 1	0.911
	1958	53	17	19 4	15	25	± 2	2 740
	1961	88	42 5	44 2	38 9	57	± 2 5	3 605
Methyl purple	1956	8	19	14 7	14 0	15 2	± 4	0 475
	1958	10	17	19 3	18	21	± 2	0 823
	1961	18	42 5	44 8	40	50	± 1 5	3 081
Mixed indicator	1958	4	17	19 5	18	20	± 3	1 000
	1961	15	42 5	42 8	39	49	± 3	3 550
Brom cresol green	1958	1	17	19	19	19	± 2	
All methods	1956	60	19	19 2	11 23	30 0	± 2	4 460
	1958	92	17	19 6	15	25	± 2	2 320
	1961	178	42 5	43 9	25	57	± 1 9	5.335

Alkalinity - The methods for alkalinity measurement varied only in the choice of indicator or pH for determining the end point of the titration. The indicators used included methyl orange, methyl purple, and mixed indicator. The data shows that as the use of electrometric end point increased, the use of methyl orange decreased.

SUMMARY

Amount Added	17 mg/l (as $CaCO_3$)
Avg. Deviation from amount added	3.1 mg/l
Standard Deviation	3.4 mg/l
50% Range	2.0 mg/l
Method Most Commonly Used	Electrometric
Method Preferred	Electrometric
Total Number of Observations	41

V PROCEDURE

The actual measurement of alkalinity is a very simple procedure requiring only titration of sample with a standardized acid and the proper indicator. For phenolphthalein alkalinity the end point and indicator are well established. For the bicarbonate titration the final end point is a function of the HCO_3^- concentration. With low amounts (<50 mg/l as $CaCO_3$) the pH at end point may be approximately 5.0. With high concentrations (>250 mg/l as $CaCO_3$) the pH at end point may be 4.5 to 3.8. For all-purpose work, in which the highest degree of accuracy is not required, an end point at pH 4.5 using methyl purple as the indicator is recommended.

The traditional methyl orange frequently proves to be unsatisfactory because of the indefinite color change at the end point and also because of the low pH (3.8 - 3.9) required to establish the change.

VI EPA ANALYTICAL METHODS

A The Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory has compiled a manual of Analytical Methods which is to be used in Federal laboratories for the chemical analysis of water and waste samples. The title of this manual is "Methods for Chemical Analysis of Water and Wastes, 1971."

B This manual lists two parameters which are related to the subject matter in this outline. They are total alkalinity and total acidity.

C For the measurement of both of these parameters, the recommended method is volumetric, with the equivalence point being determined electrometrically. The use of a color indicator (methyl orange) is recommended only for the automated method.

D The procedure references cited by the EPA Methods manual are the 13th Edition of Standards Methods and ASTM Standards, Part 23, 1970.

REFERENCES

1 Standards Methods for the Examination of Water and Wastewater, 12th Ed. APHA, Inc., New York. 1965.

2 ASTM Standards, Part 23. 1968.

This outline was prepared by R. C. Kroner, Chief, Physical and Chemical Methods, Analytical Quality Control Laboratory, Water Programs Operations, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR TOTAL ALKALINITY

I METHOD SUMMARY

A In the current methods manual[1] of the Environmental Protection Agency, the following method is specified for use in Office of Water Programs laboratories The sample is titrated with 0.02N hydrochloric or sulfuric acid to a final pH of 4 5, the end point being determined electrometrically.

1 The sample must not be filtered, diluted, concentrated or altered in any way

2 Results are reported as mg$CaCO_3$/liter

3 The procedure can be found in Standard Methods[2] and ASTM Book of Standards.[3]

B The procedure as given in the above references has been adapted for this laboratory session to accommodate group participation and to accomplish instructional objectives.

II DISCUSSION

In this manual, outline 8 considers the relationships among the 3 forms of alkalinity commonly found in water Refer to the graphs in outline 8 for the following·

For Case 1, $(OH)^-$ alkalinity only, pH values change very gradually until the approach of pH 9 Then an abrupt change occurs and the addition of a few ml of titrant causes the value to change rapidly to about pH 4

For Case 2, $(CO_3)^=$ alkalinity only, there is a gradual change of pH values as the acid titrant is added. Around pH 9 there is an abrupt change of the scale reading Around pH 6, the change is again gradual. Around pH 5, another rapid change occurs until pH 3 is reached

A pH of 8 3 represents the average pH when $(OH)^-$ has been totally neutralized and $(CO_3)^=$ has been half neutralized to $(HCO_3)^-$. A pH of 4 5 represents the average pH occurring when $(HCO_3)^-$ has been neutralized to CO_2 and H_2O. (See Case 3)

In order to obtain standardized data, the EPA method specifies stopping alkalinity titrations at pH 4 5. At this pH, any $(OH)^-$, $(CO_3)^=$ and $(HCO_3)^-$ in the sample will be neutralized. Since all three of these alkalinity forms may be present, the results are expressed as total alkalinity

It is useful to express total alkalinity as mg $CaCO_3$/liter.

III INSTRUCTIONAL OBJECTIVES

A You will use the adapted EPA method to determine total alkalinity by titrating 3 solutions of known alkalinities and one unknown to an end point of pH 4 5 You will then use the data to calculate the total alkalinity for each as mg $CaCO_3$/liter

1 By slowly adding the HCl titrant to solutions of $(OH)^-$, of $(CO_3)^=$ and of $(HCO_3)^-$ and observing the pH scale, the characterizing response of each alkalinity at different pH values can be observed.

2 Recording the volume of HCl used to reach pH 8 3 and then pH 4 5 will also illustrate the pH characteristics of the three forms of alkalinity

3 Calculating the total alkalinity of these three solutions will familiarize you with the calculations for the determination.

B You will also do titrations on the same solutions using color indicators --- phenolphthalein (colorless at pH 8) and methyl orange (amber-orange at pH 4 6) --- in order to contrast values obtained by the pH determination.

C Using experience gained in the lab session, you will estimate the types of alkalinity present in the unknown sample.

IV REAGENTS

A Carbon Dioxide-Free Distilled Water

B Anhydrous Sodium Carbonate (primary standard)

C Hydrochloric Acid Titrant (0.02N)

D Phenolphthalein

E Methyl Orange

V STANDARDIZATION of the HYDROCHLORIC ACID TITRANT

A Set the temperature reading on the pH meter dial to match the temperature of the buffer and sample solutions.

B Standardize the pH meter against a reference buffer solution.

C Weigh accurately 0.088 ± 0.001 g of the dried sodium carbonate and transfer it to a 500 ml conical flask.

D Add 50 ml of water and swirl to dissolve the carbonate.

E While stirring the solution, add the hydrochloric acid titrant from a 100 ml buret until a pH of 4.5 is attained.

F Calculate the normality of the hydrochloric acid solution as follows:

$$A = \frac{B}{0.053 \times C}$$

A = normality of the hydrochloric acid

B = g of sodium carbonate used

C = ml of hydrochloric acid consumed

0.053 = millequivalent weight of Na_2CO_3

VI TITRATIONS to pH END POINTS

For each of the solutions marked Case 1 $(OH)^-$, Case 2 $(CO_3)^=$ and Case 3 $(HCO_3)^-$ and for the assigned unknown, A or B, the following procedure is to be followed All data and the calculation results should be recorded on the Data Table provided

A Calibrating the pH meter

1 Pour about 50 ml of the buffer solution into a small beaker.

2 Set the temperature reading on the pH meter dial to match the temperature of the reference buffer.

3 Immerse the electrode(s) into the buffer solution.

4 Turn the meter to "ON"(or "Read" or "pH").

5 Using the "Balance" (or "Asymm") knob, set the scale reading to 6.84.

6 Turn the meter to "Off" (or "Hold" or "Standby").

7 Remove electrode(s) from buffer and save this solution for possible later use.

8 Rinse electrode(s) with distilled water, then immerse them in a small beaker of distilled water until time to use

B Prepare the buret by rinsing 3 times using about 15 ml HCl each time. Then fill the buret with HCl and adjust the volume so that the top of the liquid column is positioned for a reading.

C Titration Procedure:

1 Each titration will require up to 25 ml 0.02N HCl titrant. Refill the buret as needed before beginning each titration.

2 Pipet 50.0 ml of the solution to be used into a beaker

3 Remove the pH electrode(s) from the beaker of distilled water and immerse electrode(s) in the solution to be titrated. The sample solution should cover the glass bulb(s).

4 Turn pH meter to "ON" (or "Read" or "pH").

5 On the Data Table, record the ml of HCl in the buret at the start of the titration.

6 Record the initial pH of the sample on the Data Table.

7 While gently swirling either the electrode or the sample in the beaker, slowly add the HCl titrant, observing the scale on the pH meter Stop adding titrant when pH is about 8.3 and record the buret reading (This step is not part of the EPA procedure. It is included here for instructional purposes).

8 Again gently swirling either the electrode or the beaker, continue slow addition of the HCl titrant, observing the meter scale Be very cautious when the scale approaches ph 5 0. Stop titrating at pH 4.5 If a lower pH is inadvertently reached, note this on the Data Table.

9 On the Data Table, record the ml HCl remaining in the buret.

10 Set pH meter to "OFF" (or "Hold" or "Standby").

11 Rinse the pH electrode(s) with distilled water and immerse in the beaker of distilled water

12 Empty the sample solution and rinse the beaker with distilled water.

13 Using the next solution, repeat this procedure (C, steps 1 through 12) until data has been recorded for the 3 knowns and the 1 unknown sample. Then proceed to part VII.

DATA TABLE
TITRATIONS TO pH END POINTS

	CASE 1 $(OH)^-$	CASE 2 $(CO_3)^=$	CASE 3 $(HCO_3)^-$	SAMPLE ____
Initial pH				
ml@pH 4.5				
ml@pH 8.3				
ml@Start	____	____	____	____
TOTAL ml. HCl				
TOTAL ALKALINITY (mg $CaCO_3$ /liter)				

VII TITRATIONS to COLOR END POINTS

If time permits, the following procedure should be used to titrate the 3 solutions of known constituents and the assigned unknown, A or B. At least, do one of the known solutions and the unknown used for the pH titration All data and the calculation results should be recorded on the Data Table provided.

A Indicators Used

1 Phenolphthalein indicator is a deep pink around pH 10 and becomes colorless at pH 8 In the Data Table, this colorless end point is designated as P. End Pt.

2 Methyl orange indicator gives a yellow color to a colorless solution At pH 4.6 (referred to as M.O. End Pt on Data Table) it becomes amber-orange. This end point color requires practice to detect If the solution acquires a pinkish cast, you are past the desired end point.

B Titration Procedure

1 Each titration will require up to 25 ml 0.02N HCl titrant. (If pH determinations have been done, refer to these results for approximations of HCL titrant to be used). Refill the buret as needed before beginning each titration.

2 Pipet 50.0 ml of the solution to be used into a beaker

3 Place a piece of white paper under the beaker.

4 Add 3 drops phenolphthalein indicator to the sample and swirl to mix

5 On the Data Table record the ml of HCl in the buret at the start of the titration.

6 Using the known relationship of the color of phenolphthalein to different pH values, record your estimate of the initial pH of the sample on the Data Table.

7 If the solution remains colorless, record 0 0 for P End Pt and continue with step 9.

If there is pink color in the solution, slowly add HCl titrant while stirring the sample

As the end point is approached, a lingering colorless region where the titrant first contacts the sample will be observed Add the titrant very cautiously at this time, stirring the sample solution and allowing time for total mixing and contact before adding more HCl titrant.

The end point occurs when the entire solution is colorless. Stop adding the HCl titrant.

8 Record the ml of HCl in the buret for the P. End Pt. on the Data Table

9 Add 3 drops of methyl orange indicator to the same sample solution.

10 If the sample solution becomes amber-orange, record 0 0 for M.O. End Pt. and continue at step 12

If the solution becomes yellow, slowly add HCl titrant while stirring the sample.

As the end point is approached, a a lingering amber-orange region where the titrant first contacts the sample will be observed Add the titrant very cautiously at this time, stirring the sample solution and allowing time for total mixing and contact before adding more HCl titrant.

The end point occurs when the entire solution is amber orange Stop adding the HCl titrant. (If the solution develops a pinkish cast, you are past the desired end point and should note this on the Data Table).

11 Record the ml of HCl in the buret for the M.O. End Pt on the Data Table

12 Empty the sample solution and rinse the beaker with distilled water

13 Choose the next solution according to time available and repeat this procedure (B, steps 1 thru 12).

DATA TABLE

TITRATIONS TO COLOR END POINTS

	CASE 1 $(OH)^-$	CASE 2 $(CO_3)^=$	CASE 3 $(HCO_3)^-$	SAMPLE ____
initial pH (Estimate)				
ml @ M.O. End Pt				
ml. @ P. End Pt.				
ml. @ Start	____	____	____	____
TOTAL ml. HCl				
TOTAL ALKALINITY (mg $CaCO_3$ /liter)				

VIII CALCULATIONS

A Calculate Total Alkalinity for each solution titrated, using the following·

$$\text{mg } CaCO_3/1 = \frac{A \times N \times 50,000}{\text{ml. of sample}}$$

A = total ml of standard acid required for titration to pH 4 5 end point

N = normality of standard acid

50 = equivalent weight of $CaCO_3$

1000 converts ml to liters

B Record calculation results on the Data Tables.

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL, Cincinnati, OH 45268, (1971)

2 Standard Methods for the Examination of Water and Wastewater, 13th edition, APHA-AWWA-WPCF, Method 102 (1971)

3 ASTM Book of Standards, Part 23, p 154 (1970)

This outline was prepared by A. Donahue, Chemist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR TOTAL ACIDITY

I REAGENTS

For detailed discussion of reagent preparation, consult reference 2.

A Carbon Dioxide - Free Distilled Water

B Potassium Hydrogen Phthalate (primary standard)

C Sodium Hydroxide Titrant (0.02 N)

II STANDARDIZATION OF THE SODIUM HYDROXIDE TITRANT

A Set the temperature reading on the pH meter dial to match the temperature of the buffer and sample solutions.

B Standardize the pH meter against a reference buffer solution.

C Weigh accurately 0.19 ± 0.005 g of the dried potassium hydrogen phthalate and transfer it to a 500 ml conical flask.

D Add 100 ml of reagent water and swirl gently to dissolve the phthalate.

E While stirring the solution(magnetic bar and stirrer), add the sodium hydroxide from a 100 ml buret until a pH of 8.3 is attained.

F Calculate the normality of the sodium hydroxide solution as follows·

$$A = \frac{B}{0.20423XC}$$

A = normality of the sodium hydroxide

B = g of potassium hydrogen phthalate

C = ml of sodium hydroxide solution

0.20423 = milliequivalent weight of potassium hydrogen phthalate ($KHC_8H_4O_4$)

III PROCEDURE

A Pipet 100 ml of the sample into a 400 ml beaker.

B Titrate with the sodium hydroxide to pH 8.3

C Calculation

Total acidity as mg of $CaCO_3$/l =

$$\frac{A \times N \times 50000}{\text{ml of sample}}$$

A = ml of standard NaOH titrant

N = N of standard NaOH titrant

50 = equivalent weight of $CaCO_3$

1000 - converts ml to liters

REFERENCES

1 Methods for Chemical Analysis of Water & Wastes, EPA-AQCL, 1971, Cincinnati, OH 45268.

2 A.S.T.M. Book of Standards, Part 23, D 1067-70, pp. 155-158.

This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, OWP, MDS, DTTB, EPA, Cincinnati, OH 45268.

DETERMINATION OF CHLORIDE IN WATER SUPPLIES

I OCCURRENCE

In fresh waters, high chloride values may indicate the presence of animal pollution. However, the chloride test as an indication of animal pollution should be confirmed by bacteriological and sanitary analyses. A high chloride value can be due to other sources such as oil field brines and other industrial wastes, also from the passage of water through a natural salt formation, or from agricultural return wastes

II SIGNIFICANCE

Chloride compounds may break down, specially under boiler pressures, to form HCl, thus causing corrosion problems Chlorides are undesirable in ice making as they spoil the appearance of the ice. Approximately 500 mg of salt/l imparts an undesirable taste to drinking water For brewing or soft drinks, the salt content should not exceed 275 mg/l, as the concentration may be increased in the process.

The U. S. Public Health Service Drinking Water Standards for potable waters recommend a maximum chloride content of 250 mg per liter, because of taste effects

III ANALYTICAL METHODS

A Volhard

An excess of silver nitrate is added to the acidified sample to precipitate chlorides The excess silver is titrated with thiocyanate in the presence of ferric ion and nitrobenzene. At the end point, red ferric thiocyanate is formed. Iodide and bromide register as equivalent chloride. Phosphate and sulfite do not interfere, but sulfide does.

B Mohr

The neutral or weakly alkaline sample is treated with chromate indicator and titrated with silver nitrate Silver chloride precipitates and at the end point, red silver chromate is formed Iodide and bromide register as equivalent chloride. Phosphate, sulfide and cyanide interfere Sulfite interferes but can be removed with hydrogen peroxide. Color if present, can be removed with aluminum hydroxide suspension.

C Mercuric Nitrate

The sample, adjusted to pH 3.1*, is titrated with mercuric nitrate solution. Since slightly dissociated mercuric chloride is formed, no precipitation occurs At the end point, the excess mercuric ions produce a violet color with diphenylcarbazone indicator. Bromphenol blue is added to the indicator solution for pH adjustment It improves the sharpness of the endpoint by masking the pale color produced by diphenylcarbazone during the titration

Iodide and bromide register as equivalent chloride Sulfite in concentrations greater than 10 mg/l interferes, but can be removed with hydorgen peroxide. Chromate and feric ions interfere when in excess of 10 mg/l

IV ENVIRONMENTAL PROTECTION AGENCY METHOD

A The Analytical Quality Control Laboratory, Office of Water Programs, Environmental Protection Agency, has published a manual of analytical chemical procedures.

B This manual cites references (2) or (3) for the determination of chloride; the two procedures both involve titration with mercuric nitrate.

C An automated ferricyanide method is described in detail in reference (1).

V PRECISION

Using the mercuric nitrate titration, forty-two analysts in eighteen laboratories analyzed synthetic water samples containing exact increments of chloride with the following results:

Increment as Chloride, mg/l	Precision as Standard Deviation, mg/l
17	1.54
18	1.32
91	2.92
97	3.16
382	11.7
398	11.8

REFERENCES

1 Methods for Chemical Analysis of Water and Wastes, 1971, Environmental Protection Agency, Water Quality Office, Analytical Quality Control Laboratory

2 Standard Methods for the Examination of Water and Wastewater, 13th Ed., page 97, Method 112B (1971).

3 ASTM Methods, Part 23, Water; Atmospheric Analysis, page 24, Method 512-67 (1970).

This outline was prepared by D. G. Ballinger, Director, Analytical Quality Control Laboratory, NERC, EPA, and revised by C. R. Feldmann, Chemist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

DETERMINATION OF SULFATE IN WATER SUPPLIES

I DEFINITION AND OCCURRENCE

Sulfate is found in all fresh waters as a result of solvent action of water on gypsum ($CaSO_4 \cdot 2H_2O$) and other common minerals such as epsom salt ($MgSO_4 \cdot 7H_2O$). Sulfates also occur as the final oxidized stage of sulfides, sulfites, and thiosulfates. They may also occur as the oxidized state of organic matter in the sulfur cycle, and, in turn serve as sources of energy for sulfate-reducing bacteria, at lower pH these will form hydrogen sulfide, which is quite undesirable.

Industrial wastes, such as those from sulfate-pulp mills, tanneries, pickling operations, and other plants that use sulfates or sulfuric acid, contribute to the natural sulfate content of raw waters. Sulfuric acid is the heaviest tonnage chemical manufactured.

II SIGNIFICANCE

PHS Drinking Water Standards call for not more than 250 mg $SO_4^{=}$/l. Public water supplies with high sulfate content are commonly used with no adverse effects, this limit does not appear to be based on tests or physiological effects other than a laxative action for new users. The taste threshold of magnesium sulfate is 400 - 600 mg/l, calcium sulfate is reported to be 250 - 900 mg/l. Excessive concentrations (1000 - 2000 mg/l) of magnesium sulfate may have purgative effects.

Sulfates may be either beneficial or detrimental in water used for manufacturing. In the brewing industry, the presence of sulfate is advantageous, as it aids in producing desirable flavor. On the other hand, sulfates are not desirable in the ice industry because of the formation of white butts. In domestic water systems, sulfates do not appear to cause any increased corrosion on brass fittings, but concentrations above 200 mg/l do increase the amount of lead dissolved from lead pipes.

Normally, calcium sulfate scale is not encountered in once-through cooling water systems since it is quite soluble at the temperatures usually existing. In recirculating systems, where concentration takes place, the sulfate content of the circulating water may become high enough to precipitate calcium sulfate in the form of gypsum ($CaSO_4 \cdot 2H_2O$).

Treatment measures for preventing scale formation are directed primarily at calcium carbonate precipitation, since it is less soluble than the sulfate. The usual treatment uses sulfuric acid which increases the sulfate content. This, under certain conditions, may create the additional problem of calcium sulfate scale formation.

The precipitation of calcium sulfate can be hindered by surface-active agents such as the polyphosphates and organics. If necessary, sulfates can be removed by evaporation, demineralization, or precipitation with barium salts.

The publication, "Water Quality Criteria" of the California State Water Pollution Control Board, lists the recommended limits on sulfate in mg/l, as shown in Table 1.

III ANALYTICAL METHODS

The gravimetric method is recognized as the standard procedure and is the most accurate and time-consuming. It should be used for sulfate in greater concentrations than 60 mg/l. The turbidimetric procedure is rapid and more accurate for concentrations less than 50 mg/l, but can be used up to 60 mg/l. The most rapid method is the titrimetric, which is applicable to solutions containing 100 mg $SO_4^{=}$/l, or more where an accuracy of $\pm$10% is acceptable as in boiler water analysis.

This procedure is not applicable to Basic Data Network samples. Obviously, dilution

Table 1. RECOMMENDED LIMITS ON SULFATE CONTENT OF INDUSTRIAL WATER

INDUSTRIAL PROCESS	Milligrams per liter			
	SO_4	$CaSO_4$	$MgSO_4$	Na_2SO_4
Brewing	-	100 - 500	100 - 200	100
Carbonated Beverages	250	-	-	-
Concrete Corrosion	25	-	-	-
Ice Making	-	300	130 - 300	300
Milk Industry	60	-	-	-
Photographic Process	-	100	-	-
Sugar Making	20	-	-	-
Textiles	100	-	-	-

or concentration of the sample will bring most waters into the desired range for any of the methods.

A Gravimetric Procedure

The gravimetric procedure involves the addition of a dilute solution of barium chloride to the sample to precipitate barium sulfate. The precipitation is made in a solution slightly acidified with HCl and near the boiling temperature. The precipitate is filtered off, washed in water until free of chloride ions, ignited at 800° C and weighed as barium sulfate.

$$\text{mg } SO_4^{=}/l = \frac{\text{mg } BaSO_4 \times 411.5}{\text{ml of sample}}$$

B Turbidimetric Method

In the more rapid turbidimetric method, sulfate ion is precipitated with barium ion in acid solution in such a manner as to form barium sulfate crystals of uniform size. No other ions are found in normal waters that will precipitate with barium in acid solution. Light transmitted by the turbid solution is measured with a photometer and the sulfate ion concentration is read from a standard curve. Color and turbidity must be removed first. The procedure described in Standard Methods involves very careful control of stirring and the time interval before reading. A modification used by the ARS Laboratory at SEC has shown more consistent results.

IV PRECISION AND ACCURACY

The ARS Water Mineral Study of 1961 reported on a reference sample containing 259 mg $SO_4^{=}/l$ that the gravimetric procedure remains the most commonly used, with the turbidimetric method next.

The standard deviation for the gravimetric method was ± 11.9 mg/l and for the turbidimetric method it was ± 23.9 mg/l.

V FWQA METHODS

A The Federal Water Quality Administration, Division of Research, Analytical Quality Control Branch, has compiled a manual of analytical methods which is to be used in Federal laboratories when analyzing surface waters. The title of this manual is "FWQA Methods for Chemical Analysis of Water & Wastes, 1970".

B In this manual the turbidimetric method is recommended (see III B); the gravimetric procedure is not recommended.

C The 12th Edition of Standard Methods and A. S. T. M. Book of Standards, 1968 are cited in the FWQA manual as the references for the turbidimetric method.

REFERENCES

1 Standard Methods for the Examination of Water and Wastewater. 12 Ed. APHA, AWWA, WPCF. 1965.

2 Water Mineral Study of 1961. Analytical Reference Service, SEC.

3 Water Quality Criteria. State Water Pollution Control Board, Sacramento, California. 1963.

4 Public Health Service Interstate Quarantine Drinking Water Standards (Revised) 1961. Federal Register pp 6737-6740. July 27, 1961.

5 FWQA Methods for Chemical Analysis of Water & Wastes, 1970.

This outline was prepared by D. G. Ballinger, Director, Analytical Quality Control Laboratory, NERC, EPA, Cincinnati, OH 45268.

SOURCES AND ANALYSIS OF ORGANIC NITROGEN

I INTRODUCTION

A Organic nitrogen refers to the nitrogen in combination with any organic radical. For sanitary engineering the main interest is the nitrogen contained in proteins, peptides, amines, amino acids, amides and related compounds of animal or vegetable origin

II SOURCES OF ORGANIC NITROGEN

A Natural Origin

1 Dead animal and plant residues

2 Animal wastes - urea, feces

3 Autotrophic organisms - algae, s.bact

4 Heterotrophic organisms

B Industrial Origin

1 Food processing wastewater - meat, milk, vegetables

2 Pharmaceutical wastes, antibiologicals

3 Plastics - polyamides, nitriles

4 Chemical intermediates or products

5 Dye industry - azo, nitro

III TOTAL KJELDAHL NITROGEN PROCEDURE

A Organic nitrogen is determined using the Total Kjeldahl Nitrogen (TKN) method. This determination includes both organic nitrogen and free ammonia By distilling the free ammonia off the sample before the determination, organic nitrogen can be determined directly

B Scope

The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but it may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semi-carbazones and some refractory tertiary amines.

C Method Summary[1]

The sample is heated in the presence of a concentrated sulfuric acid-potassium sulfate-mercuric sulfate mixture and evaporated until sulfur trioxide fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled and diluted, then treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled off and then determined either by nesslerization or by titration.

1 Nesslerization (colorimetric) is the method used when the ammonia-nitrogen concentration is less than 1.0 mg N/liter.

2 For ammonia-nitrogen concentrations above 1.0 mg N/liter, the ammonia is determined by titration with 0.02 N H_2SO_4 in the presence of a mixed indicator.

For a detailed description of the procedure and reagent preparation, consult the EPA methods manual.

D Precision and Accuracy

1 Thirty-one analysts in 20 laboratories[1] used the Total Kjeldahl Nitrogen procedure to analyze natural water samples containing the following increments of organic nitrogen: 0.20, 0.31, 4 10 and 4.61 mg N/liter.

a Precision results for a standard deviation were 0.197, 0.247, 1.056 and 1.191 mg N/liter, respectively.

b Accuracy as bias was +0.03, +0.02, +0.04 and -0.08 mg N/liter, respectively.

2 The nature and composition of extraneous materials affect analytical recovery. High salt concentrations may raise digestion temperature. High concentrations of organic sample components may require excessive acid during digestion tending to low nitrogen yield.

3 The digestion temperature is critical. 380 to 390°C usually gives high analytical recovery on the more refractory nitrogen compounds of natural origin. Nitrogen losses occur above 420°C.

E Optimum temperature is associated with a digestion mix containing 1 gram of potassium sulfate for each ml of sulfuric acid. However, the digestion mixture usually contains a fraction of H_2SO_4 greater than this.

During heating, the water boils off first, followed by fuming as the H_2SO_4 becomes concentrated. Fuming and oxidation of the sample components lead to loss of H_2SO_4 and a progressive decrease in acid content during heating. As the acid decreases, the temperature of the heated mixture rises.

Valid nitrogen determinations require a slight excess of acid to retain NH_3 as NH_4HSO_4 rather than the more volatile $(NH_4)_2SO_4$ Concurrently, excess acid will tend toward incomplete oxidation of sample components as a result of lowering digestion temperature

IV AUTOMATED PHENOLATE METHOD

The EPA manual[1] also presents an automated (phenolate) method for organic nitrogen The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric sulfate as a catalyst, then neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent This treatment forms a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

V PRESERVATION OF SAMPLES

A Most nitrogen compounds are characterized by rapid conversion from one form to another by biological and chemical action. Hydrolysis, deamination, peptide formation, and other reactions may appreciably alter the original form of sample nitrogen within a short time Organic nitrogen tends to hydrolyze to free NH_3 during storage Therefore, valid results require prompt analysis.

B Addition of 40 mg $HgCL_2$/liter of sample and storage at 4°C are preservation measures for samples. Even when so preserved, samples are unstable and should be analyzed as soon as possible.

REFERENCE

1 Methods for Chemical Analysis of Water and Wastes, EPA-AQCL. Cincinnati, OH 45268 (1971).

This outline was prepared by Audrey E Donahue, Chemist, National Training Center, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

AMMONIA, NITRITES AND NITRATES

I SOURCES AND SIGNIFICANCE OF AMMONIA, NITRITES AND NITRATES IN WATER

The natural occurrence of nitrogen compounds is best demonstrated by the nitrogen cycle (Figure 1)

A Ammonia

1 Occurrence

Ammonia is a product of the microbiological decay of animal and plant protein In turn, it can be used directly to produce plant protein Many fertilizers contain ammonia

2 Significance

The presence of ammonia nitrogen, in raw surface waters might indicate domestic pollution. Its presence in waters used for drinking purposes may require the addition of large amounts of chlorine in order to produce a free chlorine residual. The chlorine will first react with ammonia to form chloramines before it exerts its full bactericidal effects (free chlorine residual).

B Nitrites

1 Occurrence

Nitrite nitrogen occurs in water as an intermediate stage in the biological decomposition of organic nitrogen. Nitrite formers (nitrosomonas) convert ammonia under aerobic conditions to nitrites. The bacterial reduction of nitrates can also produce nitrites under anaerobic conditions. Nitrite is used as a corrosion inhibitor in industrial process water.

2 Significance

Nitrites are usually not found in surface water to a great extent. The presence of large quantities indicates a source of wastewater pollution.

C Nitrates

1 Occurrence

Nitrate formers convert nitrites under aerobic conditions to nitrates (nitrobacter). During electrical storms, large amounts of nitrogen (N_2) are oxidized to form nitrates. Finally, nitrates can be found in fertilizers.

2 Significance

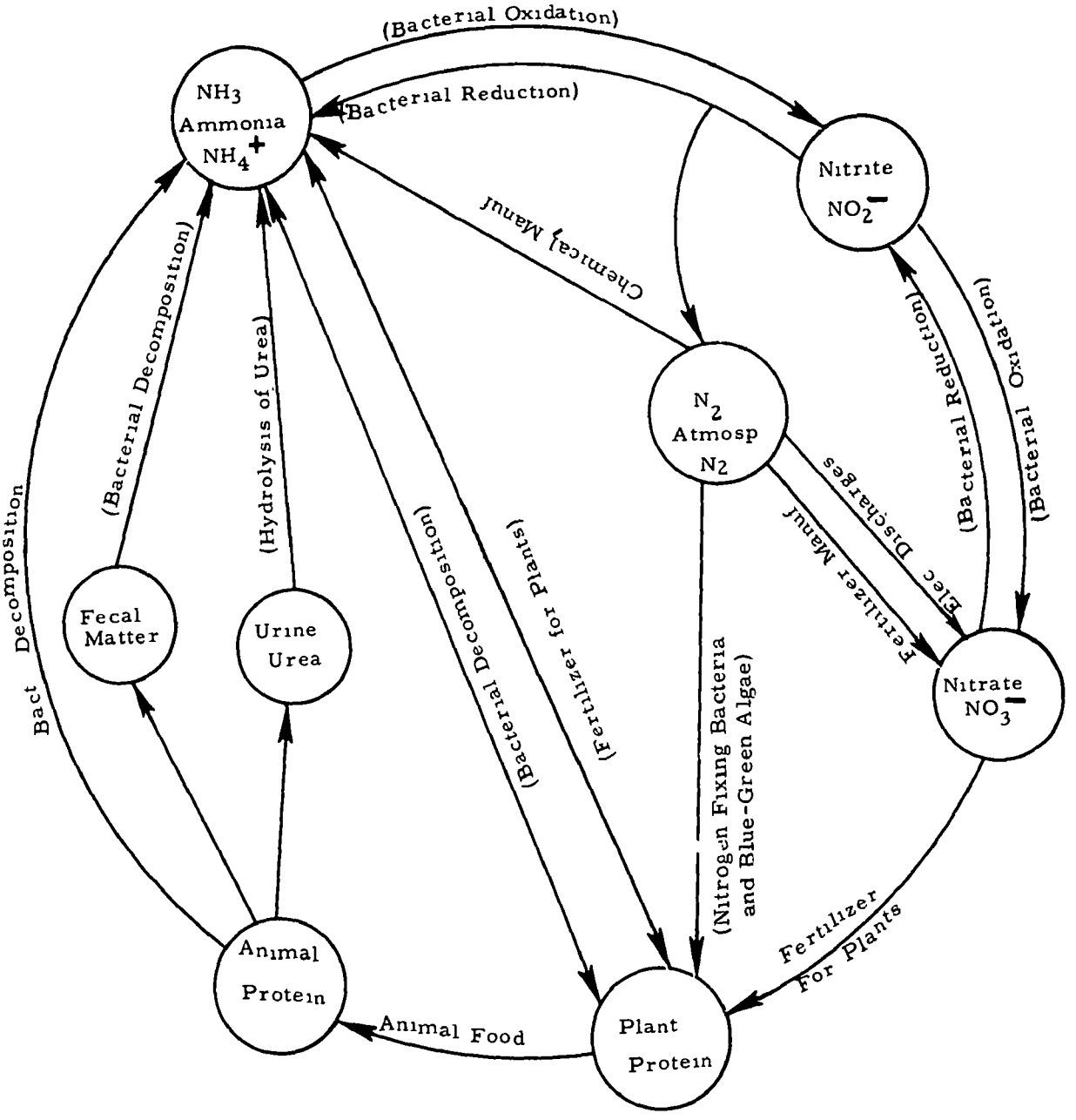
Nitrates in water usually indicate the final stages of biological stabilization. Nitrate rich effluents discharging into receiving waters can, under proper environmental conditions, cause stress to stream quality by producing algal blooms. Drinking water containing excessive amounts of nitrates can cause infant methemoglobinemia.

II ANALYSIS OF AMMONIA, NITRITES AND NITRATES IN WATER

A Sample Preservation [8]

1 If only ammonia is to be determined on the sample, it may be preserved with 1.0 ml of concentrated sulfuric acid per liter and stored at 4° C.

2 To preserve samples for any of the nitrogen procedures, add 40 mg $HgCl_2$ and store at 4° C. Even when so preserved, conversion of organic nitrogen to ammonia may occur. Preserved samples should be analyzed as soon possible.



THE NITROGEN CYCLE

Figure 1

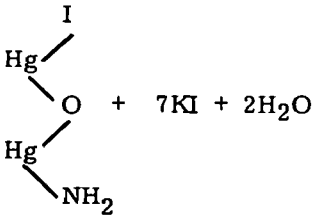
B Determination of Ammonia

1 Nesslerization

a Reaction

Nessler's reagent, a strong alkaline solution of potassium mercuric iodide, combines with NH_3 in alkaline solution to form a yellowish brown colloidal dispersion.

$$2K_2HgI_4 + NH_3 + 3KOH \longrightarrow$$



$$+ \; 7KI + 2H_2O$$

Yellow-Brown

The intensity of the color follows the Beer-Lambert Law and exhibits maximum absorption at 425 nm.

b Interferences

1) Nessler's reagent forms a precipitate with some ions (e.g., Ca^{++}, Mg^{++}, Fe^{+++}, and $S^{=}$). These ions can be eliminated in a pretreatment flocculation step with zinc sulfate and alkali. Also, EDTA or Rochelle salt solution prevents precipitation with Ca^{++} or Mg^{++}.

2) Residual chlorine indicates ammonia may be present in the form of chloramines. The addition of sodium thiosulfate will convert these chloramines to ammonia.

3) Certain organics may produce an off color with Nessler's reagent. If these compounds are not steam distillable, the interference may be eliminated in the distillation method.

4) If the turbidity and natural color of the sample cannot be eliminated with flocculation, it is then necessary to use the following distillation method.

2 Distillation

a Reaction

1) The sample is distilled in the presence of a phosphate buffer at pH 7.2 - 7.4.

$$NH_4 + \xrightarrow{\Delta} NH_3 + H^+$$

$$H^+ + Na_2HPO_4 \longrightarrow NaH_2PO_4 + Na^+$$

Buffer — pH maintained between 7.2 - 7.4

2) The ammonia in the distillate is then measured by either of two techniques.

a) Nesslerization is used for samples containing less than 1 mg/l of ammonia nitrogen.

b) Absorption of NH_3 by boric acid and back titration with a standard strong acid is more suitable for samples containing more than 1 mg NH_3 N/l.

$$NH_3 + HBO_2 \longrightarrow NH_4^+ + BO_2^-$$

$$BO_2^- + H^+ \xrightarrow[+]{\text{Methyl Red}} HBO_2$$

Methylene Blue

pH 7.8 - 8.0 Green — pH 6.8 - 7.0 Purple

b Interferences

1) Calcium reacts with the phosphate buffer releasing H^+ and lowering the pH in the distillation step. When the sample contains more than 250 mg Ca^{++}/l, it may be necessary to adjust the pH to 7.4 after adding the buffer.

2) Residual chlorine.

3) Aliphatic and aromatic amines may distill over and cause positive interference by reacting in the acid titration.

3 Precision and Accuracy

On a synthetic sample containing 0.8 mg ammonia nitrogen/l, the ARS Water Nutrients Study (1966) reported results as shown in Table 1.

Method	Standard Deviation	Mean Error
Direct Nesslerization	±0.077 mg/l	0.00 mg/l
Distillation with Nesslerization	±0.100 mg/l	+0.02 mg/l
Distillation with Titration	±0.167 mg/l	+0.04 mg/l

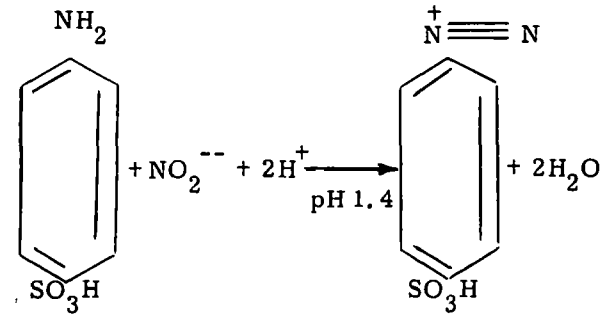
Table 1. ARS WATER NUTRIENTS, 1966

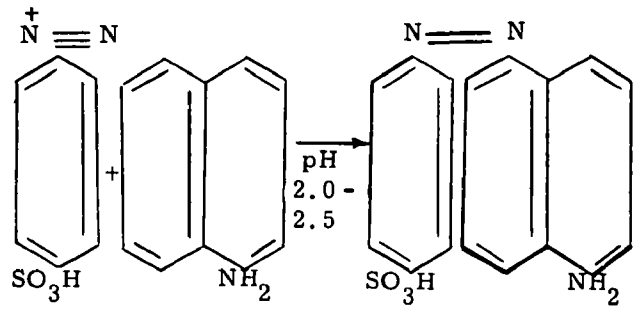
C Determination of Nitrite - Diazotization

1 Reaction

a Under acid conditions, nitrite ions react with sulfanilic acid to form a diazo compound.

b The diazo compound then couples with α-naphthylamine to form an intense red azo dye which exhibits maximum absorption at 520 nm.





2 Interferences

a Certain ions (e.g., Hg^{++}, Ag^+, Bi^{+++}, Sb^{+++}, Fe^{+++}, Pb^{++}) may interfere by precipitating during the test. A 0.5% EDTA solution may be used to complex iron.

b Cupric ion introduces negative interference by catalyzing decomposition of the diazo compound.

c Colored ions and turbidity may be removed by using $Al(OH)_3$ suspension or by flocculation with zinc sulfate and alkali.

3 Precision

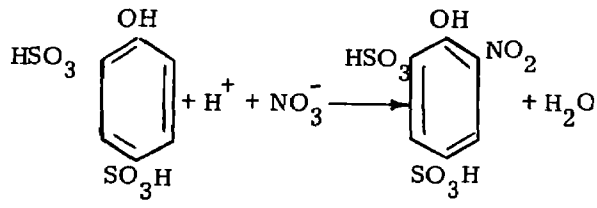
On a synthetic sample containing 0.25 mg nitrite nitrogen/l, the ARS Water Minerals Study (1961) reported 125 results with a standard deviation of ±0.029 mg/l.

D Determination of Nitrate

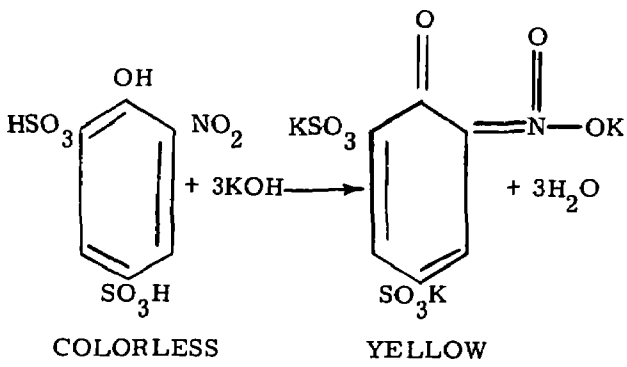
1 Phenoldisulfonic Acid

a Reaction

1) Phenoldisulfonic acid reacts with nitrate to produce a nitro derivative.



2) In alkaline solution the nitro derivative rearranges to form a yellow-colored compound which exhibits maximum absorption at 410 nm.



b Interferences

1) Chlorine ion under the acid conditions of the test introduces negative interference.

$$6Cl^- + 2NO_3^- + 8H^+ \longrightarrow 3Cl_2 \uparrow + 2NO \uparrow + 4H_2O$$

Silver sulfate can be used to precipitate Cl^-, but due to incomplete precipitation of Ag^+, an offcolor or turbidity is produced when the final color is developed. (Note· This difficulty can be overcome by using NH_4OH as the alkali.)

2) Nitrites in concentrations greater than 0.2 mg N/l introduce positive interference. However, in most waters, the concentration of nitrite is insignificant compared to nitrate.

3) Color and turbidity may be removed by using $Al(OH)_3$ suspension or by flocculation with $ZnSO_4$ and alkali.

c Precision and accuracy

On a synthetic sample containing 1.0 mg nitrate N/l, and 200 mg Cl^-/l, the ARS Water Nutrients Study (1966) reported 46 results with a standard deviation of $\pm$0.399 mg/l and a mean error of -0.31 mg/l.

2 Brucine

a Reaction

Brucine, a strychnine compound reacts with nitrate to form a yellow compound which exhibits maximum absorption at 410 nm. The reaction according to the procedure as outlined in Standard Methods (page 178) does not follow Beer's Law. However, a recent modification by Jenkins and Medsker (2) has been developed. Conditions are controlled in the reaction so that Beer's Law is followed and concentrations below 1 mg nitrate N/l can be determined.

b Interferences

1) Nitrite may react the same as nitrate but can be elim-

inated by the addition of sulfanilic acid to the brucine reagent

2) Organic nitrogen compounds may hydrolyze and give positive interference at low (less than 1mg/l) nitrate nitrogen concentrations

3) Residual chlorine may be eliminated by the addition of sodium arsenite

c Precision and Accuracy[8]

1) Twenty-seven analysts in 15 laboratories analyzed in natural water samples containing the following increments of inorganic nitrate· 0.16, 0.19, 1.08 and 1.24 mg N/liter

2) Precision results as standard deviation were 0.092, 0.083, 0.245, and 0.214 mg N/liter respectively

3) Accuracy expressed as bias was -0.01, +0.02, +0.04 and +0.04 mg N/liter, respectively.

3 Hydrazine Reduction

A method using hydrazine to reduce nitrate to nitrite followed by subsequent measurement of nitrite by diazotization was recently reported by Fishman, et al.[1] The procedure has been successfully adapted to the Auto Analyzer where a high degree of control of reaction conditions can be achieved [3]

III EPA METHODS

A The Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory, has compiled a manual of analytical methods for use by Federal laboratories. The title of this manual is "Methods for Chemical Analysis of Water & Wastes", 1971.

B The manual lists four nitrogen constituents These are Ammonia Nitrogen, Total Kjeldahl Nitrogen, Nitrate Nitrogen and Nitrite Nitrogen.

1 Ammonia Nitrogen

The recommended procedures are either an automated method or distillation followed by titration (for high concentration samples) or Nesslerization (for low concentration samples)

2 Total Kjeldahl Nitrogen

The total Kjeldahl nitrogen is determined by an automated phenolate procedure, or by mercuric sulfate-sulfuric acid-potassium sulfate digestion followed by titration or colorimetric analysis

3 Nitrate Nitrogen

The automated method of determination involves reduction of the nitrate to nitrite with hydrazine, followed by colorimetric analysis The manual method is a spectrophotometric procedure in which a yellow color is produced by the reaction the nitrate ions with brucine sulfate and sulfanilic acid

4 Nitrite Nitrogen

Nitrite is determined by diazotizing sulfanilamide with nitrite ions under acid conditions, coupling the product with N (1-Naphthyl) ethylenediamine, and reading the resultant red-purple color at 540 nm.

REFERENCES

1 Fishman, Marvin J , Skougstad, Marvin W , and Scarbio, George, Jr Diazotization Method for Nitrate and Nitrite JAWWA 56·633-638 May, 1960

2 Jenkins, David and Medsker, Lloyd L Brucine Method for Determination of Nitrate in Ocean, Estuarine and Fresh Waters Anal Chem 36·610-612 March, 1964

3 Kamphake, L J , Chemist, Engineering Section, Basic and Applied Sciences Branch, DWS & PC, Robert A Taft Sanitary Engineering Center, Personal Communication

4 Lishka, R J., Lederer, L A , and McFarren, E F Water Nutrients No. 1, Analytical Reference Service 1966

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6 Standard Methods for the Examination of Water and Waste Water APHA. AWWA. WPCF. 13th Ed. 1971.

7 ASTM Book of Standards, Part 23, 1970.

8 Methods for Chemical Analysis of Water & Wastes, EPA-AQCL, Cincinnati, Ohio 45268

This outline was prepared by Betty Ann Punghorst, former Chemist, National Training Center and Charles Feldmann, Chemist, NTC, DTTB, MDS, OWP, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR NITRATE NITROGEN

MODIFIED BRUCINE METHOD

(Range 0.1 to 2 mg nitrate nitrogen/l)

I REAGENTS

A Water

Distilled water free of nitrite and nitrate should be used in the preparation of all reagents and standards.

B Stock Potassium Nitrate
(1.0 ml = 0.1 mg NO_3^- N):

Dissolve 0.7218 g of anhydrous KNO_3 in distilled water and dilute to 1 liter.

C Standard Potassium Nitrate
(1.0 ml = 0.001 mg NO_3^- N):

Dilute 10.0 ml of the stock solution to 1 liter with distilled water. This solution should be prepared fresh weekly.

D Sulfuric Acid:

Carefully add 500 ml of concentrated H_2SO_4 (sp. gr. 1.84) to 125 ml of distilled water. Cool and keep tightly stoppered.

E Sodium Chloride (30%):

Dissolve 30 g of NaCl in distilled water and dilute to 100 ml.

F Brucine - sulfanilic acid reagent:

Dissolve 1 g of brucine sulfate, $(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7\ H_2O$, and

0.1 g of sulfanilic acid ($NH_2C_6H_4SO_3H \cdot H_2O$) in 70 ml of hot distilled water.
Add 3 ml of concentrated HCl, cool, and dilute to 100 ml. This solution is stable for several months if stored in a dark bottle at 5°C. The pink color which develops slowly does not effect the usefulness of the solution. The bottle should be marked "toxic".

G Acetic Acid:

Dilute 1 vol of glacial $HC_2H_3O_2$ with 3 vols of distilled water.

II SAMPLE PREPARATION

A In the case of highly alkaline waters, it is necessary to adjust the pH to approximately 7 with the acetic acid.

B Filter through a 0.45 micron pore size filter if necessary.

III PROCEDURE

A Pipet 0.0, 2.0, 5.0, 7.0, and 10 0 ml of the standard KNO_3 solution into 50 ml test tubes held in a suitable rack. Add sufficient distilled water to bring the volume to 10.0 ml.

B Pipet 2.0, 3.0, and 5.0 ml. of the sample into 50 ml test tubes. The purpose of using three different volumes of the sample is to ensure that when the colors are developed, at least one of the three samples will give an absorbance which lies within the range of the calibration curve Add sufficient distilled water to bring the volume to 10 ml.

C If the samples are saline, pipet 2.0 ml of 30% NaCl into the standard and sample tubes. Mix well This addition is unnecessary for fresh water samples.

D Chill all of the tubes to 0 - 10°C in a cold water bath.

E Pipet 10.0 ml of the H_2SO_4 solution into each standard and sample tube, mix well by swirling, and again chill to 0 - 10°C.

F Pipet 0.5 ml of the brucine - sulfanilic acid reagent into the standard and sample tubes, mix well by swirling, and place the tubes in a boiling water bath for 25 minutes.

G Remove the tubes from the boiling water bath and cool to 20-25°C in a water bath.

H If color or turbidity develops in the sample while the tubes are in the boiling water bath, a blank must be prepared using all reagents except the brucine - sulfanilic acid.

I Measure the absorbance values of the standards and samples at 410 nm. on a suitable spectrophotometer. Before each solution is read, rinse the spectrophotometer cell at least twice with the solution.

J Prepare a calibration curve of absorbance values for the standards vs. mg of NO_3^- N. For example: if 2.0 ml of the standard KNO_3 solution are used, and its concentration is 0.001 mg of NO_3^- N/ml, then 0.002 mg of NO_3^- is the value plotted on the calibration curve vs. the corresponding absorbance value.

K Determine the mg of NO_3^- N present in the samples using the calibration curve and then calculate the mg of NO_3^- N/l using the formula:

$$\text{mg of } NO_3^- \text{ N/l} = \text{mg of } NO_3^- \text{ N from curve} \times \frac{1000 \text{ ml/l}}{\text{ml of sample}}$$

REFERENCES

Methods for Chemical Analysis of Water & Wastes, EPA-AQCL, 1971.

This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, MDS, WPO, EPA, Cincinnati, OH 45268.

DETERMINATION OF PHOSPHORUS IN THE AQUEOUS ENVIRONMENT

I Phosphorus is closely associated with water quality because of (a) its role in aquatic productivity such as algal blooms, (b) its sequestering action, which causes interference in coagulation, (c) the difficulty of removing phosphorus from water to some desirable low concentration, and (d) its characteristic of converting from one to another of many possible forms.

A Phosphorus is one of the primary nutrients such as hydrogen (H), carbon (C), nitrogen (N), sulfur (S) and phosphorus (P).

1 Phosphorus is unique among nutrients in that its oxidation does not contribute significant energy because it commonly exists in oxidized form.

2 Phosphorus is intimately involved in oxidative energy release from and synthesis of other nutrients into cell mass via:

a Transport of nutrients across membranes into cell protoplasm is likely to include phosphorylation.

b The release of energy for metabolic purposes is likely to include a triphosphate exchange mechanism.

B Most natural waters contain relatively low levels of P (0.01 to 0.05 mg/l) in the soluble state during periods of significant productivity.

1 Metabolic activity tends to convert soluble P into cell mass (organic P) as a part of the protoplasm, intermediate products, or sorbed material.

2 Degradation of cell mass and incidental P compounds results in a feedback of lysed P to the water at rates governed by the type of P and the environment. Aquatic metabolic kinetics show marked influences of this feedback.

3 The concentrations of P in hydrosoils, sludges, treatment plant samples and soils may range from 10^2 to 10^6 times that in stabilized surface water. Both concentration and interfering components affect applicability of analytical techniques.

II The primary source of phosphorus in the aqueous system is of geological origin. Indirect sources are the processed mineral products for use in agriculture, household, industry or other activities.

A Agricultural fertilizer run-off is related to chemicals applied, farming practice and soil exchange capacity.

B Wastewaters primarily of domestic origin contain major amounts of P from·

1 Human, animal and plant residues

2 Surfactants (cleaning agent) discharge

3 Microbial and other cell masses

C Wastewaters primarily of industrial origin contain P related to:

1 Corrosion control

2 Scale control additives

3 Surfactants or dispersants

4 Chemical processing of materials including P

5 Liquors from clean-up operations of dusts, fumes, stack gases, or other discharges

III Phosphorus terminology is commonly confused because of the interrelations among biological, chemical, engineering, physical, and analytical factors.

A Biologically, phosphorus may be available as a nutrient, synthesized into living mass, stored in living or dead cells, agglomerates, or mineral complexes, or converted to degraded materials.

B Chemically, P exists in several mineral and organic forms that may be converted from one to another under favorable conditions. Analytical estimates are based upon physical or chemical techniques necessary to convert various forms of P into ortho phosphates which alone can be quantitated in terms of the molybdenum blue colorimetric test.

C Engineering interest in phosphorus is related to the prediction, treatment, or control of aqueous systems to favor acceptable water quality objectives. Phosphorus removal is associated with solids removal.

D Solubility and temperature are major physical factors in phosphorus behavior. Soluble P is much more available than insoluble P for chemical or biological transformations and the rate of conversion from one to another is strongly influenced by temperature.

E Table 1 includes a classification of the four main types of chemical P and some of the relationships controlling solubility of each group. It is apparent that no clear-cut separation can be made on a solubility basis as molecular weight, substituent and other factors affect solubility.

F Table 2 includes a scheme of analytical differentiation of various forms of P based upon:

1 The technique required to convert an unknown variety of phosphorus into ortho P which is the only one quantitated by the colorimetric test.

2 Solubility characteristics of the sample P or more precisely the means required to clarify the sample.

a Any clarification method is subject to incomplete separation. Therefore, it is essential to specify the method used to interpret the yield factor of the separation technique. The degree of separation of solubles and insolubles will be significantly different for·

1 Membrane filter separation (0.5 micron pore size)

2 Centrifugation (at some specified rpm and time)

3 Paper filtration (specify paper identification)

4 Subsidence (specify time and conditions)

G Analytical separations (Table 2) like those in Table 1, do not give a precise separation of the various forms of P which may be included quantitatively with ortho or poly P. Conversely some of the poly and organic P will be included with ortho P if they have been partially hydrolyzed during storage or analysis. Insolubles may likewise be included as a result of poor separation and analytical conditions.

1 The separation methods provide an operational type of definition adequate in most situations if the "operation" is known. Table 2 indicates the nature of incidental P that may appear along with the type sought.

Table 1

PHOSPHORUS COMPOUNDS CLASSIFIED BY CHEMICAL AND SOLUBILITY RELATIONS

Form	Water Soluble(1)	Insoluble(1)
1. Ortho phosphates $(PO_4)^{-3}$	Combined with monovalent cations such as H, Na, K, NH_4	Combined with multi valent cations such as Ca^{+2} Al^{+3} Fe^{+3}
2. Poly phosphates $(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$ $(P_3O_9)^{-3}$ and others depending upon the degree of dehydration.	as in 1 Increasing dehydration decreases solubility	(a) as in 1 (b) multi P polyphosphates (high mol. wt.) including the "glassy" phosphates
3. Organic phosphorus R-P, R-P-R (2) (unusually varied nature)	(a) certain chemicals (b) degradation products (c) enzyme P (d) phosphorylated nutrients	(a) certain chemicals (b) cell mass, may be colloidal or agglomerated (c) soluble P sorbed by insoluble residues
4. Mineral phosphorus	(a) as in 1	(a) as in 1 (b) as in 2 (c) geological P such as phosphosilicates (d) certain mineral complexes.

(1) Used in reference to predominance under common conditions.

(2) R represents an organic radical, P represents P, PO_4^{-3}, or its derivatives.

2 Total P in Table 2 includes liquid and separated residue P that may exist in the whole sample including silt, organic sludge, or hydrosoils. This recognizes that the feedback of soluble P from deposited or suspended material has a real effect upon the kinetics of the aqueous environment.

Table 2

PHOSPHORUS COMPOUNDS CLASSIFIED BY ANALYTICAL METHODOLOGY

Desired P Components	Technique[1]	Incidental P Included[2]
1. Ortho phosphates	No treatment on clear samples	Easily hydrolyzed (a) poly phosphates - (b) organic -P, - (c) Mineral -P, + or -
2. Polyphosphates (2)-(1) = poly P (hydrolyzable)	acid hydrolysis on clear samples, dilute (a) H_2SO_4 (b) HCl heated	(a) ortho-P + (b) organic -P + or - (c) mineral -P + or -
3. Organic phosphorus (3) - (2) + org P (hydrolyzable)	acid + oxidizing hydrolysis on whole sample. dilute (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + (NH_4)_2S_2O_8$ heated	(a) ortho P + (b) poly P + (c) mineral P + or -
4. Soluble phosphorus (preferably classified by clarification method)	clarified liquid following filtration, centrifugation or subsidence	generally includes (a) 1, 2, or 3 (b) particulates not completely separated
5. Insoluble phosphorus (residue from clarification)	Retained residues separated during clarification See (6)	(a) generally includes sorbed or complexed solubles.
6. Total phosphorus	Strong acid + oxidant digestion (a) $H_2SO_4 + HNO_3$ (b) $H_2SO_4 + HNO_3 + HClO_4$ (c) $H_2O_2 + Mg(NO_3)$ fusion	all components in 1, 2, 3, 4, 5 in the whole sample

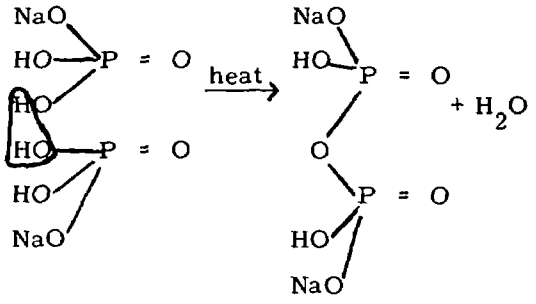
(1) Determinative step by phospho molybdate colorimetric method.

(2) Coding: + quantitative yield
- a small fraction of the amount present
+ or - depends upon the individual chemical and sample history

IV Polyphosphates are of major interest in cleaning agent formulation, as dispersants, and for corrosion control.

A They are prepared by dehydration of ortho phosphates to form products having two or more phosphate derivatives per molecule.

1 The simplest polyphosphate may be prepared as follows



mono sodium ortho phosphate (2) — disodium dihydrogen polyphosphate

2 The general form for producing polyphosphates from mono substituted orthophosphates is

$$n\ (NaH_2PO_4) \xrightarrow[200\text{-}400^\circ C]{heat} (NaPO_3)_n + n\ H_2O$$

3 Di-substituted ortho phosphates or mixtures of substituted ortho phosphates lead to other polyphosphates

$$2Na_2HPO_4 + NaH_2PO_4 \xrightarrow{heat} Na_5P_3O_{10} + 2H_2O$$

disodium hydrogen ortho phosphate + mono sodium di hydrogen ortho phosphate → pentasodium tri-phosphate + water

4 The polyphosphate series usually consist of the polyphosphate anion with a negative charge of 2 to 5. Hydrogen or metals commonly occupy these sites. The polyphosphate can be further dehydrated by heat as long as hydrogen remains. Di or trivalent cations generally produce a more insoluble polyphosphate than the same cation in the form of insoluble ortho phosphate. Insolubility increases with the number of P atoms in the polyphosphate. The "glassy" polyphosphates are a special group with limited solubility that are used to aid corrosion resistance in pipe distribution systems and similar uses.

B Polyphosphates tend to hydrolyze or "revert" to the ortho P form by addition of water. This occurs whenever polyphosphates are found in the aqueous environment.

1 The major factors affecting the rate of reversion of poly to orthophosphates include·

a) Temperature, increased T increases rate

b) pH, lower pH increases rate

c) Enzymes, hydrolase enzymes increase rate

d) Colloidal gels, increase rate

e) Complexing cations and ionic concentration increase rate

f) Concentration of the polyphosphate increases rate

2 Items a, b and c have a large effect upon reversion rate compared with other factors listed. The actual reversion rate is a combination of listed items and other conditions or characteristics.

3 The differences among ortho and ortho + polyphosphates commonly are close to experimental error of the colorimetric test in stabilized surface water samples. A significant difference generally indicates that the sample was obtained relatively close to a source of polyphosphates and was promptly analyzed. This implies that the reversion rate of polyphosphates is much higher than generally believed.

V SAMPLING AND PRESERVATION TECHNIQUES

A Sampling

1 Great care should be exercised to exclude any benthic deposits from water samples.

2 Glass containers should be acid rinsed before use.

3 Certain plastic containers may be used. Possible adsorption of low concentrations of phosphorus should be checked.

4 If a differentiation of phosphorus forms is to be made, filtration should be carried out immediately upon sample collection. A membrane filter of 0.45 μ pore size is recommended for reproducible separations.

B Preservation

1 If at all possible, samples should be analyzed on the day of collection. At best, preservation measures only retard possible changes in the sample.

a Possible physical changes include solubilization, precipitation, absorption on or desorption from suspended matter.

b Possible chemical changes include reversion of poly to ortho P and decomposition of organic or mineral P.

c Possible biological changes include microbial decomposition of organic P and algal or bacterial growth forming organic P.

2 Refrigeration at 4°C is recommended if samples are to be stored. This decreases hydrolysis and reaction rates and also losses due to volatility.

3 Addition of 40 mg $HgCl_2$/liter is recommended for longer storage periods. This chemical limits biological changes.

a $HgCl_2$ is an interference in the analytical procedure if the chloride level is low (See Part VI, B3).

VI THE EPA ANALYTICAL PROCEDURE[6]

A This is a colorimetric determination, specific for orthophosphate. Depending on the nature of the sample and on the type of data sought, the procedure involves two general operations:

1 Conversion of phosphorus forms to soluble orthophosphate (See Fig. 1).

a sulfuric-acid-hydrolysis for polyphosphates, and some organic P compounds,

b persulfate digestion for organic P compounds.

2 The color determination involves reacting dilute solutions of phosphorus with ammonium molybdate and potassium antimonyl tartrate in an acid medium to form an antimony-phosphomolybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the orthophosphate concentration.

Color absorbance is measured at 880 nm and a concentration value obtained using a standard curve.

Reagent preparation and the detailed procedure can be found in the EPA manual.

The methods described there are usable in the 0.01 to 0.5 mg/liter phosphorus range.

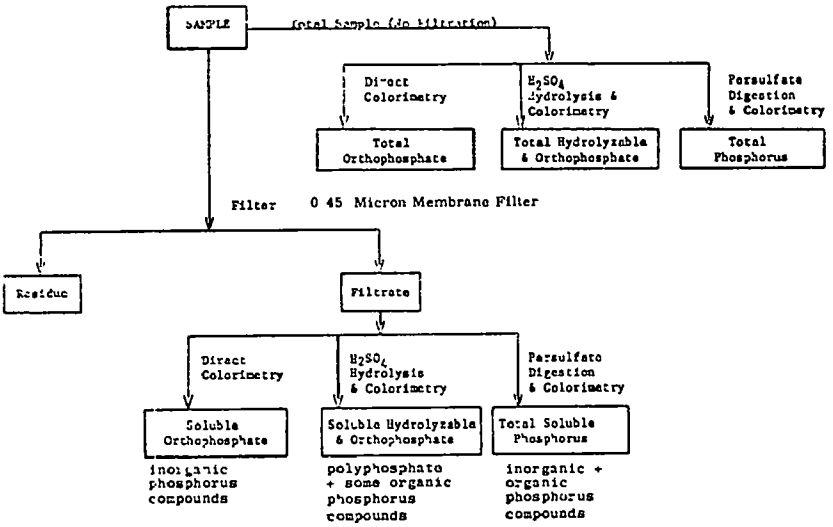


FIGURE 1

ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

B Interferences

1 Erroneous results from contaminated glassware is avoided by cleaning it with hot 1:1 HCl, treating it with procedure reagents and rinsings with distilled water. Preferably this glassware should be used only for the determination of phosphorus and protected from dust during storage. Commercial detergents should never be used.

2 High iron concentrations in samples can precipitate phosphorus.

3 If $HgCl_2$ is used as a preservative, it interferes if the chloride level of the sample is less than 50 mg Cl/liter. Spiking with NaCl is then recommended.

4 Others have reported interference from chlorine, chromium, sulfides, nitrite, tannins, lignin and other minerals and organics at high concentrations.

C Precision and Accuracy[6]

1 Organic phosphate - 33 analysts in 19 laboratories analyzed natural water samples containing exact increments of organic phosphate of 0.110, 0.132, 0.772, and 0.882 mg P/liter.

Standard deviations obtained were 0.033, 0.051, 0.130 and 0.128 respectively.

Accuracy results as bias, mg P/liter were: +0.003, +0.016, +0.023 and - 0.008, respectively.

2 Orthophosphate was determined by 26 analysts in 16 laboratories using samples containing orthophosphate in amounts of 0.029, 0.038, 0.335 and 0.383 mg P/liter.

Standard deviations obtained were 0.010, 0.008, 0.018 and 0.023 respectively.

Accuracy results as bias, mg P/liter were -0.001, -0.002, -0.009 and -0.007 respectively.

VII VARIABLES IN THE COLORIMETRIC PROCEDURE

Several important variables affect formation of the yellow heteropoly acid and its reduced form, molybdenum blue, in the colorimetric test for P.

A Acid Concentration during color development is critical. Figure 2 shows that color will appear in a sample containing no phosphate if the acid concentration is low. Interfering color is negligible when the normality with respect to H_2SO_4 approaches 0.4.

1 Acid normality during color development of 0.3 to slightly more than 0.4 is feasible for use. It is preferable to control acidity carefully and to seek a normality closer to the higher limits of the acceptable range.

2 It is essential to add the acid and molybdate as one solution.

3 The aliquot of sample must be neutralized prior to adding the color reagent.

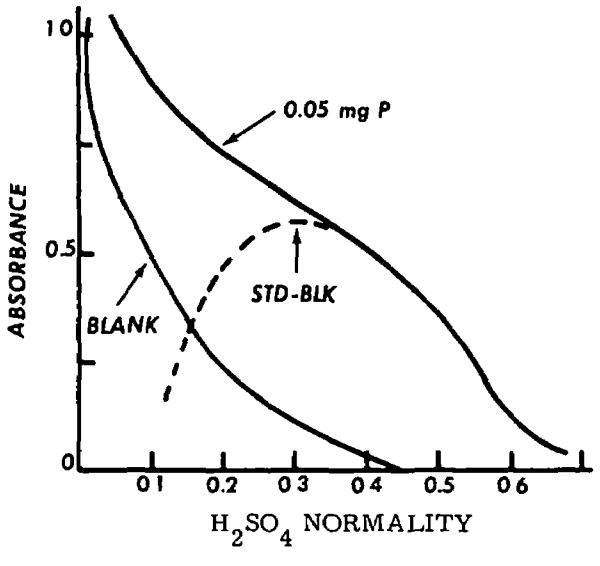


Figure 2

0-PHOSPHATE COLOR VS ACIDITY

B Choice of Reductant - Reagent stability, effective reduction and freedom from deleterious side effects are the bases for reductant selection. Several reductants have been used effectively. Ascorbic acid reduction is highly effective in both marine and fresh water. It is the reductant specified in the EPA method.

C Temperature affects the rate of color formation. Blank, standards, and samples must be adjusted to $\pm 1^{\circ}C$, preferably room temperature, before addition of the acid molybdate reagent.

D Time for Color Development must be specified and consistent. After addition of reductant, the blue color develops rapidly for 10 minutes then fades gradually after 12 minutes.

VIII DETERMINATION OF TOTAL PHOSPHORUS

A Determination of total phosphorus content involves omission of any filtration procedure and using the acid-hydrolysis and persulfate treatments to convert all phosphorus forms to the test-sensitive orthophosphate form.

B Determining total phosphorus content yields the most meaningful data since the various forms of phosphorus may change from one form to another in a short period of time. (See part V, B1)

IX DEVELOPMENT OF A STANDARD PROCEDURE

Phosphorus analysis received intensive investigation, coordination and validation of methods is more difficult than changing technique.

A Part of the problem in methods arose because of changes in analytical objectives such as:

1 Methods suitable to gather "survey" information may not be adequate for "standards".

2 Methods acceptable for water are not necessarily effective in the presence of significant mineral and organic interference characteristic of hydrosoils, marine samples, organic sludges and benthic deposits.

3 Interest has been centered on "fresh" water, it was essential to extend them for marine waters.

4 Instrumentation and automation have required adaptation of methodology.

B Analysts have tended to work on their own special problems. If the method apparently served their situations, it was used. Each has a "favorite" scheme that may be quite effective but progress toward widespread application of "one" method has been slow. Consequently, many methods are available. Reagent acidity, Mo content, reductant and separation techniques are the major variables.

C At the present time there is not sufficient data to warrant EPA endorsement of the P procedure for sediment-type samples, sludges, algal blooms, etc. Following is a procedure (not included in the EPA manual) which is useful when solids are present in samples.

1 If sample contains large particles, grind and emulsify solids in a blender.

2 Transfer 50 ml sample, or aliquot diluted to 50 ml, into a 250 ml Erlenmeyer flask

3 Add 6.0 ml of 18N H_2SO_4, 5 ml concentrated HNO_3, 2 berl saddles and digest on hot plate

4 Digest until the disappearance of nitric acid fumes and the appearance of white SO_3 fumes. Continue digestion for approximately 5 minutes. Cool before proceeding with Step 5.

5 Add 2 ml of HNO_3-$HClO_4$ mixture and 5 ml concentrated HNO_3. Continue digestion until all of the nitric acid is driven off and dense fumes of perchloric evolve. Perchloric acid requires dilution with sulfuric acid and prior destruction of most organics for safety.

6 Cool. Add approximately 40 ml distilled water and transfer to 100 ml volumetric flask.

7 Add 2-3 drops of phenolphthalein and concentrated ammonium hydroxide until a pink color is seen. Then discharge the pink color with the strong sulfuric acid. It is advisable to add an equivalent amount of salt formed during neutralization of digested samples to the calibration standards to equalize salt content during color development.

8 Determine orthophosphate according to the usual color procedure.

ACKNOWLEDGMENT:

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This outline was prepared by Audrey E. Donahue, Chemist, National Training Center, DTTB, MDS, WPQ EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR PHOSPHORUS

I OBJECTIVE

The concentration of phosphorus (including available mineral, organic, poly and ortho phosphate) in the whole sample from a wastewater treatment plant is sought.

II METHOD SUMMARY:

A The EPA Official Method is used. Certain non-official procedures are included to adapt this exercise for group participation and according to sample characteristics. Class use of 1 cm cells necessitates a stronger reference solution. Omitting the digestion of reference standards also deviates from the official method.

B Mixed phosphorus forms in the sample will be converted into ortho phosphate by digestion at boiling temperature for 30 minutes in the presence of sulfuric acid and the oxidant, ammonium persulfate.

Excess acid will be neutralized, the sample diluted to its original volume and aliquots diluted to 50 ml for comparison with a series of reference sample dilutions and a reagent blank. Both sample and reference dilutions are adjusted to the same volume and mixed reagent added at the same time for comparative color development.

All solutions used in the procedure should be at room temperature or within $\pm 1^{\circ}C$ of the same temperature.

C You may encounter 0.5 to 15 mg P/1 in a sample of the type provided. Several small sample aliquots diluted to volume should be prepared along with your reference series to obtain at least one sample aliquot within the readable absorbance range of the references. Suggested sample aliquots in the procedure will give at least one readable result for a sample range of 0.1 to 20 mg P/liter

III ANALYTICAL PROCEDURE

A The conversion of phosphorus forms to orthophosphate

1 Measure 50 ml of the well mixed sample provided into one of the 125 ml erlenmeyer flasks.

2 Add one of the berl saddles to the sample aliquot.

3 Add one ml or 20 drops of strong acid to the sample aliquot.

4 Add 1 scoopful or approximately 0.4 g of dry powder ammonium persulfate, $(NH_4)_2S_2O_8$, to the sample aliquot.

5 Mix and place the sample flask on a preheated hot plate. Adjust heat to obtain a gentle boiling rate for 30 minutes. Add distilled water if necessary to prevent the sample volume from evaporating to a volume of less than 10 ml.

6 After 30 minutes boiling remove the digestion flask from the hot plate and cool it. If you cannot complete the analysis at this time, hold it at this stage after covering the flask with aluminum foil or an inverted beaker to protect it from contamination

B Colorimetric Determination of Orthophosphate

1 Add 1 drop of phenolphthalein solution. Add NaOH solution dropwise to neutralize excess acid until the mixed digestate develops a red color. Add one drop of strong acid to eliminate the indicator color.

2 Adjust the volume of the digestate to its original volume of 50 ml by addition of distilled water. Mix.

3 Identify each of the seven remaining 125 ml erlenmeyer flasks as follows: Blank, 10-R, 20-R, 40-R, 0.5-S, 2.0-S and 10.0-S.

4 Add 50 ml of distilled water to the blank. Add 10 ml of reference solution + 40 ml of distilled water to the 10-R flask. Add appropriate amounts of reference solution or sample as indicated to the remaining flasks, plus enough distilled water to make a final volume of 50 ml in each flask. (It will not be necessary to add the indicator and adjust pH with NaOH or acid because all mixtures are approximately neutral at this stage. If you are not sure - check it).

5 Add 8 ml of mixed reagent to each of the reference and sample aliquots and to the 50 ml blank. Mix each one after addition.

6 After a 10 minute color development time (and not longer than 30 minutes), measure absorbancy of the color produced versus the reagent blank. (Adjust the instrument to zero absorbance (A) with the reagent blank in the light path).
Record results on the following data table:

Item	ml R or S	μg P/50 ml	mg P/1	A
Blank	0	0	0	0
10-R	10-R	12.5	0.25	
20-R	20-R	25.0	0.50	
40-R	40-R	50.0	1.00	
0.5-S	0.5-S			
2.0-S	2.0-S			
10 0-S	10.0-S			

These data items require measurement of the absorbancy produced versus the reagent blank (adjust instrument to zero A for the blank) to estimate the mg P/1 in the sample. Note that both the μg P/50 ml and the equivalent mg P/1 are listed in separate columns in the data record. The μg column is for check purposes to avoid calculation errors. Data is reported as mg P/liter

IV EVALUATING PHOSPHORUS CONCENTRATION

A Procedure

1 Consulting the data table for a range of values, mark absorbance vs mg P/liter scales on the coordinate paper provided. Also, mark the μg P/50 ml values below the mg P/liter scale values.

2 Plot the data for the Reference samples and draw a line of best fit

3 Plot the data for the Sample aliquots and choose the valid result(s).

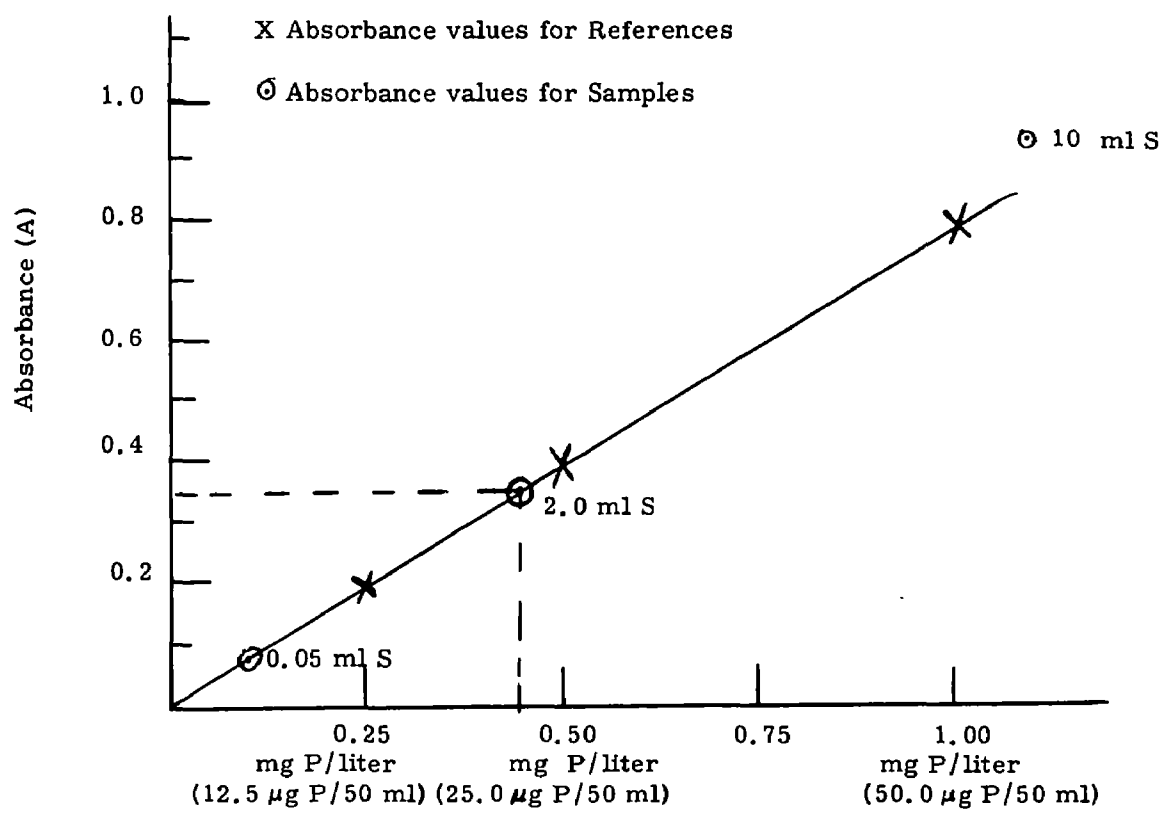
4 Evaluate the valid Sample points on the curve as mg P/liter.

5 Check calculations using the μg P/50 ml scale.

6 An example of this whole procedure follows.

B The Standard Curve

Reference and sample values have been plotted on this graph:



In this example, there is only one sample volume that falls on the most readable range of the instrument and within the calibrated range of the Absorbance plot - the 2.0 ml sample. The other two sample volumes gave invalid results.

C Using the Standard Curve as shown in B above, the next step is to find mg P/liter of the sample when absorbance on the 2 ml sample diluted to 50 ml was 0.35: Draw a dashed horizontal line from the absorbance scale reading of 0.35 to the calibration line. Draw a vertical line from that intersection to the concentration scale. This reads approximately 0.45 mg P/liter for the diluted aliquot color. Since this sample was diluted from 2 to 50 ml, the original sample concentration was $0.45 \times \frac{50}{2} = 0.45 \times 25$ or 11.25 mg P/1.

D Checking Calculations:

With the same observed values and manipulation but using the μg P/50 ml scale, the vertical intercept falls at about 22 μg P/50 ml in the sample. For a sample containing 22 μg P in the diluted 2 ml aliquot, the volume correction becomes $22 \times \frac{1000}{2} = 22 \times 500 = 11000$ μg P/1 or 11.00 mg P/1.

It is evident that the estimation of intercept positions on the small scales with infrequently marked positions yields a lack of precise agreement for the two independent calculations. The general agreement, however, does eliminate the question of a possible serious dilution error which frequently plagues the results.

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, DTTB, MDS, WPQ EPA, Cincinnati, OH 45268.

VII SOLIDS RELATIONSHIPS IN POLLUTED WATER - GREASE AND OIL

Solids control is a prime necessity and the major problem in wastewater treatment. Solids are intimately related to oxygen demand and feed back or recycle of polluting materials depending upon the degree of contact with the aqueous system. Solids separation from water is difficult and comprises a major part of treatment dynamics. Stabilization and disposal poses a two pronged difficulty of what to do with it and where to put it to minimize feedback.

Contents of Section VII

	Outline Number
Solids Relations in Polluted Water	35
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Laboratory Procedure for the Determination of Total Solids	37
Laboratory Procedure for Non-filterable (Suspended) Solids	38
Laboratory Procedure for Filterable (Dissolved) Solids	39
Laboratory Procedure for Volatile Solids	40
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SOLIDS RELATIONS IN POLLUTED WATER

I MPN, oxygen demand, and solids have been major water pollution control criteria for many years. This discussion is concerned primarily with solids and their interrelations with oxygen demand.

A Engineered treatment or surface water self-purification depends upon:

1 The conversion of soluble or colloidal contaminants into agglomerated masses that may be separated from the water.

2 Oxidation of putrescible components into stable degradation products.

3 Item 1 is the major concern in most treatment systems because item 2 requires a greater investment in time, manpower and capital costs.

B Stress on oxygen demand removal frequently results in an unduely small amount of attention to the contribution of solids in the oxygen demand picture.

1 Oxygen demand formulations generally are specified to be applicable in the absence of significant deposition.

2 Increasing impoundment, tidal estuaries, and incomplete solids removal, generally ensure that solids deposition will be significant.

3 The BOD test stresses the fraction of oxygen demand that is exerted relatively rapidly. It includes only the fraction of unstable material that is exerted under test conditions - usually short term.

4 Contributions of a bed load of solids to oxygen demand frequently are incompletely recognized because they have a more local effect, are difficult to measure, tend to move, and are incompletely understood.

II A given wastewater may have several forms of solids in changeable proportions with time and conditions.

A Solids may be classified among the charted forms according to biological, chemical or physical properties. It is not possible to precisely classify a given material into any one form because they usually are mixtures that may include or be converted into other forms.

B Interrelationships are indicated by diagram in Figure 1. Some changes occur more readily than others.

1 Settleable solids generally consist of a mixture of organic, inorganic, entrained dissolved or colloidal solids with living and dead organisms.

a They may be hydrolyzed into smaller molecules to acquire colloidal or dissolved characteristics.

b They may be converted into a larger fraction of living material or vice versa.

2 Colloidal solids also are likely to be a mixture of organic and inorganic materials, living or dead containing associated dissolved materials.

a They are likely to agglomerate to form settleable masses with time or changing conditions.

b Chemical reactions may solubilize the colloidal masses for recombination into other forms.

3 Dissolved solids are most readily available of all forms for biological, chemical or physical conversion.

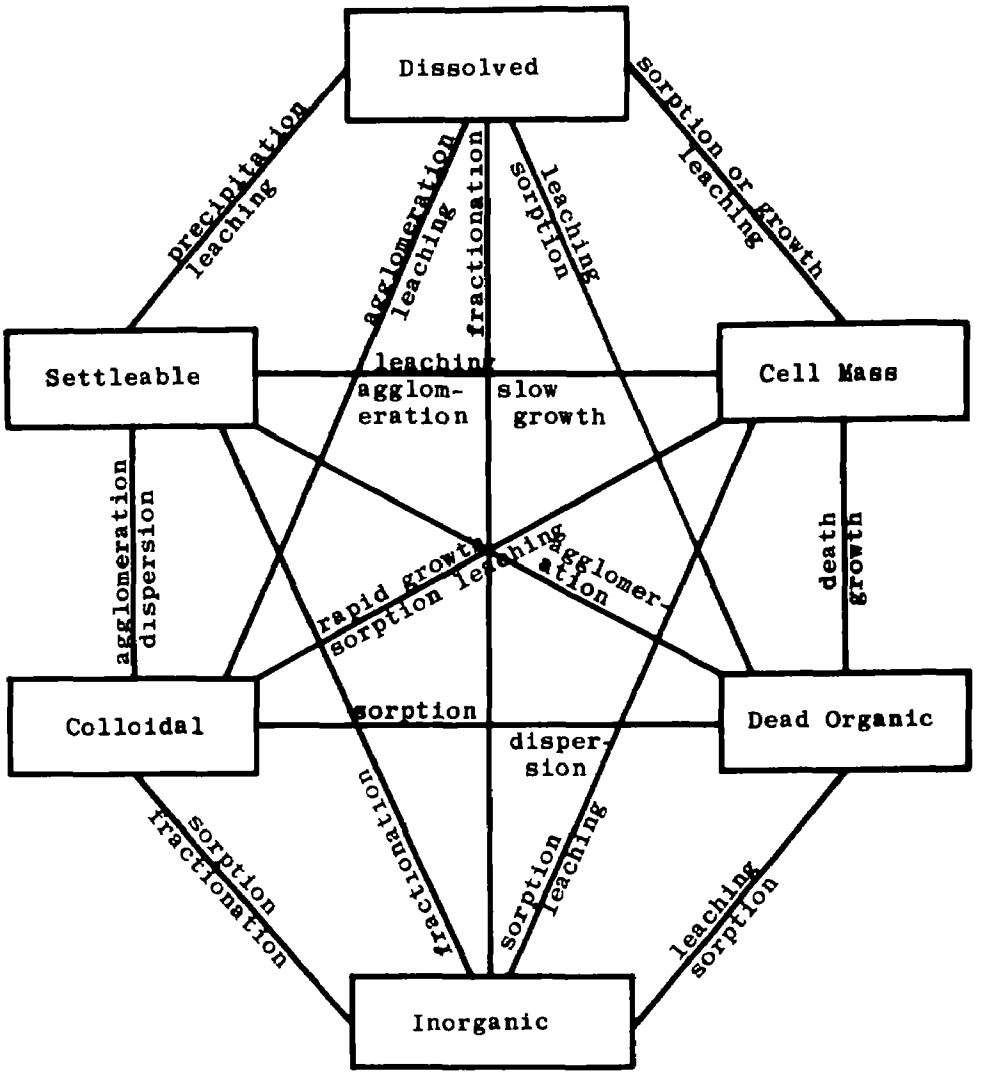

Figure 1. SOLIDS INTERRELATIONSHIPS IN WATER

a They may be assimilated into cell mass to become colloidal during a state of rapid growth or form settleable masses during slow growth. Surface phenomena are likely to encourage inclusion of other forms of solids with cell mass.

C Stabilization occurs with each conversion because energy is expended with each change.

1 Biological changes involve oxidation to obtain enough energy to synthesize cells.

a Products consist of cell mass and degradation products.

b Oxidation tends toward production of CO_2, H_2O, NO_3^-, SO_4^{-2}etc. CO_2 has limited water solubility and partially leaves the aqueous environment. More soluble constituents tend to remain with it for recycle.

c Biological residues tend to recycle. Eventually the mass consists of relatively inert and largely insoluble residues. These make up the

bed load that may decompose at a rate of less than 1% per day, but accumulates in mass until it becomes dominant in water pollution control activities.

III Treatment is an engineered operation designed to utilize events in surface water self-purification in a smaller package in terms of space and time. Effective treatment presupposes oxidation either in the plant or in facilities to minimize feedback of solids components to the aqueous environment.

A Primary treatment consists of a separation of floatable or settleable solids and removal from the used water.

1 Prompt removal is essential to minimize return of solubilized or leached materials from the sludge mass.

2 Sludge and scum are highly putrescible and difficult to drain.

3 Subsequent treatment prior to disposal serves to enhance drainability, reduce solids or volume for burial or burning.

B Secondary treatment generally involves some form of aerobic biological activity to oxidize part of the colloidal and dissolved contaminants and convert most of the remainder into a settleable sludge.

1 Aerobic systems favor assimilation of nutrients into cell mass at the expense of part of the available energy represented by oxidation to products such as CO_2 and water.

2 Cell mass and intermediate degradation products are only partially stabilized and tend to recycle with death and decay.

3 Rapid growth of cells tends to produce sludges that are highly hydrated and thin.

4 A compromise must be reached to produce a favorable balance among separation of solids and feedback of lysed materials.

a Cell growth continues as long as essential nutrients are available.

b Under limiting nutrient conditions the population may show a relatively low rate overall dieoff but variety is changing.

c Species most favored by the new conditions tend to grow while others die, lyse and release part of their stored nutrients for subsequent use.

C Anaerobic digestion of solids separated during treatment is one process used to increase solids stability and drainability while reducing total volume or mass for disposal.

1 Growth of cell mass is relatively slow under anaerobic conditions while hydrolytic cleavage is relatively large in comparison to that during aerobic metabolism.

a Feedback to the aqueous environment represents a significant fraction of the input in the form of ammonia, colloidal solids, low molecular weight acids, and other products.

b Mass of the sludge is reduced by the fraction of methane, carbon dioxide, and other gases produced in process.

c Remaining solids tend to be more concentrated, are lower in putrescibility and give up their water more readily.

2 The liquid fraction of the products remaining after anaerobic digestion contain nutrients, oxygen demand, solids, and malodorous constituents that are objectionable in surface waters if released without further aerobic stabilization.

3 Digester liquids are much more concentrated than raw sewage and nutritionally unfavorable, hence they are difficult to treat and tend to shock aerobic treatment processes.

D Suitable disposal of solids resulting from treatment operations takes various forms of which the most desirable is to oxidize it completely to gaseous products of oxidation or inert ash. The objective is to limit feedback into the aqueous environment and delay the charted interchanges.

1 Deposition of sludge as a cover on crop land is effective providing surface drainage is properly designed to limit washoff.

2 Burial in areas above the flood plain provides a suitable disposal.

3 Incineration under controlled conditions to produce complete burning of organics is preferable.

a Incineration of digested sludge implies substantial feedback in process.

b Incineration of freshly formed sludge is difficult because of drainability and concentration problems. It is preferable from the standpoint of maximum destruction of organic pollutants and minimum feedback in process. Current investigations are directed toward improving feasibility of this means of disposal of solids.

IV ANALYTICAL PROCEDURES

A The Analytical Quality Control Laboratory, Office of Water Programs, Environmental Protection Agency has published a manual titled, Methods for Chemical Analysis of Water and Wastes, 1971.

B In this manual solids are classified into four groups:

1 Total solids
2 Non-filterable (suspended) solids
3 Filterable (dissolved) solids
4 Volatile solids

C The procedure for the determination of 1, 2, and 3 above is given in the Analytical Quality Control Laboratory Manual.

Standard Methods for the Examination of Water and Wastewater, 13th Ed., page 538, Method 224D (1971) is the reference cited by the Analytical Quality Control Laboratory Manual

This outline was prepared by F. J. Ludzack, Chemist, National Training Center, Water Programs Operations, EPA, Cincinnati, OH 45268.

DETERMINATION OF SUSPENDED SOLIDS

I Wastewater stabilization includes the conversion of solid, liquid, or gaseous pollutants to (a) materials at a higher oxidation state or (b) to a more removable form. Both are interrelated, but (b) is favored during conventional treatment.

A Economics of treatment are more favorable when pollutants can be removed and treated in more concentrated form.

B Pollutant characteristics guide selection of operations applicable for contaminant conversion to suspended solids.

C Control of suspended solids removal is a major factor in treatment efficiency. Incomplete removal leads to feedback of nutrients from the unstable solids during later stages of stabilization.

II Characterization of the various forms of solids that may exist or be formed during stabilization of polluting materials necessarily depends upon operational factors. These are usually conditional, but their appreciation is essential.

A Dissolved solids include solid, liquid, or gaseous materials that are dispersed in the aqueous system in molecular or ionic form. The "solution" may be colored but it is likely to be clear unless the concentration of some items in the mixture approaches their solubility limits.

B Colloidal solids include a stage or condition of suspension where the solubility limit has been reached and the ionic or molecular dispersion tends to agglomerate to form "clumps" of insoluble materials or particulates. These particulates are large enough to scatter light and form cloudy or turbid suspensions but are not large enough to subside without further agglomeration.

C Suspended solids include the colloidally dispersed solids and the suspended solids that have agglomerated to form particulates that will subside or settle under quiescent conditions to form a solids concentrate or sludge. Size is a major criterion but density, shape, and interface characteristics among the liquid and solid are important factors.

D Total solids include A, B and C that may be present in the whole sample.

E Solids interrelationships are discussed in a prior outline. Determination of suspended solids is the object of this one.

III Sources of suspended solids include both Natural and man-made operations.

A Rainfall washes particulate material from the air and from the surfaces of forest, farmland, undeveloped land and urban or industrial centers.

B Municipal water supplies are controlled to maintain negligible suspended solids. Some may be formed in distribution systems.

C Groundwaters may contain dissolved solids that form suspended solids upon exposure to air or oxidation.

D Wastewaters are likely to contain varied suspended solids or components that may be converted to suspended solids by biological, chemical or physical transformations. Their nature varies with the origin such as household, commercial, industrial process, mining, washing, or other operations.

E Breaks below the groundwater level in partially filled sewers allow infiltration of suspended materials characteristic of the surrounding soils and water movement in them.

F Combined sewer systems, either intentional or otherwise, permit surface drainage from roofs, streets, parking lots, lawns, etc. to enter sanitary sewers

IV The determination of suspended solids relate to operational factors determining the particular result obtained.

A A fence of wire or bars of appropriate sized openings may serve as a barrier to passage of horses and cattle but fail to stop cats or dogs.

B Smaller sized particles are commonly separated by filtration, centrifugation, subsidence, or other hydraulic gradation.

1 Roots, branches, rags, etc., usually are separated by bar racks or screens with relatively large openings.

2 Grit, sand and small, but heavy, particles generally are separated hydraulically in chambers allowing 1 to 3 minutes detention time.

3 Filtration is the most commonly applied technique for determination of the relatively low density particulates that tend to remain in aqueous suspension largely as a result of turbulence. Since pore sizes of filters differ, it is essential to specify the media used to interpret results.

V Standard Methods[1] refers to residues from whole sample evaporation as total residue, suspended solids as the non-filterable residue retained on or in the filter and dissolved residue as that appearing in the filtrate.

A Many factors influence the results obtained during suspended solids determinations such as

1 Chemical and physical nature of the solids

2 Sampling techniques

3 Pore size of the filter

A membrane filter of 0.5μ pore size may retain particulates that are not retained on paper.

4 Area, condition, and consistency of filter media.

5 Technique and manipulative care in drying, weighing, storage, transfer, and temperature.

B Standard Methods includes three methods for non-filterable residue or suspended solids.

1 Gooch crucible

2 Membrane filter[2, 3]

3 Buechner funnel-aluminum dish (aerator or sludge suspended solids).

C A spectrophotometric method was described by Krawczyk and Gonglewski[4]. The importance of homogenization to improve sampling uniformity was stressed.

D Chanin, et. al.[5] described the use of glass fiber media for the determination of suspended solids.

VI Precision, sensitivity, and applicability of various methods for determination of suspended solids are indicated in the cited references.

A Table I indicates data from several references on precision of he Gooch crucible, asbestos fiber mat determination.

B Table 2 presents data published on the precision of the Gooch crucible, glass fiber determination.

C Smith and Greenberg[7] compared results of 5 replicates each of eight samples (influents, primary effluents, final effluents and industrial waste samples) by five different methods of determination including·

1 Gooch crucible, asbestos mat

2 Gooch crucible, glass fiber mat

3 Buechner funnel, wire cloth, glass fiber mat

TABLE 1

GOOCH CRUCIBLE
ASBESTOS MAT

Range of Values mg/l	Ref	Number of Replicates	Mean mg/l	Standard Deviation mg/l + or -	Sample Size Filtered ml
170-308	6	10	224	47	25
218-288	6	10	242	24	25
- -	7	5	360	13	50
- -	7	5	80	25	100
- -	7	5	104	8	100
- -	7	5	152	6	100
- -	7	5	1,023	79	25
- -	7	5	95	7	100
- -	7	5	297	27	50
- -	7	5	96	2	200
14-26	a	10	17	4	100
190-213	(d) 4	10	203	7	100
100-132	(d) 4	10	122	9	100
52-66	(d) 4	10	59	4	100

a Private communication, Lake Huron Program Office Methods Manual

TABLE 2

GOOCH CRUCIBLE
FIBER FILTER

Range of Values mg/l	Ref.	Number of Replicates	Mean mg/l	Standard Deviation mg/l + or -	Sample Size Filtered ml
13-22	a	9	18	3	100
20-24	a	9	22	1	100
60-72	a	10	67	4	100
- -	6	5	1086	14	25
- -	6	5	361	47	50
- -	6	5	306	15	50
- -	6	5	146	3	100
- -	6	5	104	1	100
- -	6	5	100	1	100
- -	6	5	84	3	100
- -	6	5	79	5	100
104-436	5	12	296	119	25
60-196	5	14	111	38	25
8-152	5	11	100	54	25
56-100	5	9	76	17	50

4 Membrane filter 0.4 to 0.5 μ pore size

5 Gooch crucible, glass fiber mat, celite filter aid

a Their conclusions based upon carefully controlled technique indicated that yield and precision were not statistically different among the five tested methods.

b Determining factors other than in (a) included· manipulation time, required skills, speed of filtration, consistence of mat quality, equipment cost and availability.

c Methods 2 and 3 more frequently gave a lower coefficient of variation among those tested and were more favorable with respect to (b).

VII Section VI indicates that good results are possible among any of the tested methods but it was easier to obtain precise results with certain methods. The Gooch Crucible-asbestos mat requires a high degree of skill and manipulative time to provide consistent filtration. The membrane filter tends toward slow filtration, sampling or manipulative difficulties, and is more expensive.

A Certain precautions essential to consistent results with Gooch crucible-asbestos mats include

1 A thin suspension of asbestos in distilled water is essential. A walnut-sized wad of fiber/1 is desirable.

2 Shake the suspension vigorously, allow it to settle 10-20 seconds. Pour the suspension into another container and discard asbestos slivers.

3 A more uniform suspension of asbestos is achieved after 10-30 seconds treatment in a Waring blendor or equivalent.

4 Allow to stand overnight or for a sufficient time to form an asbestos fiber concentrate on the bottom. Discard any milky fines in suspension and rewash, if necessary until a reasonably clear supernatant is obtained.

5 Insert the clean gooch crucible (30 ml or other selected size and diameter) into a Walters crucible adapter fitted to an 0.5 or 1 1 suction flask.

6 Shake the asbestos suspension vigorously, add the mixed suspension to the crucible until it is 1/2 to 2/3 full.

7 Allow the fiber to "mat" for 1/2 to 2 minutes, then apply vacuum slowly. If a hole forms in the mat the vacuum was applied too soon and too rapidly.

8 Add additional suspension (vacuum off) and apply vacuum to withdraw water and form a mat of 2-3 mm. Waiting period before vacuum application is not essential once a mat has formed. Water penetration rate is an effective means to judge mat quality. Good filtering is obtained if the exit stream from the Walters adapter is continuous but slow enough to be slightly off axis.

9 Wash the mat with successive portions of distilled water (200-250 ml) to clear the mat of fines.

10 Remove tne crucible, wipe the outside with clean tissue or cloth and dry and weigh.

B Precautions and aids for glass fiber mat usage

1 Gooch crucibles employing glass fiber mats of 2.4 cm diameter may encourage slow filtration, different shaped units such as the asphalt filtration crucible are designed to use larger diameter mats which may be desirable for aerator or sludge solids tests.

2 It is advisable to insert glass fiber mats with the smooth side up. A second mat may be required if the prewetting rinse

suggests unusually rapid water passage. Careful placement of the mat is essential.

C Membrane filter precautions include

1 The filters are fragile and must be carefully handled to prevent damage during use.

2 Prewashing in distilled water for 24 hours helps to remove glycerol or other softening agent that is likely to be eluted by the sample.

3 Filters tend to curl on drying. An individual desiccator is described[3] that helps to control drying at ambient temperature and curling in process.

D Beuchner funnel suspended solids determinations include the (a) aluminum dish-paper, (b) wire cloth-glass fiber and (c) paper filtration. The aluminum dish is designed to support the paper and prevent losses during drying. The wire cloth promotes more rapid filtration and support. When neither supports are used, the paper or glass fiber may be folded once with the solids inside during drying.

1 Buechner funnel filtrations always present the problem of solids adherence to funnel sidewalls instead of the filter media. Solids losses due to incomplete transfer to the filter may be minimized but not eliminated. A square ended spatula aids transfer. It is advisable to adjust sample size to yield > 100 mg of non-filterable residue to reduce percentage error for Buechner funnel tests.

E General precautions applicable for precise results on suspended solids include:

1 Filters or crucibles must be clearly identified, dried, generally at 103°C, and stored in a desiccator until weighed and used.

2 The balance should contain a desiccant to control atmospheric moisture during weighing. This should be changed regularly and the doors opened only as necessary.

3 Filters may be dried in a desiccator (preferably 24 hours).

4 For rapid, control work with Buechner funnel-paper filtering, the papers may be oven dried and equilibrated with room moisture for 10 minutes to reduce changes due to rapid moisture pickup after oven drying.

5 Manufactured filters must be handled carefully with forceps, polished if necessary to prevent puncture or damage to the filter surface.

6 Place the filter media carefully in the crucible covering all openings in the support.

a Special care must be used for membrane filter holders to clamp the filter in place firmly without rupture or displacement.

7 Wet the filter media with distilled water preferably directed at the center of the mat first. Allow the mat to become thoroughly wet before applying vacuum then prewash the filter with about 100 ml of distilled water.

8 With the vacuum on, add the selected aliquot of sample.

a The sample volume should be large enough to minimize percentage errors during weighing. Less precise technique requires more weight difference between the pre and post filtration weights. Three to five mg weight difference is adequate for Gooch crucible technique, > 100 mg required for Buechner funnel tests. Homogenize the sample if sampling uniformity is questionable.

9 Rinse the non-filterable residue with distilled water to wash sidewall solids down onto the filter mat and to minimize occluded solubles in the solids and filter mat.

10 Dry and weigh under the same conditions as used for preweighing.

11 Final weight - Initial weight (mg)

$$\times \frac{1000}{\text{ml sample aliquot}} =$$

suspended solids in mg/l
Results are usually expressed in mg/l (g X 1000).

12 It is not feasible to dry suspended solids to a constant weight because of the interchange of moisture, volatiles or changes due to oxidation in process. Weight must be defined in terms of a routine that minimizes weight change errors.

13 It is not advisable to pre-ignite glass fiber mats for the determination of sample volatile solids as recommended for asbestos fiber mats. Glass fiber generally will soften at about 400° C but is likely to tolerate ignition at 550 to 600° C without fusion. It is advisable to check a given supply of fiber glass circles by igniting 5 or 6 under carefully controlled ignition temperatures and time sequence. If the glass fiber mats are uniform in weight and composition their weight loss on ignition will be comparable. This amount of ignition loss may be subtracted from the ignition loss for samples to obtain a corrected value for sample volatile suspended solids (VSS) if the loss is acceptably consistent.

14 The aluminum dish may be fabricated from an aluminum milk dish (listed in apparatus supply catalogues) by punching or drilling 1/16 inch holes in the bottom comparable to those of the Buechner funnel into which it is inserted. The aluminum dish and filter may be weighed before and after sample filtration and drying. An O ring of silicone M rubber slightly smaller than the dish may be used to provide a vacuum seal with the Buechner funnel. Sample contents must not overfill the dish during manipulation to avoid loss of solids by adherence to the funnel surfaces.

VIII The FWPCA "Methods for Chemical Analysis of Water and Wastes", method for non-filterable solids (Storet number 00530) prescribes filtration through glass fiber media with drying at 105° C. (1969, in press)

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LABORATORY PROCEDURE FOR TOTAL SOLIDS

I INTRODUCTION

A This procedure was excerpted from methods for Chemical Analysis of Water and Wastes, 1971, Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory

B The procedure is applicable to surface and saline waters, domestic and industrial wastes.

C The practical range of the determination is 10-30000 mg/l.

D Nonhomogenous materials (large floating particles or submerged agglomerates) should be excluded from the sample. Floating grease and oil should be included in the sample and dispersed in a blender before measuring the aliquot.

E Samples should be analyzed as soon as possible.

II EQUIPMENT

A Porcelain, Vycor, or platinum evaporating dishes, 100 ml capacity; smaller sizes may be used as required.

B Muffle furnace, $550 \pm 50^{\circ}C$

C Drying oven, $103 - 105^{\circ}C$

D Desiccator

E Analytical balance

F Steam bath

III PROCEDURE

A Heat the clean evaporating dish in a muffle furnace at $550 \pm 50^{\circ}C$ for 1 hour

B Cool the dish in a desiccator.

C Weigh the dish on an analytical balance.

D Store the dish in the desiccator and weigh just before use.

E Shake the sample container vigorously.

F Measure 100 ml of the well mixed sample in a graduated cylinder. (At least 25 mg of residue should be obtained, less volume of sample may be used if the sample appears to be high in solids content. If it is low in solids content, more sample may be added to the dish after drying).

G Rapidly, but without spilling, pour the sample into the evaporating dish.

H Dry the sample on a steam bath, or at $98^{\circ}C$ (to prevent boiling and splattering) in the oven.

I Dry the evaporated sample in the oven at $103 - 105^{\circ}C$ for at least one hour.

J Cool the dish in the desiccator and then weigh it.

K Repeat the heating at $103 - 105^{\circ}C$, cooling and weighing until the weight loss is less than 4% of the previous weight, or 0.5 mg, whichever is less.

IV CALCULATIONS

$$\text{mg total solids/l} = \frac{(\text{wt dish + residue})^* - (\text{wt dish})^* \times 1000 \times 1000}{\text{ml of sample}}$$

* in grams

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LABORATORY PROCEDURE FOR NONFILTERABLE (SUSPENDED) SOLIDS

I INTRODUCTION

A This procedure was excerpted from Methods for Chemical Analysis of Water and Wastes, 1971, Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory.

B The procedure is applicable to surface and saline waters, domestic and industrial wastes.

C The practical range of the determination is 20 - 20000 mg/l.

D All nonhomogeneous particulates (such as leaves, sticks, fish and lumps of fecal matter) should be excluded from the sample.

E Sample preservation is not practical; the analysis should be done as soon as possible.

II EQUIPMENT

A Glass fiber filter discs, 4.7 cm or 2.2cm, without organic binder. Reeve Angel type 984H, 934H, Gelman type A, or equivalent.

B Membrane filter funnel (for use with the 4.7 cm disc), or

C 25 ml Gooch crucible (for use with the 2.2 cm disc).

D Gooch crucible adapter.

E 500 ml suction flask

F Drying oven, 103 - 105°C

G Desiccator

H Analytical balance, 200 g capacity, capability of weighing to 0.1 mg.

III PROCEDURE

A Assemble the filtering apparatus and suction flask (either the 2.2 cm disc, Gooch crucible and adapter, or the 4.7 cm disc and membrane filter funnel).

B Apply suction to the flask and wash the disc with three successive 20 ml portions of distilled water.

C Continue the suction until all water has passed through the disc.

D Remove the Gooch crucible plus 2.2 cm disc and dry in the oven at 103 - 105°C for one hour, or,

E Remove the 4.7 cm disc from the membrane filter funnel and dry in the oven at 103 - 105°C for one hour. In D or E, if the disc is not to be used immediately, store it in the desiccator.

F Weigh the 4.7 cm disc or 2.2 cm disc plus Gooch crucible just before use.

G Assemble the filtering apparatus.

H Apply suction to the flask.

I Shake the sample container vigorously.

J Measure 100 ml of the well mixed sample in a 100 ml graduated cylinder. (If the sample appears to be low in solids, a larger volume may be used).

K Rapidly, but without spilling, pour the sample into the funnel or crucible.

L Continue the suction until all of the water has passed through the disc.

M Remove the 4.7 cm disc from the funnel and dry in the oven at 103 - 105°C to constant weight, or

N Remove the Gooch crucible plus 2.2 cm disc and dry in the oven at 103 - 105°C to constant weight.

Note: Drying to constant weight refers to the process of:

1) drying the disc (or disc plus crucible) at 103 - 105°C, 2) cooling in the desiccator, 3) weighing, 4) repeating steps 1), 2), and 3). If there is no difference in the two

final weights, the disc (or disc plus crucible) has been dried to constant weight. In practice, the initial drying period is sometimes extended to several hours. A second heating step is then generally not carried out.

IV CALCULATIONS

mg nonfilterable (suspended) solids/1 =

$$\frac{(\text{wt of 4.7 cm disc + residue})^* - (\text{wt of 4.7 cm disc})^* \times 1000 \times 1000}{\text{ml of sample filtered}}$$

or

$$\frac{(\text{wt of 2.2 cm disc + Gooch crucible + residue})^* - (\text{wt of 2.2 cm disc + Gooch crucible})^* \times 1000 \times 1000}{\text{ml of sample filtered}}$$

*in grams

This outline was prepared by C. R. Feldmann, Chemist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

LABORATORY PROCEDURE FOR FILTERABLE (DISSOLVED) SOLIDS

I INTRODUCTION

A This procedure was excerpted from Methods for Chemical Analysis of Water and Wastes, 1971, Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory.

B The procedure is applicable to surface and saline waters domestic and industrial wastes.

C The practical range of the determination is 10-20000 mg/l.

D Samples high in calcium, magnesium, chloride, and/or sulfate may be hygroscopic and require prolonged drying and desiccation, and quick weighing.

E To insure complete conversion of bicarbonate to carbonate, samples high in bicarbonate content will require careful and possibly prolonged drying at 180°C.

F Excessive residue in the evaporating dish will crust over and entrap water that will not be driven off during drying.

G Samples should be analyzed as soon as possible.

II EQUIPMENT

A Glass fiber filter discs, 4.7 cm or 2.2 cm, without organic binder. Reeve Angel type 984H, Gelman type A, or equivalent.

B Membrane filter funnel (for use with the 4.7 cm disc), or

C 25 ml Gooch crucible (for use with the 2.2 cm disc).

D Gooch crucible adapter

E 500 ml suction flask

F Drying oven, 180 ± 2°C

G Muffle furnace, 550°C

H Drying oven, 180 ± 2°C

I Desiccator

J Porcelain, Vycor, or platinum evaporating dish, 100 ml capacity.

K Steam bath

L Analytical balance, 200 g capacity, capability of weighing to 0.1 mg.

III PROCEDURE

A Assemble the filtering apparatus and suction flask (either the 2.2 cm disc, Gooch crucible and adapter, or the 4.7 cm disc and membrane filter funnel).

B Apply suction to the flask and wash the disc with three successive 20 ml portions of distilled water.

C Continue the suction until all water has passed through the disc.

D Remove the Gooch crucible plus 2.2 cm disc and dry in the oven at 103 - 105°C for 1 hour, or,

E Remove the 4.7 cm disc from the membrane filter funnel and dry in the oven at 103 - 105°C for 1 hour. In D or E, if the disc is not to be used immediately, store it in the desiccator

F Weigh the 4.7 cm disc or 2.2 cm disc plus Gooch crucible just before use.

G Heat the clean evaporating dish in the muffle furnace at 500°C for 1 hour.

H Store the dish in the desiccator and weigh just before use.

I Assemble the filtering apparatus.

J Apply suction to the flask.

K Shake the sample container vigorously.

L Measure 100 ml of the well mixed sample in a 100 ml graduated cylinder. (A larger or smaller volume may be used if the dissolved solids content is thought to be low or high).

M Rapidly, but without spilling, pour the sample into the funnel or crucible.

N Continue the suction for at least three minutes to assure water removal from the disc.

O Using a 100 ml graduated cylinder, transfer 100 ml fo the filtrate to the evaporating dish.

P Evaporate the filtrate to dryness on the steam bath.

Q Dry the evaporated sample for at least 1 hour at 180 $\pm$ 2°C.

R Cool the dish in the desiccator.

S Weigh the dish.

T Repeat the drying, cooling, and weighing steps until two successive weighings are the same, or the weight loss is less than 0.5 mg.

Note: The filtrate from the Non-Filterable (Suspended) Solids determination may be used in this procedure.

IV CALCULATIONS

mg filterable (dissolved) solids/l =

$$\frac{(\text{wt dish + residue})^* - (\text{wt dish})^* \times 1000 \times 1000}{\text{ml of sample}}$$

*in grams

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LABORATORY PROCEDURE FOR VOLATILE SOLIDS

I INTRODUCTION

A In the manual, Methods for Chemical Analysis of Water and Wastes, 1971, Environmental Protection Agency, Office of Water Programs, Analytical Quality Control Laboratory, the reference given for the determination of volatile solids is Standard Methods for the Examination of Water and Wastewater, 13th ed., p 538, Method 224D, 1971.

B The Analytical Quality Control Laboratory manual states that the test is subject to many errors due to:

1 Loss of water of crystallization.

2 Loss of volatile organic material prior to combustion.

3 Incomplete oxidation of certain complex organics.

4 Decomposition of mineral salts during combustion.

The principal source of error is said to be failure to obtain a representative sample.

C The procedure in the 13th ed of Standard Methods involves ignition of the filter disc from the determination of non-filterable (suspended) solids at 550°C for 15 minutes, cooling the disc in a desiccator and weighing.

D The equipment and procedure paragraphs below are therefore those from the Analytical Quality Control Laboratory manual.

II EQUIPMENT

A Glass fiber filter discs, 4.7 cm or 2.2 cm, without organic binder Reeve Angel type 984H, Gelman type A, or equivalent.

B Membrane filter funnel (for use with the 4.7 cm disc), or

C 25 ml Gooch crucible (for use with the 2.2 cm disc).

D Gooch crucible adapter.

E 500 ml suction flask.

F Desiccator.

G Analytical balance, 200 g capacity, capability of weighing to 0.1 mg.

H Muffle furnace, 550°C.

III PROCEDURE

A Assemble the filtering apparatus and suction flask (either the 2.2 cm disc, Gooch crucible and adapter, or the 4.7 cm disc and membrane filter funnel).

B Apply suction to the flask and wash the disc with three successive 20 ml portions of distilled water.

C Continue the suction until all water has passed through the disc.

D Remove the Gooch crucible plus 2.2 cm disc and dry in the oven at 103 - 105°C for 1 hour, or,

E Remove the 4.7 cm disc from the membrane filter funnel and dry in the oven at 103 - 105°C for 1 hour. In D or E, if the disc is not to be used immediately, store it in the desiccator.

F Weigh the 4.7 cm disc or 2.2 cm disc plus Gooch crucible just before use.

G Assemble the filtering apparatus.

H Apply suction to the flask.

I Shake the sample container vigorously.

J Measure 100 ml of the well mixed sample in a 100 ml graduated cylinder. (If the sample appears to be low in solids, a larger volume may be used).

K Rapidly, but without spilling, pour the sample into the funnel or crucible.

L Continue the suction until all of the water has passed through the disc.

M Remove the 4.7 cm disc from the funnel and ignite in the muffle furnace at 550°C for 1 hour, or,

N Remove the Gooch crucible and 2.2 cm disc and ignite in the muffle furnace at 550°C for 1 hour.

O Cool the 4.7 cm disc or 2.2 cm disc plus Gooch crucible in the desiccator.

P Weigh the 4.7 cm disc or 2.2 cm disc plus Gooch crucible.

IV CALCULATIONS

mg volatile solids/l =

$$\frac{(\text{wt of 4.7 cm disc + residue})^* - (\text{wt of 4.7 cm disc})^* \times 1000 \times 1000}{\text{ml of sample filtered}}$$

or

$$\frac{(\text{wt of 2.2 cm disc + Gooch crucible + residue})^* - (\text{wt of 2.2 cm disc + Gooch crucible})^* \times 1000 \times 1000}{\text{ml of sample filtered}}$$

*in grams

This outline was prepared by Charles R. Feldmann, Chemist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

THE DETERMINATION OF OIL AND GREASE

I INTRODUCTION

The terms grease and oil are not clearly defined. Hydrocarbons of mineral oil sources and oxygenated hydrocarbons of plant or vegetable origin are included. Low molecular weight volatile oils and high molecular weight solid fats, esters, or waxes may be included. All have a greasy or oily feeling and tend to adhere to extranenous surfaces. Solubility is a major characteristic. If the materials are preferentially more soluble in relatively nonpolar solvents such as petroleum ether or hexane than in water, they probably will be considered as grease and oil. This type of classification is part of the problem in grease and oil analysis in wastewater treatment technology. Many materials such as coloring agents having the same solubility characteristics are included as grease and oil.

II OCCURRENCE AND SIGNIFICANCE

A Grease and oil may be present as an oil-in-water or water-in-oil emulsion, as a sorbate, on suspended or deposited particulates as a metallic soap in true solution, surface film, or mixtures of these. Usually the solubility in water is small but measureable, particularly, for low molecular weight or combination products.

B Greases and oils are of interest in wastewater treatment operations because of their widespread distribution resulting from natural and industrial activities. The produce an unusual variety of undesired effects that are readily observed, and difficult to correct during wastewater treatment.

1 Most greases and oils have a low specific gravity and tend to float on the surface to produce slicks, haze or scum.

2 Oil and grease tend to coat particulate material in water to produce a sticky mess that is unsightly, odorous, and difficult to clean.

a Floating grease balls of fiber, sticks, grease and miscellaneous solids tend to build up into unsightly smelly messes that are difficult to correct.

b Floating scum tends to collect grease, fiber, and digestible material into an impervious mass that limits surface contact and biodegradability. Anaerobic digester scum is a common operating problem and is difficult to break up.

c Grease tends to adhere to equipment surface such as pipelines, pumps, screens, etc. Reduced capacity and plugging results.

d Grease and oils in solids deposits reduces efficiency of coagulation, filterability, and drying action.

3 Grease and oil are a definite safety hazard on walkways, equipment, or other surfaces.

III ANALYTICAL METHODS

A Standard Methods (1) lists three determinations for oil and grease, based on the sample coming from water, wastewater, or sludge.

1 In the case of a water sample, an extraction is made in a separatory funnel using a suitable solvent such as trichlorofluoromethane or petroleum ether. The solvent, containing the grease and oil (and any other materials extracted by the solvent), is transferred to a previously weighed distilling flask. All but about 10 ml of the solvent is removed by distillation. The final 10 ml is removed by use of a steam or hot water bath. The distilling flask is again weighed, and the increase of weight is due to materials extracted from the sample by the solvent.

2 For sewage samples, 1 l of sample is acidified to pH 1.0 and then vacuum filtered through muslin cloth overlaid with filter paper. The filter paper is carefully transferred to a Soxhlet extraction thimble. Any grease or oil materials adhering to the inside of the funnel or the sample container are wiped with pieces of filter paper soaked in solvent (n-hexane or trichlorotrifluoroethane). These pieces are also added to the thimble. The extraction flask is weighed, the thimble dried at 103°C for 30 minutes, placed in the Soxhlet extractor, and extracted at the rate of 20 cycles per hour for 4 hours.

The solvent is distilled slowly from the flask at 85°C using a water bath or heating mantle. Final drying is accomplished by means of a steam bath and vacuum. The flask is weighed and the weight gain recorded.

3 For sludge samples, the dry solids content must be known. Magnesium sulfate monohydrate is added to the weighed, acidified sample. The mixture is stirred to form a fine paste which solidifies in 15-30 minutes. The mass is ground in a porcelain mortar and the powder transferred to a Soxhlet extraction thimble. The remainder of the procedure is essentially the same as in paragraph 2 above.

B ASTM (2) lists no specific procedure for oil and grease. A method is given for the extraction of organic matter from water. It involves extracting the neutral sample with a suitable solvent, acidifying to pH 3, reextracting, making the solution alkaline (pH 11), and carrying out a third extraction. The three portions of solvent are combined, evaporated, and the previously weighed flask reweighed.

C The Analytical Quality Control Laboratory Office of Water Programs, Environmental Protection Agency (3) method for the determination of oil and grease is essentially the same as given in paragraph A 2 above. The major difference is that n-hexane is specified as the extracting solvent.

IV REFERENCES

1 Standard Methods for the Examination of Water and Wastewater, 13th ed, APHA, AWWA, WPCF, 1971.

2 Annual Book of ASTM Standards, Part 23 Water, Atmospheric Analysis, 1971.

3 Methods for Chemical Analysis of Water and Wastes, 1971. Environmental Protection Agency, Water Quality Office, Analytical Quality Control Laboratory.

This outline was prepared by F. J. Ludzack, Chemist, and Charles R. Feldmann, Chemist, National Training Center, WPO, EPA, Cincinnati, OH 45268.

VIII CHLORINATION AND CHLORINE DETERMINATION

Increasing emphasis on disinfection of discharged effluents requires consideration of special requirements of chlorination and chlorine determinations of wastewater treatment plant samples. This section presents background information and techniques related to the situation.

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THE BASIS FOR CHLORINATION OF WASTEWATERS

I INTRODUCTION

A In a recent 1968 survey of all municipal sewage treatment plants, it was reported that an overall 30% of treatment plants were provided with facilities for introduction of chlorine in connection with treatment plant operations.

Briefly, the survey of treatment plants using chlorine may be summarized as follows.

CHLORINE USED IN TREATMENT PLANTS

Population Served	Total Number of Plants	Number Plants With Chlorination	% of Total Plants Producing Chlorination
<500	1727	323	18.7
500- 1000	2060	550	26.7
1000- 5000	4995	1903	38.1
5000- 10,000	1438	821	57.1
10,000- 25,000	1164	695	59.7
25,000- 50,000	473	318	67.2
50,000-100,000	269	187	69.5
100,000-250,000	230	170	73.9
250,000-500,000	149	97	65.1
> 500,000	73	43	58.9

B In reviewing publications relating to the use of chlorine with relation to wastewaters, the uninitiated worker quickly can gain an impression that chlorine is used in wastewater treatment operations as an almost-universal panacea, as a means of solving whatever problems seem to be plaguing the operations of the particular plant at the time.

C The purpose of this discussion is to review the common applications of chlorine to wastewaters and in the treatment of wastewaters, with special reference to the forms of chlorine that may be applied, places where chlorine may be applied, and the purposes or consequences of chlorination of wastewaters.

II USEFUL PROPERTIES OF CHLORINE

In terms of its significance in wastewater treatment processes, the important properties of chlorine include the following:

A Chlorine is a powerful oxidizing agent.

B Chlorine is poisonous to living organisms.

1 The poisonous properties of chlorine probably are exerted through its oxidizing ability, through which enzyme systems essential to life are irreversibly oxidized, or at least are inactivated.

2 According to the category of organisms for which chlorine is being used as a poison, chlorine could be called a germicide, a bactericide, a disinfecting agent, an algicide, an ovocide, a cysticide, or by any of several other "-cide" terms.

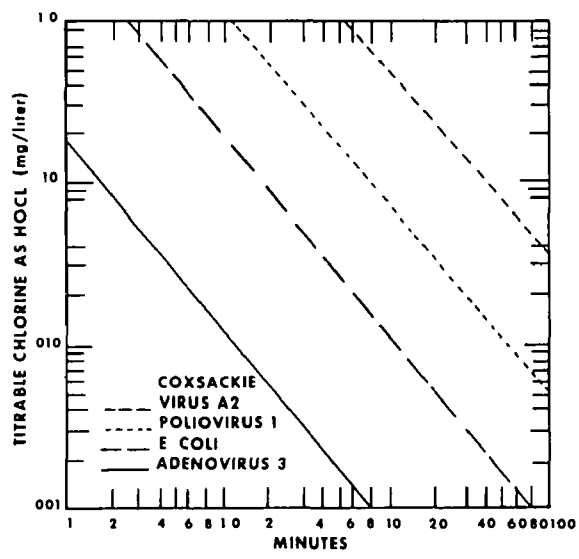
3 Different categories of organisms differ widely in their susceptibility to chlorine. Figure 1 illustrates the relative susceptibility of Escherichia coli and three different kinds of viruses, to hypochlorous acid.

4 The rate at which the disinfecting (killing) process takes place is variable, and subject to control through management of such interrelated factors as temperature, concentration, pH, and the amount, kind, and physical state of other suspended and dissolved substances present in the water.

C Chlorine is highly soluble in water, and can be introduced economically into water and wastewater with accuracy and with adequate provision to protect the health and safety of operational personnel and the population which will be exposed to contact with the chlorine-treated waters.

IV USEFUL FORMS OF CHLORINE

A Most commonly, chlorine is added to water in solution, being kept in tanks in liquid form under pressure, the liquid chlorine is converted to gas which in turn is dissolved into the water or wastewater being treated.



RELATIONSHIP BETWEEN CONCENTRATION AND TIME FOR 99% DESTRUCTION OF E COLI AND 3 VIRUSES BY HYPOCHLOROUS ACID (HOCL) AT 0–6°C

FROM Butterfield, C T, Wattie, E Megregian, S & Chambers, C W [12] Clarke, N A & Kabler P W [15] Clarke, N A Stevenson R E & Kabler P W [16] and Weidenkopf, S J [17] Composited from Data and Figures

FIGURE 1

B Molecular chlorine, Cl_2, in the concentrations applied, is quickly converted into other forms, characterized by the following examples:

1 Hydrolysis occurs:

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^-$$

2 Ionization of the hypochlorous acid occurs with increasing pH:

$$HOCl \rightleftharpoons H^+ + OCl^-$$

At pH 5 and lower, practically all the chlorine is in the form of hypochlorous acid; at higher pH levels, increasing ionization occurs until practically all the chlorine is in the form of the hypochlorite ion at pH 9. Hypochlorous acid is far more active chemically than the hypochlorite ion.

C Chlorine also may be added in the form of sodium or calcium hypochlorite, as chloramine, or as chlorine dioxide.

1 Hypochlorite ions enter into the same sort of equilibrium reaction in water as indicated above·

$$Ca(OCl)_2 \longrightarrow Ca^{++} + 2OCl^-$$

$$H^+ + OCl^- \rightleftharpoons HOCl$$

2 Chlorine dioxide ($Cl\,O_2$) is a gas, and is extremely active chemically and as a germicide.

3 Chloramines are formed in water containing ammonia; when chlorine is added, a mixture of monochloramine (NH_2Cl), and dichloramine ($NHCl_2$), are formed.

If enough chlorine is added to react with all the available NH_3 and still leave an excess of chlorine, then nitrogen trichloride (NCl_3) may be formed. Chloramines are less reactive chemically and less effective as germicides, than are the free forms of chlorine.

V REASONS FOR APPLYING CHLORINE TO WASTEWATERS

A Odor Control

1 If sewage becomes anaerobic, whether in the collecting system of sewers, in any unit of a wastewater treatment plant, or in a long outfall line, then unpleasant odors may be developed. These odors are due to the formation of such foul-smelling substances as hydrogen sulfide, indole, skatole, mercaptans, and cadaverin.

Hydrogen sulfide usually is regarded as the forerunner of the other foul-odor substances. Accordingly, odor-control measures are quite likely to result in control of unpleasant odors from all the substances listed.

2 Role of chlorine. Chlorine, when applied, reacts with H_2S, thus·

$$Cl_2 + H_2S \longrightarrow 2\,HCl + S$$

In addition, the addition of chlorine results in a slowing of biological activity, thereby reducing the rate at which oxygen is removed from the sewage.

3 Mode of application

a Any of the forms of chlorine indicated above may be added to sewage for odor control. However, since chlorine also reacts indiscriminately with other materials present, some workers prefer to add ferrous chloride for a more selective removal of H_2S, thus:

$$H_2S + FeCl_2 \longrightarrow FeS + 2\,HCl$$

Chlorinated benzols also have been applied in sewer systems for odor control.

b To control odors in sewer lines, chlorine is added as far up-sewer from the treatment plant as possible.

4 Chlorine treatment is highly regarded for odor control. In the concentrations used (usually 4 - 5 mg/liter, and sometimes over as wide a range as 3 - 30 mg/liter), a residual chlorine should not be produced, and treatment processes should not be impaired.

B Corrosion Control

1 Besides being an important contributor to unpleasant odors, H_2S formation can result in damage to concrete, mortar, and iron pipes. H_2S, when oxidized, results in production of sulfuric acid. Therefore, any measures taken to prevent formation of H_2S also contribute to corrosion control.

2 Dosages sufficient to prevent formation of H_2S, as above, are indicated.

C Control of Operational Problems

1 Types of operational problems

a Ponding, on trickling filters, sometimes results from formation of surface growths of organisms. These organisms partially or completely occlude the passageways for sewage through the trickling filters.

b Bulking, or failure of satisfactory settling of activated sludge, sometimes results when filamentous organisms, such as Sphaerotilus natans, and possibly other kinds of organisms, become quite prominant in the biota of activated sludge systems.

c Foaming sometimes occurs in Imhoff tanks.

d Septic, or anaerobic, conditions in various parts of a treatment plant may occur when the rate of flow or holding time in tanks or conduits is excessive and where reaeration is not provided.

2 The application of chlorine often is a useful and practical means of solving the above types of problems. The principle of chlorine application is to add a limited amount of chlorine, sufficient to inactivate or kill (selectively) the troublesome organisms, but not to sterilize the unit. In this respect, it is pointed out that there is a protection to the organisms most important in sewage treatment due to (a) the tremendous numbers of organisms present, (b) inherently greater resistance of the treatment organisms than the nuisance organisms to chlorination (debatable), and (c) treatment organisms usually are deeply embedded in a slime matrix or activated floc, and thereby are somewhat less accessible to chlorine than other forms.

D Disinfection of Sewage Effluents

1 Sewage is known to contain tremendous numbers of microorganisms of intestinal origin. When individuals harboring pathogenic organisms, whether bacteria, viruses, protozoa, and other forms of disease-causing organisms, will be present. Such organisms, when present, constitute a hazard to the health and safety of individuals coming into contact with such sewage or treatment plant effluents unless measures are taken to destroy such organisms.

a In recent years, an epidemic of infectious hepatitis was traced to shellfish harvested from polluted waters in Raritan Bay. A pollution study of some magnitude was initiated, and considerable numbers of bacteria attributable to waste treatment plants adjoining Raritan Bay were demonstrated in the study. During the period when wastewater treatment plants chlorinated the treatment plant effluents, enteric

pathogenic bacteria could not be recovered. However, when chlorination of treatment plant effluents was discontinued, Salmonella were discovered with some regularity at sampling points related to the above waste treatment plant discharges.

b In studies of the Red River of the North, Salmonella has been discovered with great regularity in waters polluted through discharge of inadequately treated wastewaters. In some cases, Salmonella has been discovered where the fecal coliform count was only a few hundreds per 100 ml.

2 Chlorine functions as a disinfectant in the sense that it is applied in dosages considered sufficient to destroy the pathogenic (disease-causing) organisms. Disinfection is not construed to mean total destruction of all living organisms present in the sewage effluents (sterilization).

3 For disinfection, chlorine must be added in sufficient concentration, and with a sufficient contact time, to ensure destruction of the pathogenic bacteria.

a One basis of chlorine application for disinfection, widely noted in textbooks of sanitary engineering practices, provides recommendations for adequate disinfection by adding chlorine in an amount sufficient to kill 99.9% of all coliform bacteria in the sewage, or sewage effluent, after a contact time of 15 minutes, and to have a chlorine residual of 0.5 mg/liter (according to one recommendation, the residual chlorine should be 2 mg/liter). A widely-quoted table of chlorine application follows

Table. Amounts of chlorine required for disinfection of sewage and sewage effluents, with chlorine residual 0.5 mg/liter after contact time of 15 minutes.

Type of Sewage or Effluent	Probable Chlorine Requirements		Chlorinator Capacities*	
	mg/liter	lb/day/1000 persons	mg/liter	lb/day/1000 persons
Raw sewage, depending on strength and staleness	6 - 25	5 - 21	30	25
Settled sewage	5 - 20	4 - 17	25	20
Chemically precipitated sewage	3 - 20	3 - 17	25	20
Trickling filter effluent	3 - 20	3 - 17	25	20
Activated sludge effluent	2 - 20	2 - 17	25	20
Intermittent sand filter effluent	1 - 10	1 - 3	15	12

*For sewage flow of 100 gallons per capita per day.

b It is known that the time-concentration-temperature relationship of disinfection can be formulated in a manner analogous to demonstration of the progressive exertion of the biochemical oxygen demand. Accordingly, there has been some interest in determining amounts of chlorine to be added, based on the bacterial results to be achieved in the receiving water at some point downstream from the discharge of the treatment plant effluent.

c The attached figure, based on chlorine requirements for potability treatment of clear water, with a 30 minute contact time, are quite at variance with the requirements for producing a relatively safe effluent from a wastewater treatment plant.

E Reduction of BOD

1 It is emphasized that the action of chlorine is indiscriminate when introduced into wastewaters and treatment plant effluents. Chlorine simultaneously is killing or inactivating living organisms with which it comes into contact in sufficient concentration, for a sufficient time, and it reacts with inanimate inorganic and organic matter which also is present.

In reacting with organic material, chlorine has been stated to reduce from 10% to 35% of BOD when sufficient chlorine has been added to provide a residual after 15 minutes of contact time.

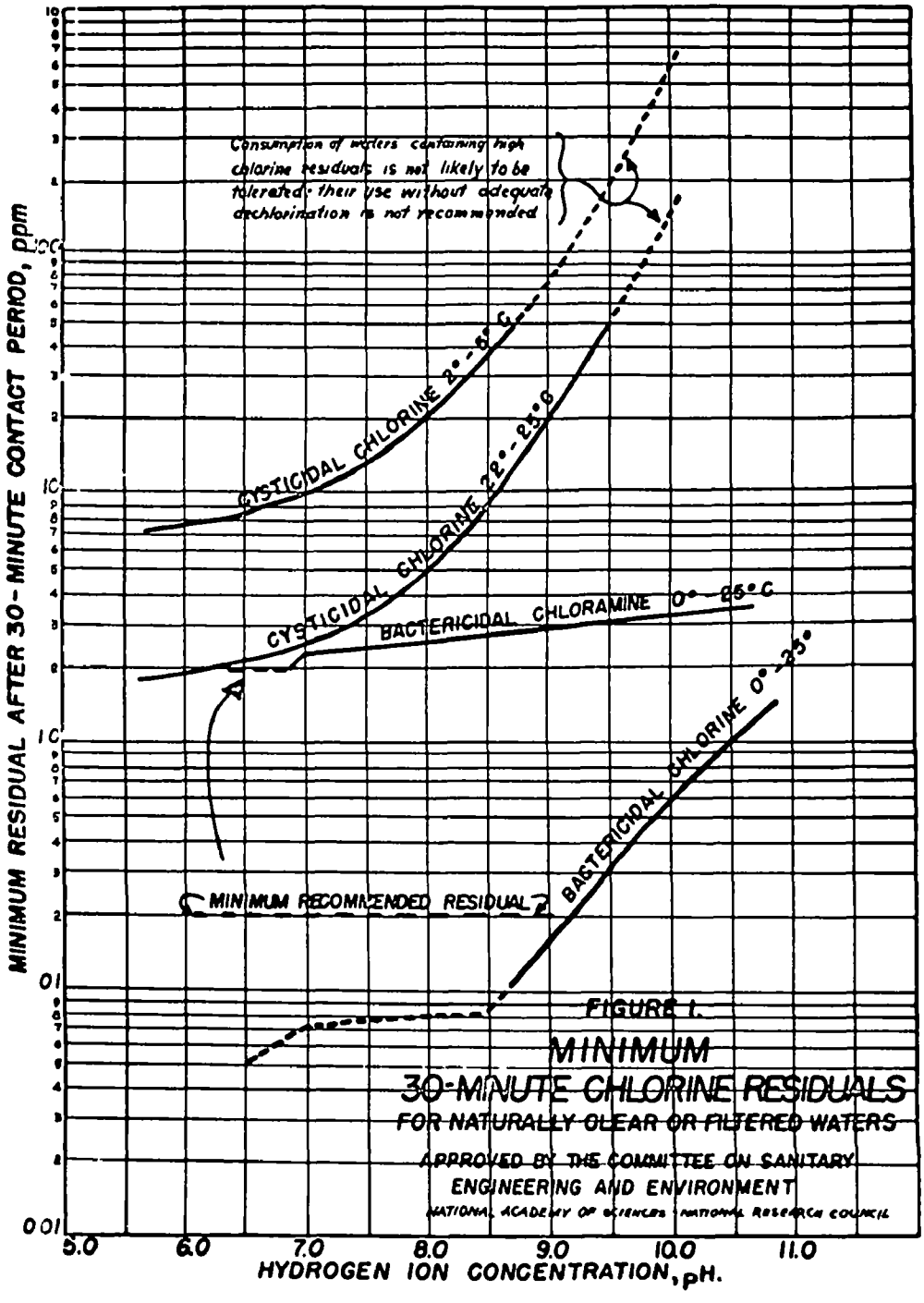
2 In reducing BOD through chlorination, the action has been attributed to

a Outright chemical oxidation of the organic material,

b Combination of chlorine with organic matter to form inherently toxic compounds (such as organic chloramines), and

c Reaction of chlorine with organic matter to form chlorinated compounds which are biologically oxidized more slowly than their predecessors.

3 Chlorination of sewage for the purpose of reducing BOD is not a recommended practice, except possibly in emergencies, pending installation of adequate treatment. The principal need for chlorination for BOD reduction is to delay (and hopefully, to diffuse) the oxygen-depleting material in the receiving water to an extent such that anaerobic conditions will not develop in the aquatic environment.


Consumption of waters containing high chlorine residuals is not likely to be tolerated; their use without adequate dechlorination is not recommended
CYSTICIDAL CHLORINE 2°-8° C
CYSTICIDAL CHLORINE 22°-25° C
BACTERICIDAL CHLORAMINE 0°-25° C
BACTERICIDAL CHLORINE 0°-25°
MINIMUM RECOMMENDED RESIDUAL
MINIMUM RESIDUAL AFTER 30-MINUTE CONTACT PERIOD, ppm
HYDROGEN ION CONCENTRATION, pH.
5.0
6.0
7.0
8.0
9.0
10.0
11.0
FIGURE 1.
MINIMUM
30-MINUTE CHLORINE RESIDUALS
FOR NATURALLY CLEAR OR FILTERED WATERS
APPROVED BY THE COMMITTEE ON SANITARY
ENGINEERING AND ENVIRONMENT
NATIONAL ACADEMY OF SCIENCES NATIONAL RESEARCH COUNCIL

REFERENCES

1 The Federation of Sewage and Industrial Wastes Association. Sewage Treatment Plant Design. 1959.

2 Great-Lakes-Upper Mississippi River Board of Sanitary Engineers. Recommended Standards for Sewage Works. 1960.

3 Cosens, Kenneth W. The Operation of Sewage Treatment Plants. A reprint from Public Works Magazine.

4 Imhoff and Fair. Sewage Treatment. 1956, Book.

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CHLORINE DETERMINATIONS AND THEIR INTERPRETATION

I INTRODUCTION

Chlorine normally is applied to water as a bactericidal agent, it reacts with water contaminants to form a variety of products containing chlorine. The difference among applied and residual chlorine represents the chlorine demand of the water under conditions specified. Wastewater chlorination is particularly difficult because the concentration of organisms and components susceptible to interaction with chlorine are high and variable. Interferences with the chlorine determination in wastewater confuse interpretation with respect to the chlorine residual at a given time and condition, its bactericidal potency, or the future behavior.

II CHEMISTRY OF CHLORINATION

A Chlorine compounds (Cl_2) dissolve in water, and hydrolyze immediately according to the reaction.

$$Cl_2 + H_2O \rightleftharpoons HOCl + H^+ + Cl^-$$

The products of this reaction are hypochlorous and hydrochloric acid. The reaction is reversible, but at pH values above 3.0 and concentrations of chlorine below 1000 mg/l the shift is predominantly to the right leading to hypochlorous acid (HOCl).

Hypochlorous acid is a weak acid and consequently ionizes in water according to the equation·

$$HOCl \rightleftharpoons H^+ + OCl^-$$

This reaction is reversible. At a pH value of 5.0 or below almost all of the chlorine is present as hypochlorous acid (HOCl) whereas above pH 10.0 nearly all of it exists as hypochlorite ion (OCl^-). The pH value that will control is the pH value reached after the addition of chlorine. Chlorine addition tends to lower the pH and the addition of alkali hypochlorites tends to raise the pH.

B The initial reactions on adding chlorine to wastewaters may be assumed to be fundamentally the same as when chlorine is added to water except for the additional complications due to contaminants and their concentration.

Hypochlorous acid (HOCl) reacts with ammonia and with many other complex derivatives of ammonia to produce compounds known as chloramines. Formation of the simple ammonia chloramines includes:

1 $NH_3 + HOCl \rightarrow NH_2Cl + H_2O$

monochloramine

2 $NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O$

dichloramine

3 $NH_2Cl + NHCl_2 \rightarrow N_2 + 3\ HCl$

The distribution of the ammonia chloramines is dependent on pH, as illustrated below·

pH	Percentage of Chlorine Present as Monochloramine	Dichloramine
5	16	84
6	38	62
7	65	35
8	85	15
9	94	6

The formation of the ammonia chloramines are dependent on pH, temperature, and chlorine-ammonia ratio. Chlorine reactions with amino acids are likely, product disinfecting powers are lower than those of chlorine or of ammonia chloramines.

III TERMINOLOGY

A Terms used with Respect to Application Site

1 Pre-chlorination - chlorine added prior to any other treatment.

2 Post-chlorination - chlorine added after other treatment.

3 Split chlorination - chlorine added at different points in the plant - may include pre- and post-chlorination.

B Terms used in Designating Chlorine Fractions

1 Free available residual chlorine - the residual chlorine present as hypochlorous acid and hypochlorite ion.

2 Combined available residual chlorine - the residual chlorine present as chloramines and organic chlorine containing compounds.

3 Total available residual chlorine - the free available residual chlorine + the combined available residual chlorine - may represent total amount of chlorine residual present without regard to type.

In ordinary usage these terms are shortened to free residual chlorine, combined residual chlorine and total residual chlorine. In the chlorination of wastewaters only combined residual chlorine is ordinarily present and is often improperly termed chlorine residual.

C Breakpoint chlorination specifically refers to the ammonia-chlorine reaction where applied chlorine hydrolyzes and reacts to form chloramines and HCl with the chloramines eventually forming N_2 + HCl as in I. B. 3. Assuming no other chlorine demand, the total chlorine residual will rise, decrease to zero and rise again with increasing increments of applied chlorine. Other substances may produce humps in the applied chlorine vs residual chlorine plot due to oxidation of materials other than ammonia. Sometimes these are erroneously considered as a breakpoint.

IV ANALYTICAL METHODS

The o-Tolidine color test and Iodometric titration methods are the basis for numerous modifications for determining chlorine residuals in water. The relative advantages of a specific determination depends upon the form in which the reactable chlorine exists and the amount and nature of interferences in the water.

Iodometric titration using the amperometric endpoint appears to be the most accurate residual chlorine method available (See current editions of Standard Methods APHA[1] and ASTM Standards [2]). The 0-Tolidine and o-Tolidine Arsenite methods require little apparatus, and are readily adapted as a field or control test. The Starch Iodine color titration endpoint for iodometric titration is suitable for use on clean water or stock solutions and may be useful on certain types of wastewater residuals. Selection of a suitable method of determining chlorine residuals depends upon the correlation of the determined residual and the bacterial kill in the presence of existing interferences under applied conditions.

A Iodometric Method

1 Scope and application

This method is applicable to the determination of total chlorine residual in wastewaters, polluted waters and some industrial wastewaters.

2 Summary of method

When a sample is treated with a measured excess of standard phenylarsine oxide

solution, or a standard thiosulfate solution, followed by the addition of iodide, the iodine liberated at the proper pH is stoichiometrically proportional to the total chlorine present. The liberated iodine reacts with the phenylarsine oxide or thiosulfate before any is lost to other extraneous reactions. The excess phenylarsine oxide or thiosulfate is titrated with standard iodine solution in the presence of starch until the phenylarsine oxide or thiosulfate is completely oxidized. The end-point of the titration is the next addition of standard iodine solution that causes a faint blue color to persist in the sample.

3 Interferences

a Organic matter - reacts with liberated iodine.

b Manganic manganese - liberates iodine from iodide at pH 4.0.

c Ferric iron, ferricyanide and nitrites up to 100 mg/l do not interfere at a pH of 4.0.

d Chromates - reduce phenylarsine oxide or thiosulfate to an appreciable extent before the excess can be titrated with standard iodine.

e Excessive color and turbidity

B Iodometric Method with Amperometric End-Point

1 Scope and application

This method is applicable to the determination of total chlorine residual in wastewaters, polluted waters and some industrial wastewaters. The back-titration method is essential for wastewaters in contrast to the direct titration with phenylarsine oxide in clean waters.

2 Summary of method

When a sample is treated with a measured excess of standard phenylarsine oxide solution followed by the addition of iodide, the iodine liberated at the proper pH is stoichiometrically proportional to the total chlorine present. The iodine liberated reacts with the phenylarsine oxide before any is lost to other extraneous reactions. When the cell is immersed in a sample so treated, no current is generated due to halogens nor is any further current generated, as the excess phenylarseneoxide is titrated with standard iodine solution until the phenylarsine oxide is completely oxidized. The end-point of the titration is the next addition of standard iodine solution that causes further current to be generated and a microammeter response or pointer deflection.

NOTE: As the end-point is approached each increment of standard iodine solution causes a temporary deflection of the microammeter, but the pointer drops back to about its original position. The true end-point is reached when a small addition of standard iodine solution gives a definite and permanent pointer deflection.

3 Interferences

a Organic matter - reacts with the liberated iodine.

b Manganic manganese - liberates iodine from iodide at a pH of 4.0.

c Ferric iron, ferricyanide and nitrites up to 100 mg/l do not interfere at a pH of 4.0.

d Chromates - reduces phenylarsine oxide to an appreciable extent before the excess can be titrated with standard iodine solution.

e Cupric ions may cause erratic behavior of the apparatus.

f Cuprous and silver ions tend to poison the electrode.

C Orthotolidine Method

1 Scope and application

This method is applicable to the determination of total chlorine residual in wastewaters, polluted waters and some industrial wastewaters.

2 Summary of method

When a sample is treated with a measured amount of orthotolidine reagent, the orthotolidine is oxidized in the resulting acid solution by chlorine and chloramines and other oxidizing agents to produce a yellow-colored compound (Holoquinone). The color produced at pH values of less than 1.8 is proportional to the amount of chlorine present and is suitable for quantitative measurement. The chlorine residual in mg/l is read directly from the colored glass disks or sealed colored liquid standards or is calculated from a previously prepared standard curve. NOTE: Particular attention is called to the importance of warming the sample to 20°C after the addition of orthotolidine reagent in order to complete the reaction.

3 Interferences

a Organic matter - oxidizes orthotolidine to produce a yellow color (Holoquinone).

b Manganic manganese - in concentrations above 0.01 mg/l oxidizes orthotolidine to produce a yellow color (Holoquinone).

c Ferric iron - in concentrations above 0.3 mg/l oxidizes orthotolidine to produce a yellow color (Holoquinone).

d Nitrites - in concentrations above 0.10 mg/l of nitrite nitrogen oxidizes orthotolidine to produce a yellow color (Holoquinone).

e Excessive color and turbidity.

D Orthotolidine- Arsenite Method

1 Scope and application

This method is applicable to the determination of free residue chlorine and combined residual chlorine in wastewaters, polluted waters and industrial wastewaters, but as normally carried out for wastewaters, etc. total residual chlorine is measured.

2 Summary of method

A sample is split into two fractions (a) and (b). Sample (a) is treated with a measured amount of arsenite reagent, followed by the addition of orthotolidine reagent. The arsenite reacts with chlorine while orthotolidine reacts with ferric iron, manganic manganese and nitrite nitrogen to produce additional color (represents interfering color). Sample (b) is treated with a measured amount of orthotolidine reagent. The orthotolidine is oxidized in the resulting acid solution by chlorine, chloramines and other oxidizing agents to produce a yellow-colored compound (Holoquinone) as described in the orthotolidine method (represents total amount of residual chlorine present and interfering color). Mg/l total residual chlorine = b - a if color compensation is not made directly. NOTE: more accurate readings may be obtained if cells containing sample fractions (a) and (b) are placed in the comparator in such relative positions that color compensation is made directly.

3 Interferences

a High color and turbidity

V INTERPRETATION

In general residual chlorine concentrations obtained by the iodometric titration method (starch iodide end-point and amperometric

end-point) will be higher than the concentrations obtained from the orthotolidine and the orthotolidine-arsenite methods. Some of the reasons for the difference have been listed under the individual methods. Extensive studies have indicated that consistently better correlation between bacterial kill and chlorine residual found is possible when the titration method is used.

REFERENCES

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IX COMPUTATION AIDS AND GLOSSARY

The chart presented as a computation aid is intended for use to provide quick and rough estimates for conversion purposes and to check decimal point control for other calculations.

The Glossary is an attempt to bridge the terminology gap for recent recruits in wastewater treatment or those interested in enhancing understanding or expression of a larger fraction of the field.

Contents of Section IX

Powers Data Sheet No. 397

Glossary - Wastewater Treatment Technology

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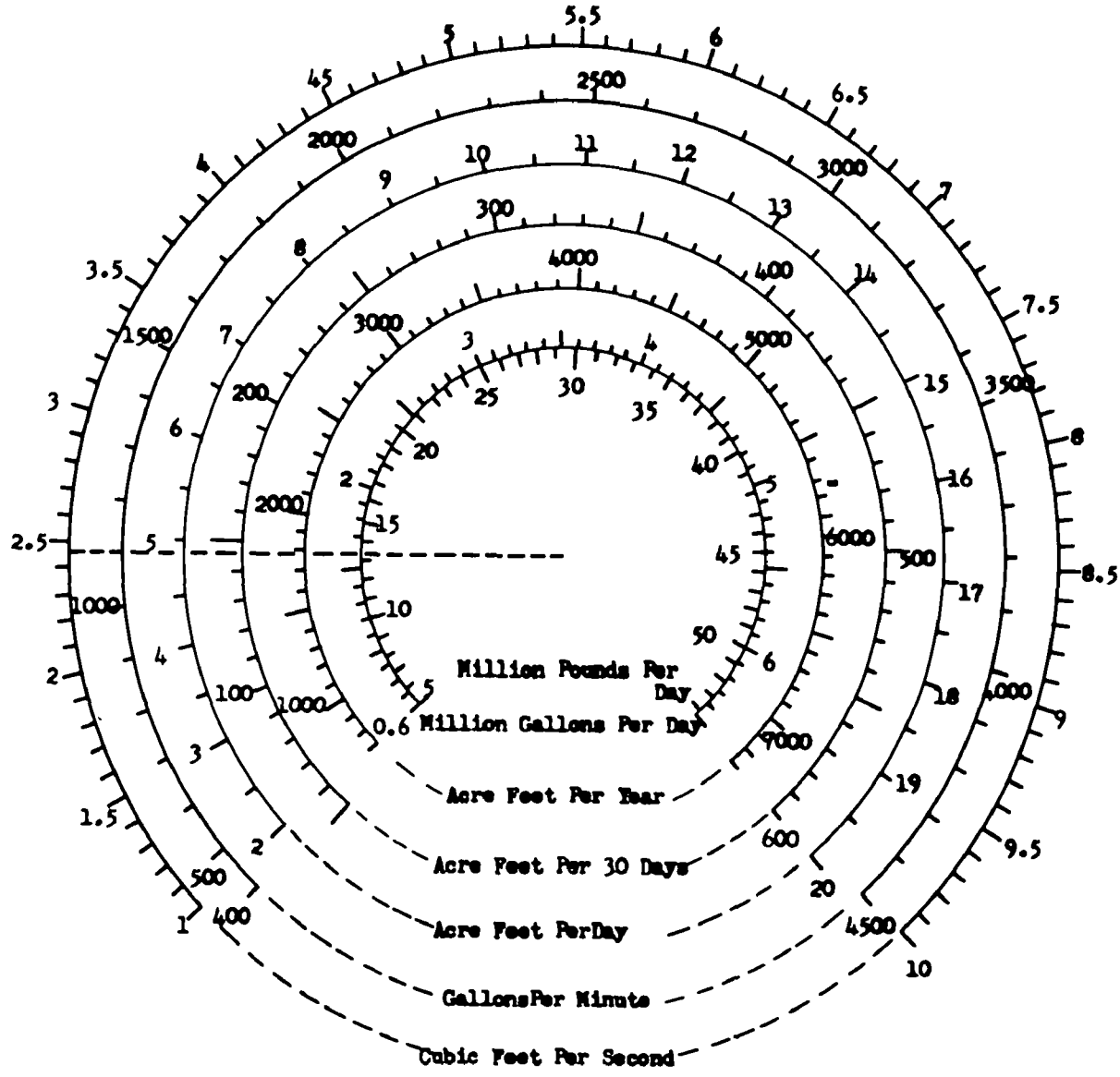

Flow of Fluids Conversion Chart

With this chart you can conveniently determine equivalent units of discharge for fluids. Merely line up a straight edge with the middle of the chart and a known discharge; read the equivalent on the other scales.

Example: Discharge from a given pipe is 1100 gallons per minute. How many gallons per day are discharged? Lining up 1100 on the gallons per minute scale with the chart center, we read 1,580,000 gallons per day on the appropriate scale. On other scales we can determine that the flow is equivalent to 2.45 cu ft per sec.

The scales cover sufficient range to allow you to find values of any magnitude by multiplying or dividing the scales by factors of ten. With a little ingenuity, you can determine several units not shown directly.

S. R. Ross, Denver, Colorado

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GLOSSARY - WASTEWATER TREATMENT TECHNOLOGY

This is a selected list containing the key ideas of terms likely to be encountered in treatment technology.

For more scientific definitions or unlisted terms, consult the list of references at the end of the glossary.

ABSORPTION - The taking up of one substance into the body of another.

ACRE FOOT - A volume term referring to that amount of liquid 1 acre in area and a depth of 1 foot. 43,560 cu.ft.

ACID - Most commonly refers to a large class of chemicals having a sour taste in water, ability to dissolve certain metals, bases or alkalies to form salts and to turn certain acid-base indicators to their acid form. Characterized by the hydrated H^+ ion.

ACTIVATED SLUDGE - A process used for purification and stabilization of wastewaters by mixing of the solids concentrate from previous contact with raw or settled wastewaters under turbulent oxygenating conditions for sufficient time to permit transfer of nutrients to the solids phase, partial biodegradation and clarification of the water before discharge.

ADSORPTION - The taking up of one substance upon the surface of or interface zone of another substance.

ADVANCED WASTE TREATMENT - Renovation of used water by biological, chemical or physical methods that are applied to upgrade water quality for specific reuse requirements. May include more efficient cleanup of a general nature or the removal of components that are inefficiently removed by conventional treatment processes.

AERATION - The operation of adding oxygen to, removing volatile constituents from, or mixing a liquid by intimate contact with air.

AERATION PERIOD - A theoretical time usually expressed in hours equal to the volume of the tank divided by the volumetric rate of flow.

AEROBIC - A condition characterized by an excess of dissolved oxygen in the aquatic environment.

AEROBIC BACTERIA - Organisms that require dissolved oxygen in the aquatic environment to enable them to metabolize or to grow.

AGGLOMERATION - An action by which small particles gather into larger particles that are more readily separated from the liquid by sedimentation or other means. May be the result of biological, chemical or physical factors.

AIR LIFT - A pump consisting of a vertical pipe immersed in a liquid into which air is mixed to reduce specific gravity of the air-liquid mixture. The net effect is to raise the liquid level in the discharge pipe.

ALGAE - Primitive plants, one or many celled, usually aquatic and capable of growth on mineral materials via energy from the sun and the green coloring material, chlorophyll. Generally considered as the primary source of food for all other organisms.

ALKALINITY - A term used to represent the sum of the effects opposite in reaction to acids in water. Usually due to carbonates, bicarbonates and hydroxides; also including borates, silicates and phosphates.

AMPEROMETRIC CHLORINE RESIDUAL - A means of determining residual available chlorine with phenyl arsene oxide (PAO) titration using current response as an indicator of equivalence. For wastewater, the PAO preferably is used in excess with iodine backtitration.

ANAEROBIC - A condition in which dissolved oxygen is not detectable in the aquatic environment. Commonly characterized by the formation of reduced sulfur compounds from the use of bound oxygen from sulfates as an hydrogen acceptor.

ANAEROBIC BACTERIA - Organisms that can metabolize and grow in the absence of dissolved oxygen. Their oxygen supply is obtained from the bound oxygen such as in sulfates, carbonates, or other oxygen-containing compounds.

ANION - A negatively charged ion in water solution. May be a single element or a combination of elements, such as the Cl^- ion in a water solution of NaCl (common table salt) or $SO_4^=$ ion in a sulfuric acid solution.

ASSESSMENT - A legal financial obligation of the property owner in an irrigation, water, drainage or sanitary district, created for the purpose of financing the construction and operation of facilities required to protect and enhance public benefit within the district.

ATTACHED GROWTH - Plant or animal growth that tends to seek a solid surface for a point of attachment from which to grow, in contrast with free-swimming or suspended organisms.

ATOM - An extremely small unit or particle of an element consisting of a positively charged nucleus and one or more negatively charged electrons. Atoms of different elements are different in mass and the number of electrons. Electrons may be located in the nucleus or externally. The external electrons determine chemical combining power.

ATOMIC WEIGHT - A relative mass of an atom of an element compared to carbon-12. May be expressed in grams (g), pounds or other consistent weight units when used for process control.

AVAILABLE RESIDUAL CHLORINE - Generally refers to that part of the chlorine that will react with ortho - tolidine or amperometric tests and exhibits significant germicidal activity.

AWWA (AMERICAN WATER WORKS ASSOCIATION) - An organization composed of individuals engaged in research, design, operation and control in the advancement of knowledge related to potable water supply

BACTERIA - Primitive organisms having some of the features of plants and animals. Generally included among the fungi. Usually do not contain chlorophyll, hence commonly require preformed organic nutrients among their foods. May exist as single cells, groups, filaments, or colonies.

BACTERIACIDE - Any component that will kill or destroy bacteria.

BACTERIOSTATIC - A condition during which the normal metabolic functions of bacteria are arrested until favorable conditions are restored.

BACTERIOLOGY - See Microbiology.

BAFFLE - A deflector or check such as a vane, guide boards, plates, grids, grating, or similar devices used to control the flow distribution or velocity of liquid in a channel or basin.

BAR RACKS (SCREENS) - A coarse screen usually consisting of bars spaced with 1 to 5 inch openings to trap roots, branches, rocks, rags, and other large materials that may be encountered in the flow of a channel or conduit.

BASE - A foundation, plate, natural or engineered support upon which a structure, channel, machine, or other device is mounted.

Chemical: A base includes a large variety of chemicals opposite in reaction to acids (alkali).

BED LOAD - Generally refers to the oxygen demand requirements of benthic deposits, sludge, muck, attached growths, moving materials, living or dead that are exerted upon waters as a result of bottom or boundary dynamics.

BENTHIC DEPOSIT (BENTHOS) - Refers to the accumulated deposition of cell mass living or dead that collects at the bottom of a stream impoundment where velocity or catchment permits.

BIO-CHEMICAL - Resulting from the combined activities of biological and chemical transformations. Usually measured in terms of the ensuing chemical changes.

BIODEGRADATION - The stabilization of wastewater contaminants by biological conversion of pollutants into separatable materials at a higher oxidation state.

BIO FILTER - See Trickling Filter.

BIOLOGICAL PROCESSES - Activities of living organisms to sustain life, growth, and reproduction. Commonly the processes by which organisms degrade complex organic material into simpler substances at a higher oxidation state to obtain energy for life processes and growth of new cell mass.

BIOLOGY - The science and study of living organisms, characteristics and behavior.

BOD - Biological or biochemical oxygen demand. A test for estimation of wastewater polluting effects in terms of the oxygen requirements for biochemical stabilization under specified conditions and time.

BRIDGING - A condition in which particulates or solids concentrates that would normally seek the lowest level of a restricted channel or basin, tend to hang up on sidewalls. The bridged material commonly may settle again with vibration, agitation, a change in flow direction, or increased flow velocity.

BTU (BRITISH THERMAL UNIT) - That amount of heat that will raise the temperature of one pound of water one degree Fahrenheit.

BUFFER ACTION - An action exhibited by certain chemicals that limits the change in pH upon addition of acid or alkaline materials to the system. In surface water, the primary buffer action is related to carbon dioxide, bicarbonate and carbonate equilibria.

BULKING - A condition, usually related to activated sludge processing, in which the sludge solids separation from the liquid is inhibited. Rapid growth, filamentous organisms, and certain other factors that are but vaguely understood, tend to produce a low density thin sludge that settles very slowly and has limited compactability.

BURNER, WASTE GAS - A device for burning the excess gas from sludge digestion.

CALORIE - That amount of heat required to raise one gram of water one degree Centigrade, or Celsius.

CARBOHYDRATES - Naturally occurring compounds consisting of carbon, hydrogen and oxygen, that are considered as energy foods and precursors of proteins and fats in the natural food chain.

CATALYST - A substance that influences the rate of chemical change but either remains unchanged during the reaction or is regenerated thereafter. Generally applies to acceleration of reaction rates.

CATCH BASIN - A chamber, well or other enlargement of a channel, designed to retain grit and detritus below the point of liquid overflow.

CATION - A positively charged ion in water solution. May be a single element or a combination of elements. such as Na^+ in a water solution of NaCl (common table salt).

CENTI - An expression used to indicate 1/100 of a given standard unit centimeter (cm). 1/100 meter.

CENTIGRADE - A temperature measurement scale in which the freezing point of pure water at sea level is designated as 0^oC and the temperature of boiling water is designated as 100^oC. This is more properly termed the Celsius scale.

CENTRIFUGAL PUMP - A pump consisting of a rotating impeller within a casing having an inlet near the center and an outlet or discharge at the tip of the impeller where centrifugal force is greatest.

CENTRIFUGE - A device for separation of solids or liquids of different densities by rotational energy; heavy materials move outward, less dense materials move toward a central take-off port.

CHANNEL - A natural or artificial waterway which continuously or periodically contains flowing water. A connecting link between two bodies of water with a definite bed and sidewalls to confine the flow.

CHANNELING - A condition in which certain portions of the flow within a channel or basin tend to seek a more limited distribution than that resulting from the confining bed or sidewalls, i.e., the flow may channel along the top, bottom or mid channel depth due to density, temperature, or some form of obstruction to uniform cross sectional flow.

CHECK VALVE (FLAP GATE) - A device to control flow in a pipe or channel limiting it to one direction. Commonly a gate hinged at the top that is limited in movement by a seat in a near vertical position so that it can open for flow in one direction but closed by reverse flow.

CHEMISTRY - A science that deals with the composition and characteristics of substances and their behavior, i.e., the transformations that they undergo.

CHLORAMINES - Products of the combination of chlorine and ammonia. Commonly classified as combined available chlorine.

CHLORINE - A greenish yellow gaseous element having strong disinfecting and oxidizing properties in water solution. It is commercially available as compressed gas, liquid, or in combined form as a powder. It is highly toxic and irritating to skin, eyes, and lungs in significant concentrations.

CHLORINATION - The application of chlorine to water or wastewater for the purposes of disinfection, oxidation, odor control, or other effects. Pre-chlorination - before treatment; post-chlorination - after treatment; in-process chlorination - during treatment.

CHLORINATION CHAMBER - A basin or tank where chlorine is applied to the liquid.

CHLORINE TEST - Commonly refers to one of two methods separately listed: see Ortho tolidine test; see Amperometric test.

CHLORINE DEMAND - The difference between applied chlorine and residual available chlorine in aqueous media under specified conditions and contact time. Chlorine demand varies with dosage, time, temperature, nature and amount of the water impurities.

CHLORO ORGANIC COMPOUNDS - A broad group of compounds containing chlorine, carbon, hydrogen and sometimes other elements. Generally originating from or associated with living or dead organic materials. This group shows a wide range of toxicities but usually have relatively little oxidizing energy compared to chlorine.

CHLOROPHYLL - The green coloring material or pigments in plants that promotes the photosynthetic reactions forming organic materials from inorganic nutrients and light energy within the living cells.

CLARIFIER - A basin or chamber serving as an enlargement of a channel to reduce flow velocity sufficiently to permit separation of settleable or floatable materials from the carrier water (a sedimentation basin).

COAGULANT - A chemical, or chemicals, which when added to water suspensions will cause finely dispersed materials to gather into larger masses of improved filterability, settleability, or drainability.

COAGULATION - The process of modifying chemical, physical, or biological conditions to cause flocculation or agglomeration of particulates.

COD - A test for the estimation of the contamination of a wastewater in terms of oxygen requirements from a strong chemical oxidant under specified conditions, i.e., Dichromate, 50% sulfuric acid and 145°C for 2 hours.

COLIFORM GROUP - A group of bacteria that inhabits the intestinal tract of man, warm-blooded animals, and may be found in plants, soil, air and

the aquatic environment. Includes aerobic and facultative gram negative non-spore forming bacilli that ferment lactose with gas formation.

COLLECTION SYSTEM - The sewerage collection system is comprised of the conduits controlled by public agencies to intercept house, commercial or industrial discharges and transport them to a treatment facility or discharge point.

COLLOID, COLLOIDAL STATE - A state of suspension in which the particulate or insoluble material is in a finely divided form that reamins dispersed in the liquid for extended time periods. Usually cloudy or turbid suspensions requiring flocculation before clarification.

COMBINED AVAILABLE CHLORINE - Generally refers to chlorine-ammonia compounds exhibiting a slower reaction with ortho tolidine, determinable with phenyl arsene oxide after addition of potassium iodide under acid conditions and usually requires higher concentration and longer time to kill in comparison with free available chlorine.

COMBINED SEWER - A sewer designed to carry wastewaters and storm waters in the same channel.

COMBINED SEWAGE - Consists of household, commercial or industrial wastes in combination with roof and surface storm drainage.

COMMINUTION -

a) The act of cutting and screening materials contained in wastewaters.

b) To reduce the size of fibrous or amorphous materials.

COMMINUTOR - A device for cutting sewage solids until they pass through an acceptable screen opening to improve pumping and wastewater processing.

COMPOUND -

a) A combination of two or more atoms having definite physical and chemical characteristics and mutually attracted to each other.

b) Atoms in the elemental state are electrically neutral but the number of external electrons may be increased or decreased in response to conditions and nature of the atom. An atom that becomes electrically charged may combine with another atom of opposite charge to form an electrically neutral compound.

CONCENTRATION -

a) The act of increasing the mass per unit volume of one substance with respect to another, such as concentrating the solids in a sludge from 3% to 6%.

b) A means of designating the ratio of one substance with respect to another, such as 15 mg of suspended solids per liter of water.

CONDITIONING - An action of improving possibilities for subsequent processing such as chemical treatment to improve sludge dewatering or filtering.

CONING - A condition in a clarifier sludge hopper where the solids concentrate or sludge is partially withdrawn to form a cone or channel through which clarified liquid is pumped out while most of the solids remain behind around the cone. Infrequent sludge pumping tends to encourage this condition where the sludge tends to solidify and is resistant to fluid flow.

CONTAMINATION - A general term referring to the introduction of materials into water that make the water less desirable for its intended use. Also introduction of undesired substances into air, solutions, or other defined media (chemical or biological)

CRITERION (pl. CRITERIA) - Something which can be measured. Commonly used as a basis for standards.

CROSS CONNECTION - In plumbing, a physical connection between two different water systems, such as between potable and polluted water lines.

CUBIC FOOT PER SECOND (c.f.s.) - A unit of discharge rate such as one cubic foot of gas per second past a given point.

CURVE -

a) A graphic plotted to represent changes in value of one quantity in reference to another.

b) A deviation from a straight line without a sharp break or angularity.

DATA - Records of observations or measurements of facts, occurrences and conditions in written, graphical or tabular form.

DEBRIS -

a) The remains of something broken down or destroyed.

b) An accumulation of fragments of rocks.

DECAY -

a) To undergo decomposition.

b) Implies a slow change from a state of soundness or perfection.

c) To decay.

DEGRADE - To reduce the complexity of a chemical compound.

DEIONIZED WATER - Water that has been treated by ion exchange resins or compounds to remove cations and anions present in the form of dissolved salts.

DENITRIFICATION -

a) The conversion of oxidized nitrogen (nitrate and nitrite-N) to nitrogen gas by contact with septic wastewater solids or other reducing chemicals.

b) A reduction process with respect to oxidized nitrogen.

DETENTION PERIOD - The theoretical time required to displace the entire volume of a tank or basin at a given rate of discharge. Tank volume ÷ rate of discharge.

DETENTION PERIOD, ACTUAL - The actual time required for a given unit of liquid to flow through the tank or process unit. Usually determined by tracer method and depends upon inlet and outlet geometry, temperature, specific gravity, stratification, and other factors.

DETERGENT - Something used for cleaning. Commonly consists of soap or surfactant plus various additives or associated materials.

DETRITUS - The heavier material moved by natural flow, usually along the channel bed. Sand, grit or other coarse material.

DIAPHRAGM PUMP - A pump consisting of a rubber diaphragm (generally) fastened to a cylindrical casing having inlet and outlet valves. When the diaphragm is raised, liquid enters to be forced out the discharge valve on the reverse stroke.

DIFFUSED AERATION - Aeration produced by introducing air through a dispersing mechanism into a liquid. Sufficient air pressure must be applied to overcome hydrostatic head and diffusor or pipe back pressure.

DIFFUSOR - A porous plate, tube, bag, or other device, through which air is forced into a liquid in the form of small bubbles.

DIGESTED SLUDGE - Solids concentrates stabilized under aerobic or anaerobic conditions to preferentially decompose the more unstable fractions and produce a residue of satisfactory disposal characteristics. To reduce the volatile fraction of the sludge.

DILUTION -

a) To make thinner or more liquid.

b) A ratio, volume or weight of a more concentrated sample or effluent flow compared to that into which it is discharged.

DISINFECTION -

a) To make free of infectious organisms.

b) To kill disease organisms.

DISPOSAL -

a) The discarding or throwing away.

b) For wastewaters, this may represent any method of disposing, but usually involves some degree of degradation and discard in a non-pollutional manner.

DISSOLVED -

a) Those materials dispersed in water in ionic, atomic, or molecular form; an homogenous mixture or solution.

b) Generally clear but may be colored.

c) Present in true solution form.

DISSOLVED OXYGEN (D.O.) - Dissolved molecular oxygen usually expressed in mg DO/l or percent of saturation.

DISTILLED WATER - A purified water resulting from heat vaporization followed later by vapor condensation to separate the water from non-volatile impurities.

DISTRIBUTOR -

a) A device to control flow into some desired direction or place.

b) A device used to spread the flow evenly across a trickling filter surface or other process unit.

DIVERSION CHAMBER - A basin or tank that may be used to divert part of the flow from a channel. May or may not contain treatment capabilities or a means of returning the diverted flow to the treatment plant when a shock load has passed.

DOSING SIPHON - A device to permit intermittent dosing, such as for a trickling filter. Consists of a chamber that will fill gradually to a fixed level before starting a siphon that permits rapid drainage to the filter or other treatment unit.

DRAINAGE TILE (Filter, or bottom tile) - A vitrified tile underdrainage system laid on the bottom to support trickling filter stone, sand, or other filter media, including sludge drying beds. These are specially prepared blocks or half-tiles containing slots for passage of water or air but restricting bed media penetration.

DRYING - The removal of water by natural or engineered means.

DYNAMIC HEAD, TOTAL - The difference in pressure at the elevation of the pump discharge and the elevation at the pump suction flange, plus the velocity head at the discharge minus the velocity head at the suction flange, all corrected to the same units and datum points.

ECOLOGY - The relation of an organism to its environment, i.e., how is an organism affected by his surroundings such as air, water, heat, noise, contamination, etc.

EFFICIENCY - The ratio of materials out of a process to those into that process usually expressed as a percentage.

EFFLUENT - A liquid or product water discharged from a chamber, basin or other treatment operation.

ELEMENT -

a) Elementary substance.

b) A substance or kind of matter in which all atoms are alike in that they will have the same average relative weight and the same number of external electrons.

ELUTRIATION - A washing operation. Sludge elutriation is an action where digested or process sludge is washed with sewage or effluent to remove fine particulates or certain soluble components. The elutriate is recycled to process waters, the elutriated solids are more readily filtered.

ENDOGENOUS METABOLISM - A diminished level of metabolism in which various materials previously stored by the cells are oxidized.

ENTERIC ORGANISMS - Those organisms commonly associated with the intestinal tract.

ENTRAINMENT - A condition or action that will cause an immiscible substance to be mixed with another. Usually the result of turbulence or entrapment, i.e., air bubbles in aqueous media.

ENZYME - A soluble or colloidal organic catalyst produced by a living organism. Usually they are simple or conjugated proteins that catalyze specific reactions.

EQUIVALENT -

a) Equal in force, amount, or value.

b) Chemical The atomic or molecular weight of one substance that will react with one unit of weight of another substance, i.e., that weight of an

alkali necessary to precisely equalize 1 gram atomic wt. of H^+ ion.

EUTROPHIC - Well nourished, rich in dissolved nutrients.

EUTROPHICATION - An action involving the aging of lakes characterized by nutrient enrichment and increasing growth of plant and animal organisms. The net effect is to decrease depth until the lake becomes a bog and eventually dry land. Man-made pollution tends to hasten the process.

FACULTATIVE BACTERIA - Bacteria that can adapt themselves to growth and metabolism under aerobic or anaerobic conditions. Many organisms of interest in wastewater stabilization are among this group.

FAHRENHEIT - A temperature scale in which pure water at sea level has a freezing point at 32 ^{o} and the boiling point is 212 ^{o}.

FATS - Naturally occurring compounds functioning as storage products in the living organisms. Consist of carbon, hydrogen and oxygen in the form of fatty acid esters. Generally semi-solid or oily at normal temperatures.

FECAL COLIFORM - A group of organisms belonging to the coliform group and whose presence denotes recent fecal pollution from warm-blooded animals. Standard tests are available to differentiate the fecal coliform group from the other members of the group which have a lesser sanitary significance.

FERMENTATION - A form of respiration by organisms which requires little or no free oxygen, yielding alcohol and carbon dioxide as end products and releasing only part of the food energy available; i.e., the conversion of sugars into alcohol by enzymes from yeasts.

FILTER - A porous media through which a liquid may be passed to effect removal of suspended materials. Filter media may include paper, cloth, sand, prepared membranes, gravel, asbestos fiber, or other granular or fibrous material.

FILTER CLOTH - Fabric, wire or other material stretched over the drum of a vacuum filter and accessories to support the solids during cake formation and discharge the solids when and where desired.

FILTER FLOODING - The filling of a trickling filter with liquid to a level above the media by closing all outlet parts. Generally to control nuisance organisms such as flies.

FILTER FLY - Small black flies commonly found in or near the trickling filter. Commonly the Psychoda group.

FILTER LOADING - The mass (or volume) of applied oxygen demand or solids per unit of filter area or volume. See load ratio.

FILTER MEDIUM - Any material over which water sewage or other liquid is passed for purification purposes by chemical, biological or physical processes.

FILTER PONDING or CLOGGING - The effect of fine particles on sand filters or organic growth on trickling filters that restricts normal passage of liquid through the filter as a result of filling void spaces.

FILTER RESIDUE - That material which is retained on or in a filter.

FILTER UNLOADING - A phenomenon in which normally attached growth or slime on trickling filter media becomes detached and either partially or completely sloughs off.

FILTRATE - That liquid which has passed through a filter.

FILTRATION RATE - A rate of application of water or wastewater to a filter. Commonly expressed in million gallons per acre per day or gallons per square foot per min.

FINAL SETTLER, CLARIFIER - A settling basin or chamber for the mixed liquor following secondary treatment.

FLAME ARRESTOR - A safety device in the handling of flammable gases. Usually consists of an enlargement in a pipe line containing a metallic

grid that allows passage of gas but acts as a barrier to the passage of flame.

FLIGHTS - A cross member of a conveyor system used for collection and transport of the collected material, i. e., the boards fastened to a chain loop on either side of a primary clarifier that pushes scum along the surface to a collector trough and sludge along the bottom to the sludge collector.

FLOAT CONTROL - Commonly a device to control a pump or pumps according to the water level in a chamber or well as indicated by the float. Usually operates a relay to control pump power, number, or speed of pumps in operation.

FLOATATION - A process for separation of solids from clarified liquid that causes particulates to be floated to the surface by means of attached air globules.

FLOATING COVER - A gas tight cover with a water seal supported by digester gas pressure and capable of moving upward or downward with liquid and gas content of the digester.

FLOC - Gelatinous or amorphous solids formed by chemical, biological or physical agglomeration of fine materials into larger masses that are more readily separated from the liquid.

FLOCCULATION - The gathering together of fine particulate materials in a suspension to form loosely associated larger masses of solids agglomerates.

FLUME - A long narrow channel for gravity flow of liquid from one point to another.

FLY AWAY BOD - Wastewater stabilization operations such as trickling filters encourage the development of insect larvae that serve as scavengers during their development. If the adult form of the larvae have functionable wings, the equivalent of oxygen demand consumed during development becomes fly-away BOD.

FREE AVAILABLE CHLORINE - Generally includes that chlorine existing in water as the hypochlorous acid. Characterized by rapid color formation with ortho tolidine. Can be titrated in neutral solution with phenyl arsene oxide and produces a rapid organism kill in low concentrations.

FREE BOARD - The vertical distance from the normal water level in a flume, conduit, channel, basin, or other water enclosure, to the top of the confining structure.

FRESH SLUDGE - Recently deposited sludge from sedimentation tanks that has not been conditioned, processed, or progressed materially into the anaerobic action stage.

FUNGI - Simple or complex organisms without chlorophyll. The simpler forms are one-celled, higher forms have branched filaments and complicated life cycles. Examples are molds, yeasts and mushrooms.

FWPCA - Federal Water Pollution Control Administration, U. S. Department of the Interior.

GAS DOME - A chamber usually mounted on top of the digester cover for separation of gas from scum, foam or liquid.

GAS HOLDER - A tank used for storage of gas from sludge digestion units for the purpose of meeting the gas demand for burners, engines, or other use during non-steady production or use periods.

GATE CHAMBER or GATE HOUSE - A chamber installed for housing devices for controlling flow to various parts of a collection, treatment or distribution system, including valves, gates, and automatic or manual controls.

GAUGE -

a) The action of measuring some item such as flow, level, size, rate, etc.

b) A device for gauging.

c) A size designation or indicator for some definite item such as 20-gauge wire, 16-gauge sheet steel.

GERMICIDE - An agent that kills microorganisms.

GRAVITY SYSTEM - A system of open or closed conduits in which the liquid flows by gravity (without pumping).

GRIT - The heavy material in water or sewage such as sand, gravel, cinders, etc.

GRIT CHAMBER - An enlargement of a channel designed to reduce flow velocity adequately to permit differential separation of sand or grit from organic suspended material. Usually approaches a linear flow velocity of 1 to 3 ft/sec.

GRIT COLLECTOR - A device placed in a grit chamber to collect and to convey the more coarse and dense grit particles out of the chamber and permit return of most of the organic or liquid materials.

HARDNESS - Commonly refers to the chemicals interfering with soap action or producing scale in boilers or heating units. Specifically refers to Calcium and Magnesium salts, sometimes including iron, aluminum, and silica.

HEAD LOSS - The difference in pressure between the inlet and outlet pressure of a given process unit arising as a result of flow resistance within the process unit, such as the head loss due to friction of a filter media and filter residue.

HEAT EXCHANGER COILS - A piping layout designed to circulate a liquid media within the contents of the process unit but without mixing with the process media for the purpose of adding or removing heat, i.e., hot liquids may be circulated within a digester to raise digester temperature.

HUMUS - A brown or black complex and variable material resulting from decomposition of plant or animal matter.

HYDROLYSIS - The addition of water to any chemical compound. Commonly involved in splitting complex substances by addition of water to form more simple compounds.

HYDROSTATIC HEAD - The pressure exerted by a given height of liquid above a given datum point. May be listed in feet of head, pounds per square inch, or other criteria.

IMPELLER - A rotating set of vanes to impart motion to a fluid, commonly within a casing where dynamic energy of fluid increases from the center to the tip of the vanes. May be closed or open depending on a tube or paddle configuration.

IMHOFF CONE - A conical glass container commonly one liter capacity, having the upper larger diameter end open and the closed apex downward with graduations to assist estimation of the volume of settleable solids after an arbitrary time interval for settling (usually one hour).

IMHOFF TANK - A deep two-story tank originally patented by Karl Imhoff. The floor of the upper chamber is slotted for transfer of settleable solids from the settling chamber. The lower chamber serves for anaerobic digestion and storage of solids.

INDICATOR - May include the color change of a dye, electronic sensor response, or other means of estimating the equivalence point of a reaction between two different materials.

INFILTRATION -

a) The entrance of ground water into a sewer through breaks, defective joints, or porous walls.

b) The penetration of water through the soil from surface precipitation, stream or impoundment boundaries.

INFLUENT - That material entering a process unit or operation.

INORGANIC - Being composed of material other than plant or animal materials. Forming or belonging to the inanimate world.

INTERCEPTOR - An intercepting sewer designed to carry the dry weather flow from a community to a treatment plant, but not large enough to carry storm water above some preset ratio to dry weather flow. May be used to collect lateral sewer flows.

LAGOON - A natural or artificial basin used for storage and/or stabilization of wastewater or sludge. Sometimes used for indefinite storage for disposal purposes. Commonly the lagoon depth is greater than a wadable depth but not greater than twenty feet.

LATERAL SEWER - A sewer that discharges into a branch or main sewer and has no other tributaries other than individual house connections.

LIQUID SLUDGE - An organic solids concentrate usually formed by deposition from wastewaters. The water content varies with the origin and nature of the sludge, usually has enough water to permit pumping but does not contain significant separatable free water.

LOAD - The load to a process is that which is contained in the inflow to that process. It may be expressed as hydraulic, oxygen demand, solids, or other criteria.

LOAD RATIO - An index of loading, including mass input per unit of capacity per unit of time. Mass may be expressed in lbs., BOD, COD, Susp. or volatile solids, capacity in volume, weight of solids or volatile solids in process, and time, usually in days.

LYSIS - To decompose, loosen, or separate into component parts.

MANHOLE - An opening by which access may be achieved for inspection, maintenance, or repair of a sewer, conduit, or other buried structure or appurtenance.

MANOMETER - An instrument for measuring pressure. Usually consists of a U-shaped tube containing a liquid, the surface of which moves proportionally in one open end with changes in pressure exerted upon the other end.

MECHANICAL AERATION - Aeration produced by mechanical energy of the turbine, pump, paddle, or other device that imparts an intimate mixture of liquid and air.

MEMBRANE FILTER - A flat, highly porous flexible plastic disc, commonly about 0.15 mm in thickness and 47-50 mm in diameter. Membrane filters having a pore size of 0.45 μ are used in water microbiology to trap organisms, and, by use of standard media and conditions, direct enumeration by colony count of selected organisms.

MENISCUS - The curved upper surface of a liquid in a tube that is concave upward when the containing walls are wetted by the confined liquid, and convex upward when they are not.

MESOPHILLIC - Medium temperature loving. Organisms capable of optimum metabolic activities at temperatures from about 80°-110°F, 26°-42°C.

METER - The length of a reference platinum bar used as a standard unit of measurement of length in the metric system. 1 meter = 39.37 inches.

MICRO - 1/1,000,000 of a unit of measurement, such as microgram, microliter.

MICROBIOLOGY - The science and study of microbiological organisms and their behavior. Commonly related to the study of pathogenic organisms.

MICRO ORGANISM - Commonly an organism too small to be observed individually by the human eye without optical aid.

MILLI- - An expression used to indicate 1/1000 of a standard unit of weight, length or capacity (metric system).

Milliliter	(ml)	1/1000 liter	(l)
Milligram	(mg)	1/1000 gram	(g)
Millimeter	(mm)	1/1000 meter	(m)

MILLI EQUIVALENT WEIGHT - 1/1000 of the equivalent weight, usually expressed in milligrams (mg).

MG/L - A unit of concentration on a weight/volume basis: Milligrams per liter. Equivalent to ppm when the specific gravity of the liquid is 1.0.

MIXED LIQUOR - A mixture of return sludge and wastewater in the aerator of an activated sludge plant. Also may be used in reference to mixed aerobic or anaerobic digesters.

MIXING ZONE - An area where two or more substances of different characteristics blend to form a uniform mixture; i.e., chlorine application, heated water, or other discharged materials entering a water mass will show significant differences of chlorine residual, temperature or other criteria, depending upon the sampling location throughout the mixing zone and approach uniform results with respect to lateral,longitudinal,and vertical sampling positions when mixing has been completed.

MOISTURE CONTENT - The content of water in some other material. Commonly expressed in percentage of moisture in soil, sludge or screenings.

MOST PROBABLE NUMBER (MPN) - A statistical method of determining microbial populations. A multiple dilution tube technique is utilized with a standard medium and observations are made for specific individual tube effects. Resultant coding is translated by mathematical probability tables into population numbers.

NITRIFICATION - The biochemical conversion of unoxidized nitrogen (ammonia and organic N) to oxidized nitrogen (usually nitrate).

NORMALITY -

a) A means of expressing the concentration of a standard solution in terms of the gram equivalents of reacting substances per liter.

b) Generally expressed as a decimal fraction, such as 0.1 or 0.02 N.

NUTRIENTS -

a) Anything essential to support life.

b) Includes many common elements and combinations of them. The major nutrients include carbon, hydrogen, oxygen, nitrogen, sulfur, and phosphorus.

c) Nitrogen and phosphorus are of major concern because they tend to recycle and are hard to control.

ODOR CONTROL - In wastewater treatment this generally refers to good housekeeping in the plant and aeration, chlorination or other operations to prevent onset of malodorous septicity in the wastewater flow.

OILS -

a) Liquid fats of animal or vegetable origin.

b) Oily or waxy mineral oils.

ORGANIC - Substances formed as a result of living plant or animal organisms. Generally contain carbon as a major constituent.

ORGANIC CHLORINE - Compounds containing chlorine in combination with carbon, hydrogen and certain other elements.

ORIFICE METER - A device consisting of a flange set in a pipe section containing an opening smaller than the pipe. Pressure readings above and below the orifice may be related to flow.

ORTHO TOLIDINE CHLORINE TEST - The dye, ortho tolidine, under highly acid conditions, produces a yellow color proportional in intensity to the concentration of available residual chlorine and certain other oxidants or interfering materials.

OUTFALL SEWER - The outlet or channel through which sewage effluent is discharged.

OXIDATION - Chemically: The addition of oxygen, removal of hydrogen, or the removal of electrons from an element or compound.

OXIDATION POND - A shallow basin employed for the stabilization of wastewaters.

OXYGEN AVAILABLE - That part of the oxygen available for aerobic stabilization of organic matter. Includes dissolved oxygen and that available in nitrite or nitrates, peroxides, ozone, and certain other forms of oxygen.

OXYGEN BALANCE - Refers to the dynamic relationship among the available oxygen assets and the oxygen requirements for stabilization of oxygen demanding materials in a treatment plant or receiving water.

OXYGEN DEPLETION - The loss of oxygen from water or sewage due to biological, chemical or physical action.

PARASITE - A living organism deriving its nutrients at the expense of another living organism, giving nothing in return.

PARSHALL FLUME - A device for estimation of the flow in an open conduit. Consists of a constricting section, a throat, and an expanding section. The throat contains a sill over which the liquid passes. The pressure change over the sill can be related to quantity of flow.

PARTICULATE MATERIAL - Refers to detectable solid material dispersed in a gas or liquid. Small sized particulates may produce a smoky or hazy appearance in a gas; milky or turbid appearance in a liquid. Larger particulates are more readily detected and separated by sedimentation or filtration.

PARTICULATES - Pertaining to small suspended solids in a gaseous or liquid media.

PARTS PER MILLION (PPM) - A unit of concentration signifying parts of some substance per million parts of dispersing medium. Equivalent numerically to mg/l only when the specific gravity of the solution is 1.0.

PATHOGENIC ORGANISMS - Bacterial, fungal, viral, or other organisms directly involved with diseases of plants, animals, or man, are included among this group.

PERCENTAGE TREATMENT - The ratio expressed as a percentage of the material removed from process water in terms of the material entering. Sometimes referred to as reduction.

pH - An index of hydrogen ion activity. Defined as the negative logarithm (base 10) of H^+ ion concentration at a given instant. On a scale of 0 to 14 pH 7.0 is neutral, pH less than 7.0 indicates a predominance of H^+ or acid ions, pH greater than 7.0 indicates a predominance of OH^- or alkaline ions.

PNEUMATIC EJECTOR - A device for pumping sludge, sewage, or other liquid by admitting the fluid into a chamber through one check valve and forcing it out of another by air pressure in the chamber above the liquid.

POLLUTION - Anything appearing in water that renders it unacceptable in terms of established water quality standards. Commonly conditions or contaminants that interfere with subsequent beneficial uses of the water.

POND - A basin or catchment used for retention of water for equalization, stabilization, or other purposes. Commonly less than five feet in depth.

PONDING - With reference to trickling filtration, ponding refers to a plugging of the filter media by slimes or solids to restrict downward movement of wastewater sufficiently to cause surface accumulation of liquid either partially or completely.

PRECIPITATE - The formation of solid particles in a solution, or the solids that settle as a result of chemical or physical action that caused solids suspension from solution.

PRESSURE - The total load or force acting upon a surface. In hydraulics, the term commonly means pounds per square inch of surface, or kilograms per square cm above atmospheric pressure on site. (Atmospheric pressure at sea level is about 14.7 pounds per square inch.)

PRIMARY SLUDGE - Sludge obtained from a primary sedimentation tank.

PRIMARY TREATMENT - Commonly the separation of settleable or floatable materials from carrier water. Usually preceded by pretreatment such as coarse screens, grit separation, comminution.

PROCESS - A series of operations or actions that lead to a particular result. A combination of unit operations that may be assembled and used for a given treatment objective.

PROTEINS - Naturally occurring compounds containing carbon, hydrogen, nitrogen, and oxygen, with smaller amounts of sulfur and phosphorus, and trace components essential to the living cells. An essential food associated with meat and eggs.

PROTOZOA - Single cell or multiple cell organisms, such as amoeba, ceiiates, and flagellates. Commonly aquatic and generally derive most of their nutrition from preformed organic food.

PSYCHROPHILLIC ORGANISMS - Low temperature loving organisms, or having a competitive advantage over other organisms at lower temperatures; i. e., from about 10°C downward to the freezing point.

PUTREFACTION - Biological decomposition of organic matter with the formation of ill-smelling products, such as H_2S, amines, mercaptans. Associated with anaerobic conditions.

QUIESCENT - Characterized by a lack of or negligible movement of the suspending media, such as liquid or gas. Still or absence of turbulence.

RAW WASTEWATER (SEWAGE) - Used wastewater prior to treatment.

RECIPROCATING PUMP - A pump device using a piston within a casing fitted with suction and discharge valves. Movement of the piston in one direction fills the casing, the reverse movement forces liquid into the discharge line. May be vertical or horizontal.

RECIRCULATION - The return of effluent to the influent of a process unit to reduce influent concentration, stabilize the system, maintain hydraulic flow, to reprocess, or for other beneficial reasons.

RECORDER -

a) A device to keep a continuous or intermittent record of some measured item such as flow, velocity, applied power, etc.

b) An individual who tabulates or maintains records of events, actions or measurements.

REDUCTION -

a) To make smaller or to remove from a given amount of material

b) Chemistry: The removal of oxygen, addition of hydrogen, or the addition of electrons to an element or compound.

RELIEF SEWER - A sewer built to carry the flow in excess of the capacity of an existing sewer.

RESIDUAL CHLORINE - See Available Residual Chlorine.

RETURN SLUDGE - Sludge returned from process to the influent flow. Commonly return activated sludge from a secondary clarifier. Also may include sludge from a clarifier after trickling filtration.

ROTARY DISTRIBUTOR - A device usually mounted on a center post with horizontal arms extending to the edge of a circular trickling filter for distribution of flow over the entire bed surface.

SALT - A chemical compound formed as a result of the interaction of an acid and an alkali (base). The commonest salt is sodium chloride formed from hydrochloric acid and sodium hydroxide. This ionizes in water solution to form Na^+ and Cl^-.

SANITARY SEWER - A sewer designed to receive and to convey household, commercial or industrial wastewater mixtures.

SAPROBIC, SAPROPHYTIC - Organisms living upon dead or decaying organic matter. Organisms that utilize nonliving organic matter as a food.

SATURATION - Commonly refers to the maximum amount of any material that can be dissolved in water or other liquid at a given temperature and pressure. For oxygen, this commonly refers to a percentage saturation in terms of the saturation value, such as about 9 mg O_2/l at 20°C.

SCAVENGERS - Organisms that feed habitually upon refuse or carrion. In water pollution, this commonly refers to worms, insect larvae, bloodworms, sow bugs, and crustaceans. Or, more properly, oligochaetes, chironomids and isopods.

SCREEN - A device with openings, generally having a relatively uniform size, that permit liquid to pass but retain larger particles. The screen may consist of bars, coarse to fine wire, rods, gratings, paper, membranes, etc., depending upon particle size to be retained.

SCREENINGS - Material removed by the screens.

SCUM BOARD - A vertical baffle, above and below the liquid surface of a basin or tank, designed to prevent the passage of or to contain floating material within designated limits.

SCUM BREAKER - A device installed in a sludge digestion tank to disperse surface accumulations. Generally accomplished by means of mechanical agitation, gas or liquid recirculation, to promote mixing and destratification.

SCUM COLLECTOR - A mechanical device for skimming and removing scum or floatable material from the surface of a tank.

SECOND FOOT - An abbreviation for cubic foot per second. A rate term.

SECONDARY TREATMENT - Processes used to convert dissolved and colloidal materials in wastewater to a form that may be separated from the water. Commonly consists of biodegradation and conversion to cell mass in a separatable form with partial oxidation, such as in activated sludge, trickling filtration, or oxidation ponds.

SEDIMENTATION - The process of subsidence and deposition of suspended matter from wastewater by gravity. Also called clarification, settling.

SEPTIC SLUDGE - That sludge which has reached a stage of anaerobic putrefaction (sulfate reduction). Includes that from Imhoff, septic, or sludge digestion tanks.

SEPTIC WASTEWATER (SEWAGE) - Wastewater in which available oxygen has been depleted and the reduction of sulfates has begun. A result of anaerobic putrefaction.

SETTLEABLE SOLIDS -

a) Includes materials that will settle by gravity under low flow velocities.

b) Commonly expressed in terms of the volume of solids accumulating in an Imhoff cone after one hour on a volume basis.

SETTLING BASIN - A natural or engineered enlargement of a channel that reduces velocity sufficiently to permit sedimentation of settleable particulates.

SEWAGE - See Wastewater.

SEWAGE GAS, DIGESTER GAS - The gas produced from anaerobic (septic) sewage solids. Generally contains marsh gas (methane) and carbon dioxide with hydrogen sulfide and other components in minor proportions.

SEWER - A pipe or conduit, generally covered, for the purposes of conveying wastewaters from the point of origin to a point of treatment or discharge.

SEWERAGE SYSTEM - A system of sewers and appurtenances for the collection, transportation and pumping of used waters for a given area or basin. Any treatment device or facility and its outfall conduit are a part of the system.

SHORT CIRCUITING - Hydraulic: A condition in which one part or unit of flow into the basin reaches the outlet in much less time than that required for a uniformly mixed flow. Electrical: A situation in which an electric current is out of place in relation to its controlled pathway.

SLIME - SEWAGE SLIMES - Consisting of organisms growing on wastewater nutrients with the formation of mucilaginous covering, streamers or clumps. May consist of bacteria, molds, protozoa or algae.

SLOUGHING - A phenomenon associated with trickling filters and contact aeration units where slimes build up to a varying degree, then slip off into the effluent.

SLUDGE - Accumulated or concentrated solids from sedimentation or clarification of wastewater. Contains varying proportions of solids in wastewater depending upon source, process, and nature.

SLUDGE BANKS - An accumulation of solids including silt, mineral, organic, and cell mass particulate material, that is produced in the aquatic system characterized by low current velocity, Generally refers to gross deposits of appreciable depth.

SLUDGE CAKE - The solids remaining after dewatering sludge by vacuum, filtration, or sludge drying beds. Usually forkable or spadable, with a water content of 30 to 80%. Also may occur on the boundaries of surface water.

SLUDGE COLLECTOR - A mechanical device, including rake, drag, or suction, for collecting settled sludge from the bottom of a clarifier into a sump or other withdrawal system.

SLUDGE DIGESTION - A process by which organic matter in sludge is converted into more stable or separatable form through the action of living organisms. May be the result of aerobic or anaerobic digestion.

SLUDGE DRYING BED - An area used to discharge wet sludge for drainage and drying. Generally prepared of porous bed material surrounded by sidewalls to contain the sludge while the liquid percolates into an underdrain system. May be covered or uncovered.

SLUDGE FILTER - A device to effect partial water removal from wet sludge,usually with the aid of vacuum or pressure of preconditioned sludge.

SLUDGE SYNTHESIS - The net gain in sludge mass in a process over a period of time as a result of simultaneous growth of cell mass and endogenous oxidation within it.

SLUICE GATE - A gate constructed for adjustment to control the flow in a channel by gate position.

SOLUTION -

a) An homogenous mixture, commonly gas, liquid, or solid in a liquid that remains clear indefinitely.

b) Generally an atomic, ionic, or molecular dispersion in a liquid (may be colored).

c) A water solution of dissolved material.

SPECIFIC GRAVITY (Sp. Gr.) -

a) The weight of a material per unit volume in reference to the weight of water at maximum density.

b) Water at 4°C has a weight of 1 g per ml. The weight ratio of any substance divided by the weight of water is the specific gravity.

SQUEEGEE -

a) A device, generally rubber, used for dislodging and removing solids, scum or other materials from a surface.

b) Metal or wood blades to move sludge solids along the bottom of a clarifier.

STABILITY -

a) The ability of any substance to resist putrefaction.

b) Ability of an engineered structure to resist distortion or overturn when loaded.

STABILIZATION -

a) The activity proceeding along the pathway to stability.

b) In organic wastes, generally refers to oxidation via biochemical pathways and conversion to gaseous or insoluble materials relatively inert to further change.

STANDARD - Something set by authority. Having qualities or attributes required by law and defined by minimum or maximum limits of acceptability in terms of established criteria or measurable indices.

STANDARD METHODS - Methods of analysis prescribed by joint action of APHA, ASCE, AWWA, and FWPCA. Methods accepted by authority.

STEP AERATION - A procedure for adding increments of wastewater at various points along the line of flow in an activated sludge aerator.

STERILIZATION - The process of making a medium free of living organisms such as by killing them, filtering through a porous medium fine enough to be a barrier to the passage of organisms, etc.

STORM OVERFLOW - A device such as a weir, dam, or orifice, in a combined sewer that will intercept design flow but permit excess storm flow to discharge directly. The overflow contains a mixed discharge of storm and other sewer components.

STORM SEWER - A sewer which carries storm water from roofs, surface wash and street drainage.

STUCK DIGESTER - Any of a series of events that results in serious malfunction of the digester. Commonly refers to anaerobic digestion where overloading, temperature control, toxicity, or other factors, result in an excessive acid production with serious limitations of gasification, stabilization, and solids concentration.

SUBSTRATE -

a) The base or media in which an organisms lives.

b) The liquid in an activated sludge aeration tank.

SUPERNATANT LIQUOR - The liquid overlying deposited sludge. Commonly that fraction of liquid in an anaerobic digester located over the deposited material and beneath possible surface floating material.

SURFACTANT -

a) A chemical that, when added to water, will greatly reduce the surface tension of the solution.

b) The surface active component in a detergent mixture.

SUSPENDED SOLIDS - The concentration of insoluble materials suspended or dispersed in waste or used water. Generally expressed in mg/liter on a dry weight basis. Usually determined by filtration methods.

SYNERGISM - Refers to the action produced when two or more substances in combination have a greater effect than that produced by the additive effects of each one separately.

TAPERED AERATION - A procedure for adjusting air input along the line of flow of an activated sludge aerator according to need. Usually requires addition of more air per unit of volume at the inlet end of the aerator.

TERTIARY TREATMENT - See Advanced Waste Treatment.

THERMAL POLLUTION - Refers to heated discharges to surface waterways. The largest contributor of heated discharges is associated with power production.

THERMOPHILLIC - High temperature loving organisms. Generally considered to include organisms having a favorable competitive advantage at temperatures above 110°F or 42°C.

THIEF - A term applied to a sampling tube used to remove a core of sample from a bag or bulk material.

TITRATION - The careful addition of a standard solution of known concentration of reacting substance to an equivalence point to estimate the concentration of a desired material in a sample.

TOC - Total Organic Carbon. A test expressing wastewater contaminant concentration in terms of the carbon content.

TOTAL SOLIDS - Refers to the solids contained in dissolved and suspended form in water. Commonly determined on a weight basis by evaporation to dryness.

TRICKLING FILTER - A treatment process employing downward flow of wastewater over the surfaces of a rock or grid system with a large void space for upward movement of air. Slime organisms accumulate to effect biological stabilization.

UNIT OPERATION - A particular kind of a physical change that is repeatedly and frequently encountered as a step in a process such as filtration, aeration, evaporation, mixing, or pumping.

UNITS OF MEASUREMENT -

English - foot, pound, second.
Metric - centimeter, gram, second.
Abbreviations: ft., lb., sec.
cm., g., sec.

USPHS - United States Public Health Service, Department of Health, Education and Welfare.

USPHS DRINKING WATER STANDARDS - A list of standards prescribed for potable water acceptable for use on interstate carriers. Deal with sources, protection, and bacteriological, biological, chemical and physical criteria--some mandatory, some desired. Official for municipal use only upon acceptance by State and local authorities.

VELOCITY (FLOW) - A rate term expressed in terms of linear movement per unit of time. Commonly expressed in ft per sec (English) or cm/sec (Metric).

VENTURI METER - A device for estimating flow of fluid in closed conduits or pipes. Generally based upon constricting and enlarging pipe sections with pressure at the full size and the point of maximum constriction. Differences in pressure can be related to flow.

VIRUS - A term generally used to designate organisms that pass filtration media capable of removing bacteria. Technically described as a collective term covering disease stimuli held by some to be living organisms and by others to be nucleic acids capable of reproduction and growth.

VOLATILE ACIDS - A group of low molecular weight acids such as acetic and propionic, that are distillable from acidified solution.

VOLATILE MATERIAL -

a) Refers to those chemicals having a vapor pressure low enough to evaporate from water readily at normal temperatures.

b) With reference to dry solids, the term includes loss in weight upon ignition at 600°C.

VOLATILE SOLIDS - The quantity of solids in water that represents a loss in weight upon ignition at 600°C.

WASTEWATER - Refers to the used water of a community. Generally contaminated by the waste products from household, commercial or industrial activities. Often contains surface wash, storm water and infiltrations water.

WASTE SLUDGE -

a) Commonly refers to activated sludge produced in excess of that required for return process.

b) Any solids concentrate to be routed for disposal.

WATER QUALITY CRITERIA - Includes selected analytical measurements with limits designated to be acceptable or unacceptable in reference to water quality standards.

WATER QUALITY STANDARDS - Limits set by authority on the basis of water quality criteria required for beneficial uses.

WEIR - A device used for surface overflow from a tank, basin or chamber. Generally designed to smooth out discharge flow to minimize turbulence within the detention basin. May be used to measure discharged flow.

WEIR BOX - An enlargement of the channel upstream of a weir to reduce the velocity and turbulence before reaching the weir.

WELL -

a) An artificial excavation or shaft that collects water from interstices of the soil or rock.

b) Also an engineered structure for the housing of pumps or other equipment below ground level.

WET OXIDATION - Oxidation of substances such as organic contaminants in water media. Includes biological oxidation and physical chemical oxidation, such as that obtained at elevated temperature, pressure, catalyst or other promoters.

WPCF - Water Pollution Control Federation. An organization composed of individuals engaged in the advancement of knowledge in research, design, operation, and control of water pollution in relation to man and his environment.

YIELD -

a) To give up or relinquish.

b) To bear or bring forth as a result of cell division.

c) To produce as a result of investment of energy, materials, or time.

d) The amount or quantity produced per unit of raw material.

YIELD FACTOR - A decimal fraction or percentage of product per unit of input.

ZOOGLOEA - A jelly-like matrix developed by certain microorganisms at some stage in their life cycle. Commonly associated with sludge flocculation in biochemical treatment operations.

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