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Waste Resources Utilization Program
Interim Report
June 30, 1976

Waste Management and Environmental Programs Department

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PREFACE

This is an interim report on the effects of the combined use of heat and ionizing radiation (thermoradiation) as a treatment for ridding sewage sludge of pathogenic organisms as well as its effect on the physical-chemical properties. This activity couples two major environmental problems, disposition of human and of nuclear waste, in an attempt to provide a framework in which both will become useful resources. This combined treatment might be chosen to inactivate both heat labile (but possibly radiation resistant) and radiation labile (but possibly heat resistant) organisms. The cost-effective analyses of such a treatment are being examined.

Sludge treated with thermoradiation offers considerable potential for use as a fertilizer in agriculture or a soil conditioner for land reclamation free of the potential health hazards associated with conventional methods of land disposal. Treated sludge may also provide a low-cost substitute for high-nutritional components in ruminant diets.

In order to determine the feasibility of treating sewage sludge with thermoradiation, a number of parameters have to be examined. These objectives include determining (1) the amount of thermoradiation needed to inactivate the major biological systems (i.e., bacteria, viruses, and parasites) found in sludge that are potentially harmful to humans; (2) the cost of such a treatment versus the value of the benefits from sludge usage; (3) any additional benefits to physical-chemical properties accruable by a treatment process involving irradiation; and (4) the design of optimal treatment facilities whereby actual sludge assessment could be determined.

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IRRADIATION SYSTEMS

Progress reported in this section relates to the development of a series of thermoradiation treatment systems for use with sewage sludge. The first of these, the "milliliter system" was designed primarily to facilitate small-scale biological experimentation. It involves an agitated batch process of a small quantity of sludge in which process parameters are highly controlled. In its design there was no intent that any ultimate treatment plant should operate in the same way.

Indeed, the philosophy guiding ultimate treatment plant design has been one favoring a real-time flow-through processing system in which sludge residence time is a matter of only a few minutes. In order to progress from a small experimental batch processer to a full-scale treatment facility, two intermediate steps were planned.

The first was the development of a real-time flow-through thermoradiation system capable of processing one to several liters of sludge per minute (the "liter system"). This system was intended as both a research facility for studying biological effects and engineering characteristics and as a system capable of processing sufficient sludge for studies of the application of sludge to land and to animal feeding programs.

The second step is to pilot plant level capable of processing up to 20,000 gallons of sludge per day (some ~15 gallons per minute).

Milliliter System

The milliliter thermoradiation system was developed to study the microbiology of sewage sludge for the purpose of establishing heat, radiation and thermoradiation treatment criteria for the elimination of pathogens.

The source elements used for the construction of the irradiator for this system consist of 26 pins of cesium-137 chloride that are double-encapsulated in stainless steel. Each pin is 2.79 cm in diameter by 30.5 cm in length. The total activity of the 26 pins is 208 KCi. The irradiator that uses these pins is illustrated in Figs. 1 and 3. The pins are placed two deep in an annular array so as to produce a fairly uniform field in the interior of the annular region. The effective volume of uniform field strength is 10 cm in diameter by 40.6 cm in length. The field strength in air in this region is approximately 850 rads/sec and is fairly uniform.

The apparatus used to heat the sample of sludge is shown schematically in Fig. 1. A 160 mℓ sludge sample is heated in a coaxial assembly with heat being applied to both the inner and outer annular surface of the liquid. The inner coaxial surface is a plunger that is alternately raised and lowered to provide mechanical agitation. Water from a water bath is recirculated through the outer jacket and the plunger. A sample temperature *versus* time curve for the apparatus is shown in Fig. 6. For most temperatures, the heat-up time is several minutes.

Six spring loaded syringes that are activated sequentially by remotely located timers take the sludge samples through stainless steel tubing to an ice cooled reservoir at selected times.

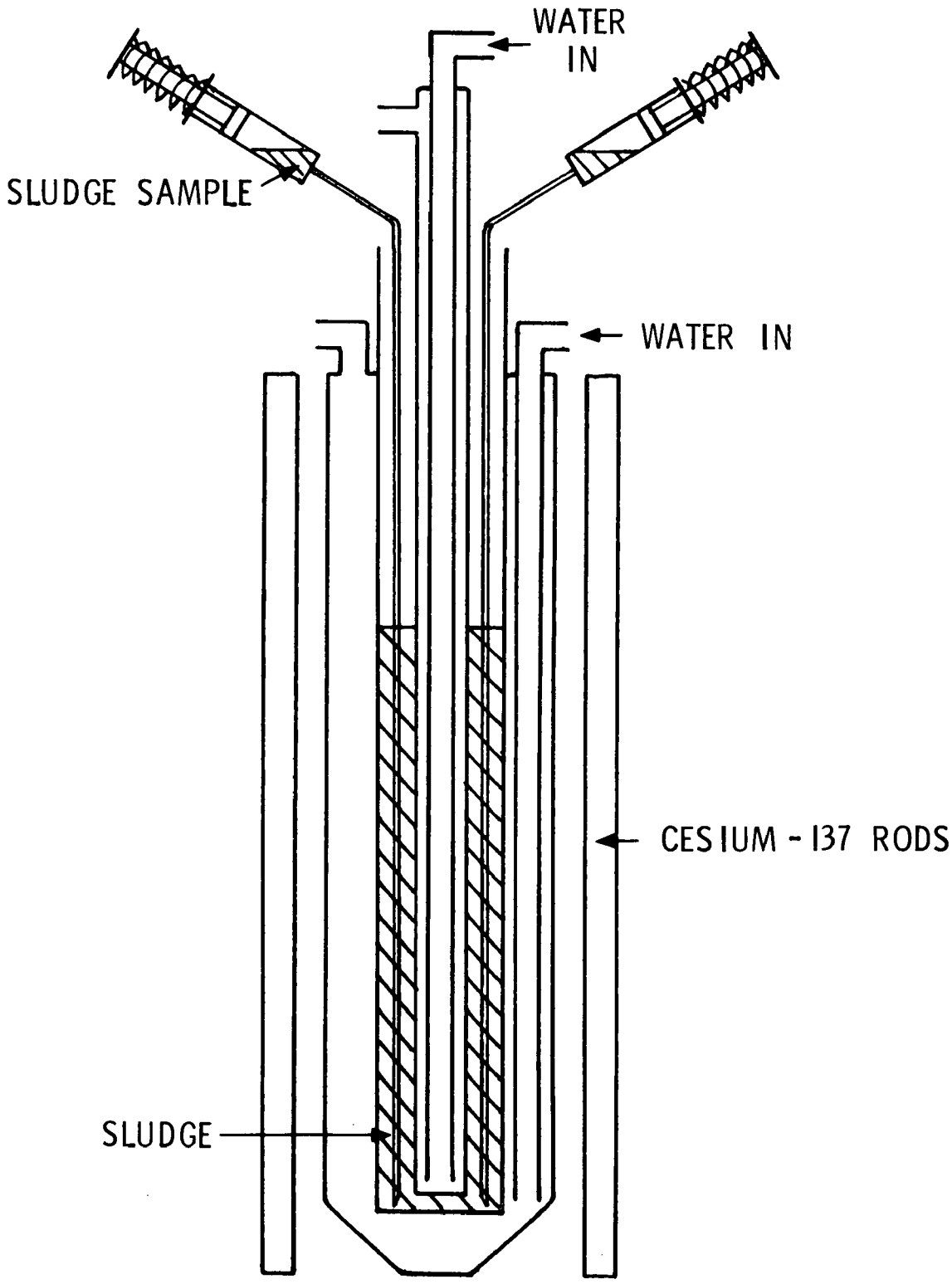


Figure 1. Schematic Drawing of Milliliter System

The apparatus described above is used in the following manner in an experiment. First, the water bath is turned on and the temperature of the water jacket allowed to reach a predetermined level with the water recirculating. Control of this temperature can be exercised within ±0.2° C over the range from approximately 5° C to 90° C. The irradiator is stored in approximately 6 m of water. To initiate the test, the irradiator is raised into a concrete shielded room. The heating apparatus described above is surrounded by the annular shaped irradiator. As the irradiator is raised, the sludge, or other type sample, is injected into the coaxial heating assembly. The temperature is elevated to the preset value before the sample begins receiving treatment (dose rate is 30 krads/min). Lead shields allow this dose rate to be reduced by a factor of four when desired. At preselected times, the sludge is remotely sampled using electrical signals fed in by timers located outside the shielded room. After the test, the irradiator is lowered into the pool and the sludge samples removed for analysis.

This apparatus has been the mainstay of the biological experimentation using thermoradiation at Sandia Laboratories. Hundreds of experiments on fecal strep, fecal coliform, Salmonella and poliovirus have been performed with this system.

Liter/Minute Flow-Through System

The flow-through system was designed to treat larger quantities (10,000 to 20,000 ℓ) of sewage sludge. After treatment it was then dried to ~25 percent solids. The dried sewage sludge is being used for animal refeeding experiments and fertilizer trials at New Mexico State University. The information gained from this system aided in the design of a pilot plant for the City of Albuquerque.

An effective pasteurization treatment was developed during experimentation using the milliliter system. Sludge is irradiated to 150 krads at a temperature of 65° C with a 5 minute residence time in the irradiator. Hardware was designed using the irradiator described in the last section to treat approximately 1 ℓ/min to these specifications. The irradiator and the treatment unit are shown in Fig. 3.

A flow schematic for the system is shown in Fig. 2. Sludge from Albuquerque's Water Reclamation Plants No. 1 and 2 was brought by truck in containers to the irradiation area. The sludge was then transferred in the plenum shown at the start of the system (Fig. 2). A recirculating pump was used to stir the sludge and to provide a small positive pressure for the inlet to the tubing pump that metered the sludge flow. The sludge was then pumped through counterflow heat exchangers. A 36 kW hot water heater was the heat source for the heat exchangers. Heated sludge was then pumped into the irradiator chamber, through the irradiator, and back out of the chamber.

The counterflow heat exchangers were fabricated from 1.25 cm stainless steel tubing and 1.9 cm galvanized pipe. At the low flow rate of the sludge (~1 ℓ/min) a point of concern was the adequacy of heat transfer across the stainless steel tubing. The equation describing an idealized counterflow heat exchange is given by

$$G = UA \left[\frac{\Delta t_2 - \Delta t_1}{\ln(\Delta t_2 / \Delta t_1)} \right]$$

where:

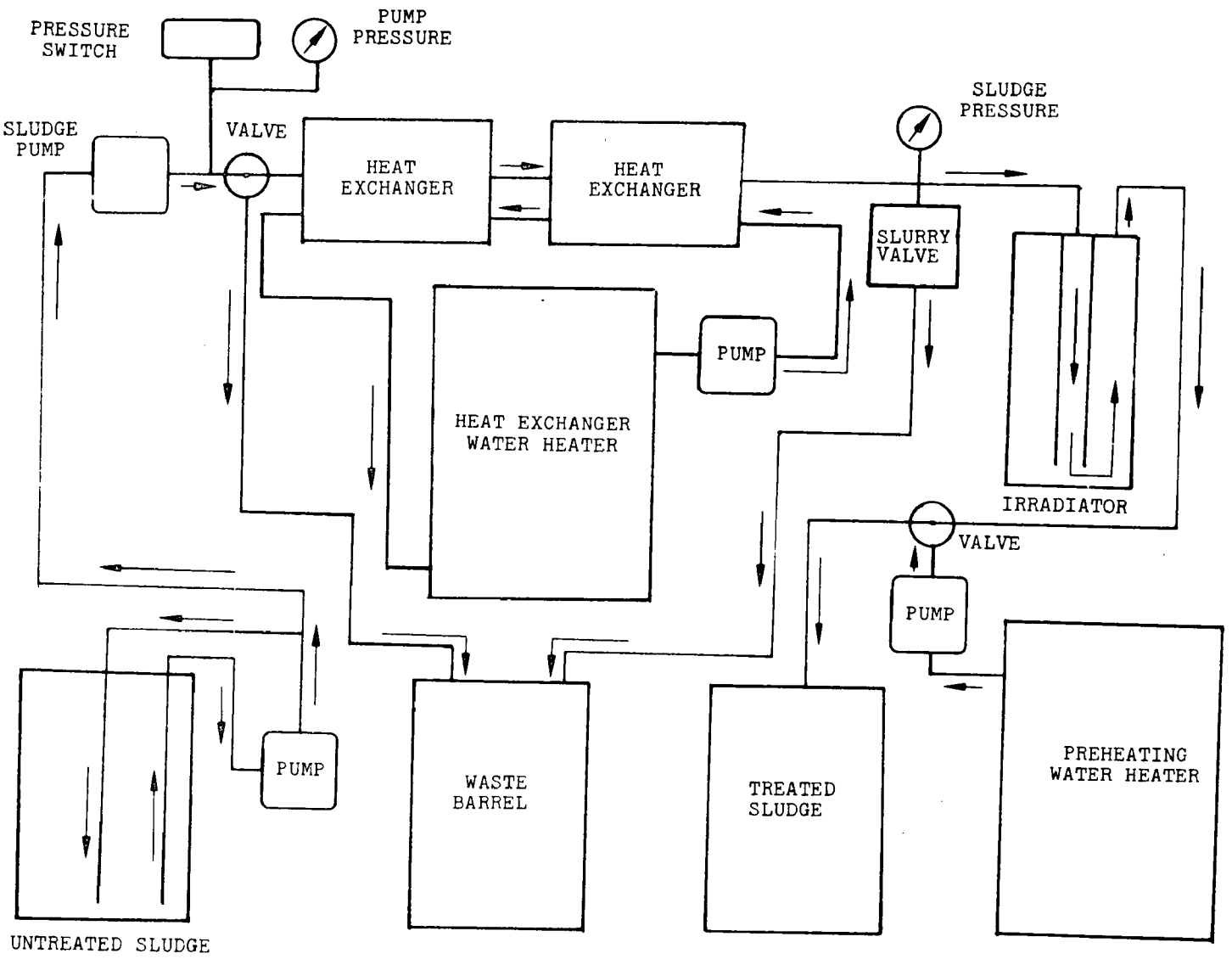


Figure 2. Flow-Through Thermoradiation System

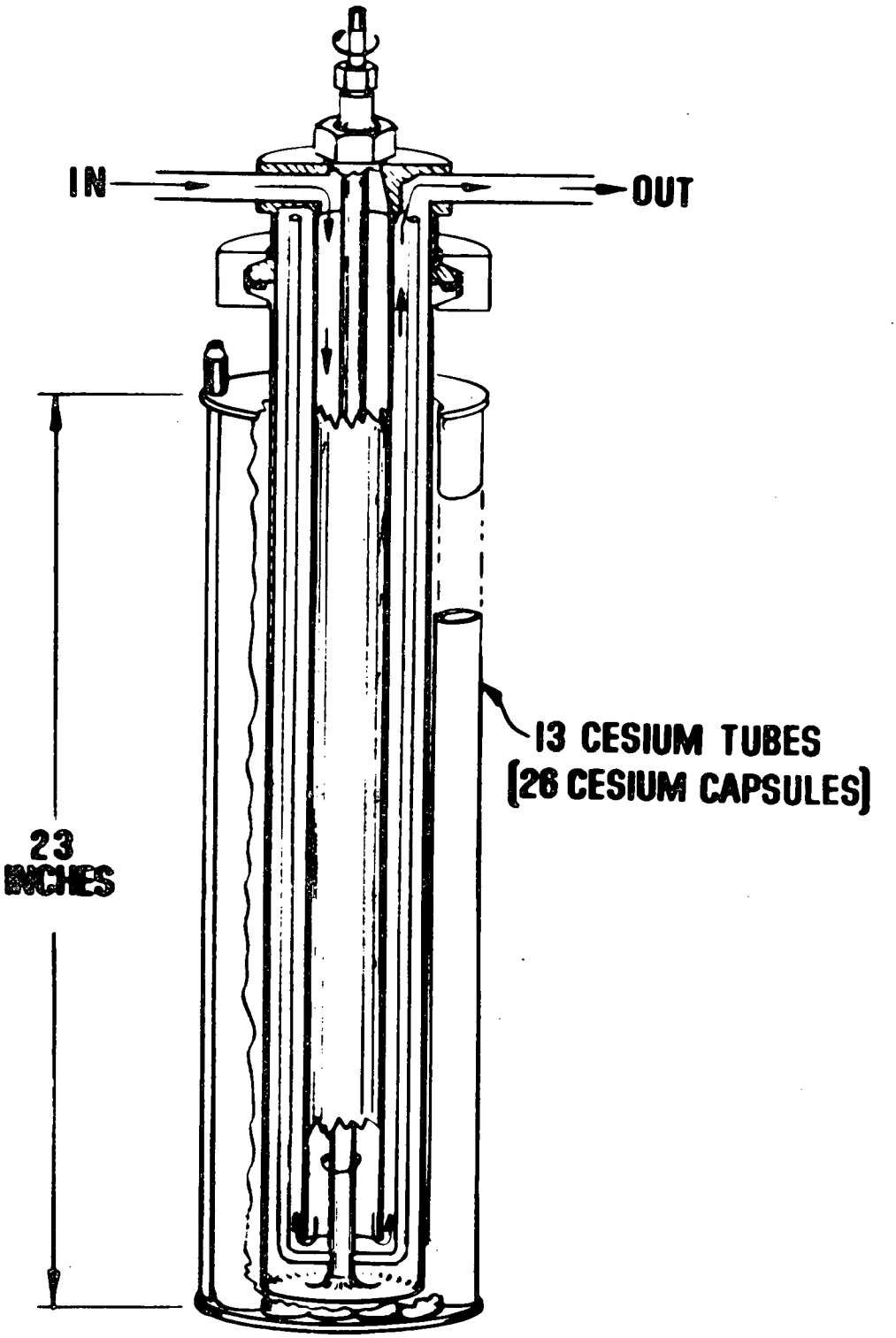


Figure 3. Irradiator

G = heat transfer rate for the entire heat exchanger

U = heat transfer rate/unit area - unit temperature

A = area of exchanger

Δt_1 = temperature difference at one end of exchangers

Δt_2 = temperature difference at other end of exchangers

For a flow situation similar to what was used in practice, G, Δt_1, and Δt_2 were measured and A was taken to be the area of the stainless steel tubing. The result

$$U = 1.1 \text{ cal/°C - cm}^2 \text{ - min}$$

was obtained. For very turbulent flow, one can obtain a theoretical maximum of

$$U = 28.6 \text{ cal/°C - cm}^2 \text{ - min}$$

for stainless steel. Therefore the low flow rates with consequent nonturbulent flow, quite effectively reduce the heat transfer in heat exchangers, and this effect must be accounted for in plant design. The easiest way to correct the situation is to increase the area of the heat exchangers, although later the problem of increasing U will be addressed. Using exchangers of this type, heat recoveries of over 50 percent have been measured at flow rates similar to those of the flow-through system.

A fiberglass holding tank held the sludge until it was carried to the centrifuge. The sludge was then dewatered using a Sharples P-660 Super-D-Canter Centrifuge. Polymers from Hercofloc were used to enhance dewatering by the centrifuge. The moist cake was then dumped into drying tanks for final drying.

Approximately 24,000 liters of sewage sludge (both digested and raw) have been treated with the thermoradiation system described above.

In addition to the biological inactivation parameters studied with this system, it has provided sufficient quantities of treated sludge for some physical and chemical studies.

Proposed Pilot Plant

The progression from "bench-model" thermoradiation apparatus to a system of treating larger quantities of sewage sludge has made the design of a full-scale facility necessary. The logical choice appears to be a pilot plant located near an existing sewage treatment plant.

A pilot plant would provide the capabilities for studying the following: definition of parameters for demonstration facility; economics of thermoradiation treatment; additional microbiological inactivation and regrowth studies; sludge dewatering and transport; irradiator design; cattle feeding and/or land application on a larger scale; and treatment of digested, undigested and dry sludges.

With the above considerations in mind, a preliminary pilot plant design has been completed with the help of the City of Albuquerque, Molzen-Corbin & Associates, and Kramer-Callahan & Associates. Tentatively, plans are to locate the pilot plant in Albuquerque at the Waste Water Treatment Plant No. 2. A 1.4 megacurie cobalt-60 radiation source valued at $700,000 would be supplied by Sandia Laboratories.

A pasteurization treatment using thermoradiation up to 65° C and 500 krads with a residence time in the irradiator up to 5 minutes will be utilized for wet sludges. Three different sludge process streams will be possible: (1) Primary clarifier sludge, (2) Digested sludge, and (3) Waste activated sludge. All of these would be thickened as necessary to approximately 10 percent solids for the thermoradiation treatment. The process fundamentals are described below. A fourth process for treating dried digested sludge is described separately.

1. Primary Clarifier Sludge

Approximately 40,000 gallons of 4 percent solids primary clarifier sludge would be treated per day. The sludge will be initially thickened using a gravity sludge thickener to 8 to 10 percent solids. The supernatant from the thickening operation will be fed to the waste activated sludge line which goes to the primary clarifier influent distributor. The 20,000 gallons/day of thickened sludge will be pumped through a hot water heat exchanger (from digester heat supply) to raise the temperature to 50° C. A steam injection system will raise the temperature another 15° to 65° C, at which point the sludge will pass through the irradiator. The treated sludge will then be dewatered to 25 percent solids using a centrifuge or filter press, or piped directly to the drying beds.

2. Digested Sludge

Digested sludge can be handled at the rate of 20,000 gallons/day. The process is identical to the primary sludge treatment except thickening will not be necessary.

3. Waste Activated Sludge

The waste activated sludge process is made more difficult by the low solids content of the sludge (< 1 to 2 percent) coupled with the ineffectiveness of the gravity thickener. The activated sludge will first be pumped to the centrifuge where it will be dewatered to about 5 percent solids. The supernatant will be pumped to the waste activated sludge line for return to the plant flow. The thickened sludge will be pumped to the heat exchangers and then steam injection used to raise the final temperature to 65° C before being passed through the irradiator. At this point, the activated sludge can be dewatered using a filter press or pumped to the drying beds.

4. Dried Digested Sludge

The dried digested sludge from the drying beds will be ground to a nominal 1/4" size, then transported to the processing building by front end loader. The milled sludge will be put into a hopper on the side of the building from which a screw type feed conveyor will move the sludge to the irradiator. After treatment to 1 Mrad, the dried sludge will be conveyed to a storage bin outside the building.

A preliminary layout of the proposed irradiation facility is given in Figs. 4 and 5. The design basis for each component cost is summarized as follows:

Item	Design Basis	Installed Cost
Building, Crane & Pools	Mechanical, electrical, 20 ton crane, and radiation tank w/16 ga. stainless steel lining; 55' x 36'	$ 182,000

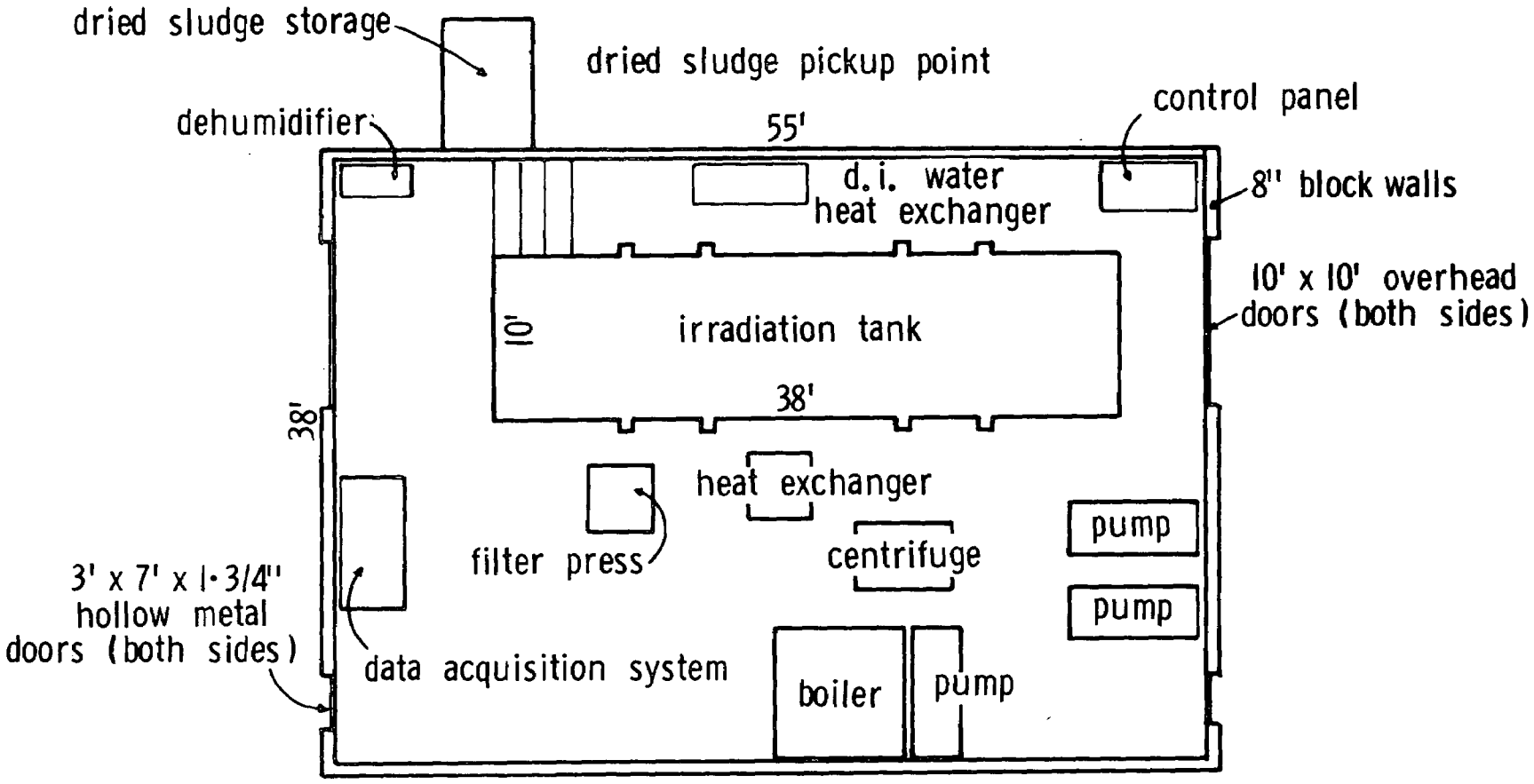


Figure 4. Schematic of Pilot Plant Irradiation Room Layout

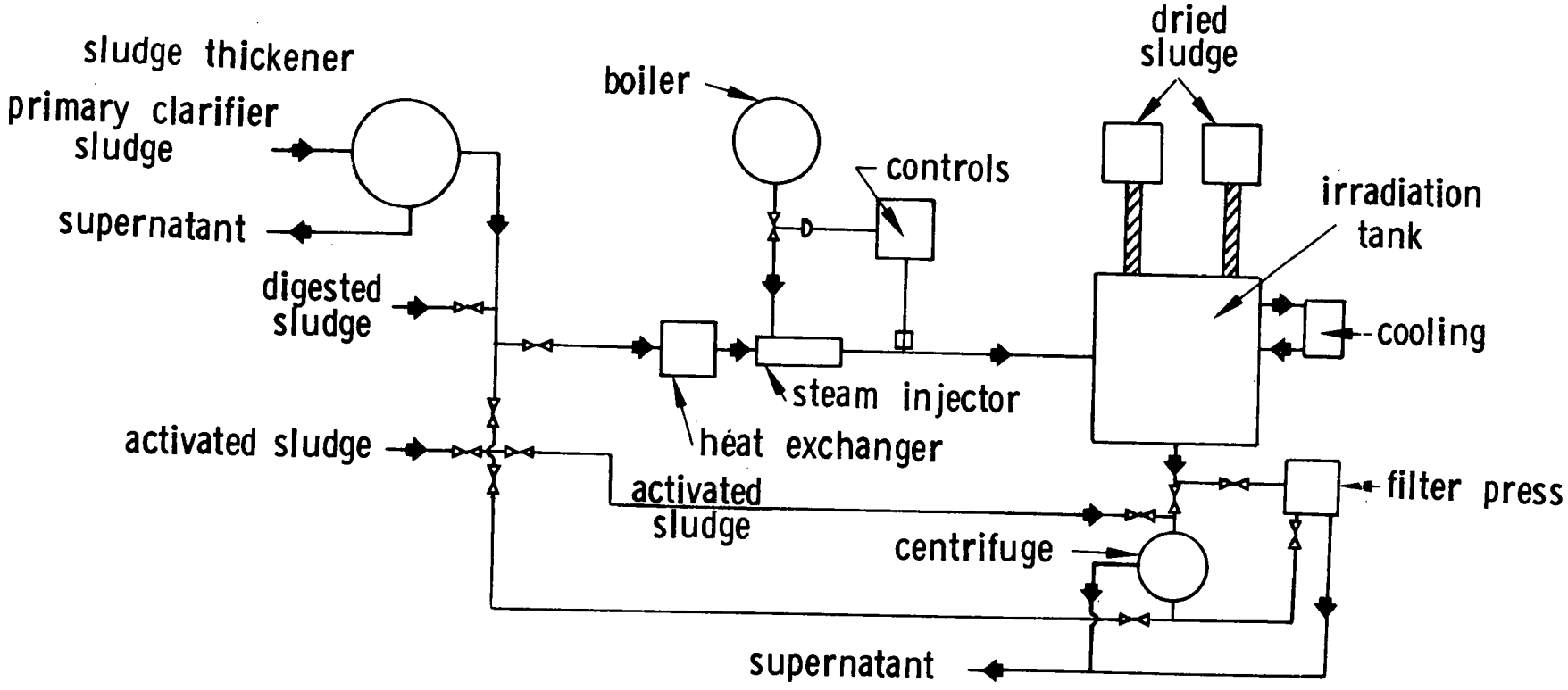


Figure 5. Proposed Sludge Processing Flow Schematic for Pilot Plant

Item	Design Basis	Installed Cost
Irradiator	By Sandia Laboratories, in pool	$ 100,000
Auger Dried Sludge Conveyors and Hopper	20 yd^3 per day, 50 ft conveyor with motors, 20 yd^3 storage	6,000
Deionized Water Heat Exchanger	Existing unit at Sandia Laboratories, 22 kw	2,500
Deionized Water Makeup System	Existing unit at Sandia Laboratories, 22 kw	500
Centrifuge	12" bowl with polymer feed system and motor control accessories (Dorr Oliver)	80,000
Data Acquisition System for Monitoring and Control	Existing unit at Sandia Laboratories	30,000
Sludge Heat Exchanger w/Controls	20,000 gallons/day to 120° F	10,000
Sludge Thickener	Covered, vented to flare, 40,000 gallons/day	55,000
Boiler and Steam Injection Equipment w/Temperature Controls	ASME 125 psi 15 HP w/pump and controls	6,500
Filter Press	1000 lb dried cake per 24 hrs, polymer-on-paper precoatings	20,000
Oxygen Injection Equipment with Controls	20,000 gpd to be oxygenated	4,000
Miscellaneous Piping, Valves, Meters, Controls, Electrical Lighting	--	30,000

Item	Design Basis	Installed Cost
Pumps and in Line Sludge Grinder	4 variable speed progressing cavity pumps (Moyno) with controls, 1 in-line sludge grinder	$ 20,000
Primary Sludge Supply	Variable speed progressing cavity pump, valves, controls, 4" pipeline taps	9,200
Digested Sludge Supply	Valves, controls, 4" pipeline tap, (pump previously listed)	5,700
Waste Activated Sludge Supply	Valves, controls, 4" line tap (pump previously listed)	1,900
Waste Sludge Return	Valves, 4" line, line tap (pump previously listed)	1,900
Waste Sludge Line to Drying Beds	Valves, 4" pipe (pump previously listed)	13,500
Washwater Supply Line	Valves, 4" line, line tap	1,200
Hot Water Supply and Return Line	Insulated 6" line, valves	4,100
Dried Sludge Supply	Dried sludge grinder (Royer Model 16) pavement	4,800
Paving of Access Area	--	4,000
Sub-Total:		$ 598,800

Subtotal:	$ 598,800
Contingencies @ 10%	59,900
Subtotal:	$ 658,700
Engineering, Legal, Administrative, etc. @ 15%	98,800
Subtotal:	$ 757,500
Inflation (12 months @ 1%)	90,900
TOTAL:	$ 848,400

Cesium-137 Trailer

A trailer mounted cesium-137 irradiator was obtained during the past year from Sandia Laboratories at Livermore, California. The source was acquired in order that low dose rate (~50 rads/sec) thermoradiation studies might be performed.

The gamma source consists of 140 KCi contained in 38 strips; each having an active length of 30.5 cm and an active width of 2.5 cm. The strips are arranged to form a rectangular source plaque 56 cm x 69 cm. Each source strip is doubly encapsulated in stainless steel.

The irradiation unit is a lead-filled steel shell which contains the fixed source plaque. The product to be irradiated is moved into the irradiator by means of a motor driven shuttle. The shielding from the source plaque is provided by a series of two mechanically and electrically interlocked doors.

A safety analysis report was prepared for and approved by the Operational Safety Division of ERDA/ALO before transfer of the trailer to Sandia Laboratories at Albuquerque, New Mexico. The irradiator will be tested and made available for general use within Department 5440.

BACTERIOLOGY

Introduction

An excellent survey of the problems associated with the existence and persistence of pathogens in sewage and sewage sludge has been published.[1]

Sensitivities of various microorganisms to heat and to radiation as reported in the literature are listed in Table I. The viruses listed tend to be generally radiation resistant but heat sensitive. The coliforms and staphylococci tend to be relatively sensitive to either treatment. Streptococci (and possibly Salmonella) appear to be the most heat and radiation resistant groups (aside from spores). The majority of the bacteriological work in sewage sludge has been geared to either the coliform group, since it is almost a universal indicator in monitoring effectiveness of wastewater treatment processes, or to the fecal streptococcus bacteria, which appear to be the most resistant in terms of both heat and radiation.

Research areas described in this report include (1) inactivation rates of coliform and fecal streptococcus bacteria for treatment by heat and by ionizing radiation, over the temperature range of 20° C - 70° C; (2) dose rate effects in inactivation of these organisms in sludge; (3) possible "protective" effects exerted by the sludge; (4) regrowth curves in sludge, measured for both coliforms and strep; (5) inactivation of Salmonella in sludge; (6) effects of

TABLE I

Sensitivities of Various Microorganisms to Heat and Radiation

Organism	D-Value*		Reference
	krads	min @ 60° C	
Adenovirus	450	0.15	2,3
Poliovirus	300	1.5	4,5
Coliforms	20	2	6
Staphylococci	22	3.3	7,8
Salmonella	45	7.5	8,9
Streptococci	120-200	15	10

*Treatment required per log (base 10) reduction in population.

oxygenation on the radiation-induced inactivation of coliforms, Salmonella, and fecal strep.

Experimental

The majority of the inactivation rate studies were made using the remote sampling system described in section I of this report. Briefly, spring-loaded syringes allowed sampling of sludge during a treatment process, or "run." The rise time (to within a degree of final temperature) following injection of the sludge ranged from 1 to 2 minutes, depending on temperature (Fig. 6). A "fast-rise" chamber (rise time ≈20 seconds) was used to measure heat inactivation rates of bacteria at 70° C (Fig. 7). Regrowth of coliform and fecal strep bacteria was carried out in spinner flasks in an incubator at 37° C. Samples were oxygenated simply by bubbling the pure gas through the sample prior to and during irradiation. Oxygen concentration was not measured.

Inactivation is measured by the "colony forming ability" of the bacteria following treatment. Initial counts are approximately 10^5 and 10^4/ml for coliform and fecal strep, respectively. Appropriate dilutions are plated out on selective media (agar) in petri dishes. Coliform colonies are distinguishable as those having a green metallic sheen.[11] Fecal streptococci (this plate count technique is a standard method[12]) are distinguishable as bright red lens-shaped colonies embedded in the agar medium. Approximately 150 to 200 plates are required for a single determination of each inactivation curve. EPA-approved MPN methods[13,14] as well as plate-count techniques (described in the appropriate section) were used in the determination of Salmonella presence or inactivation rates in sludge.

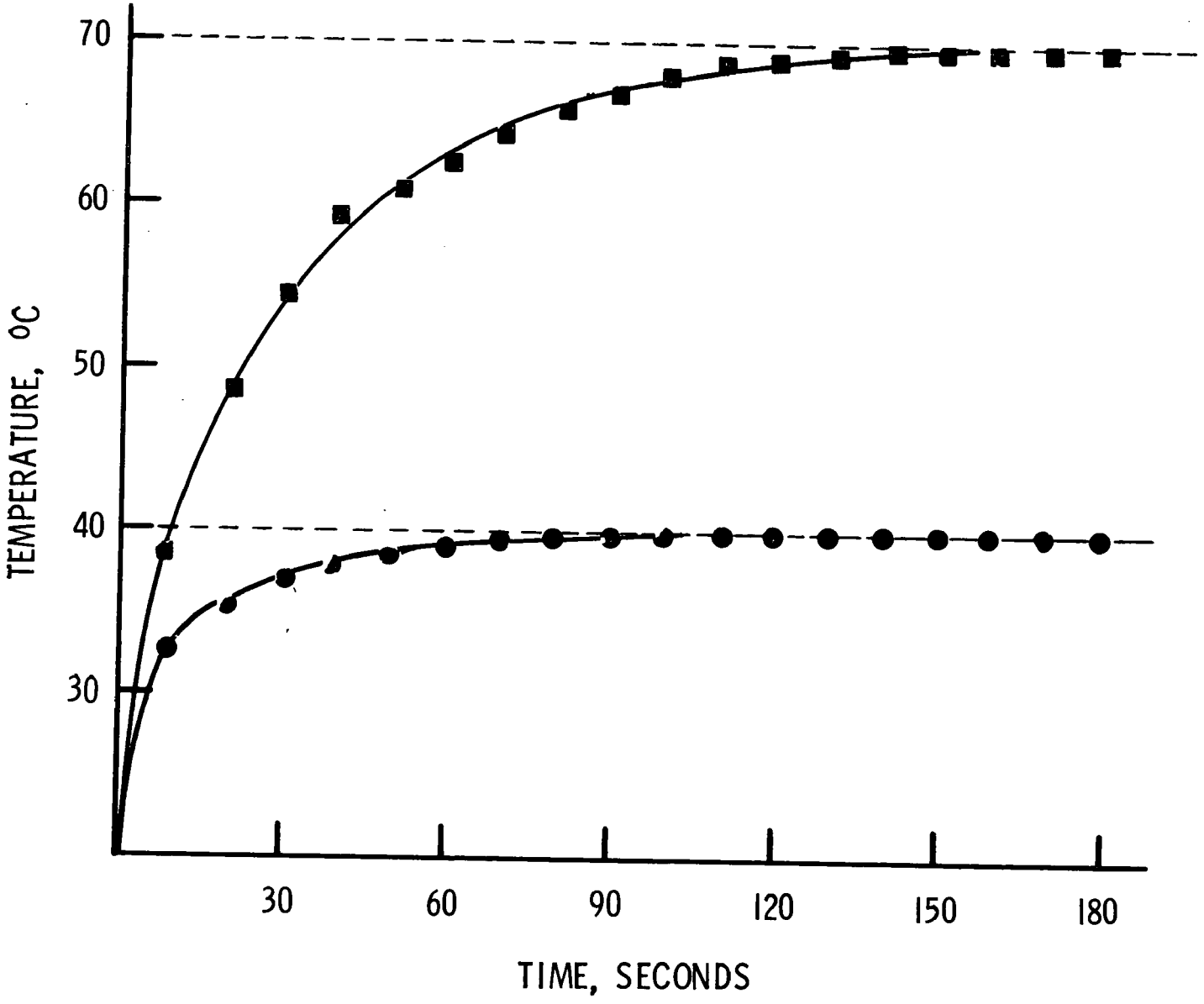


Figure 6. Heat-up Profiles for Remote Sampling System (70° and 40° C)

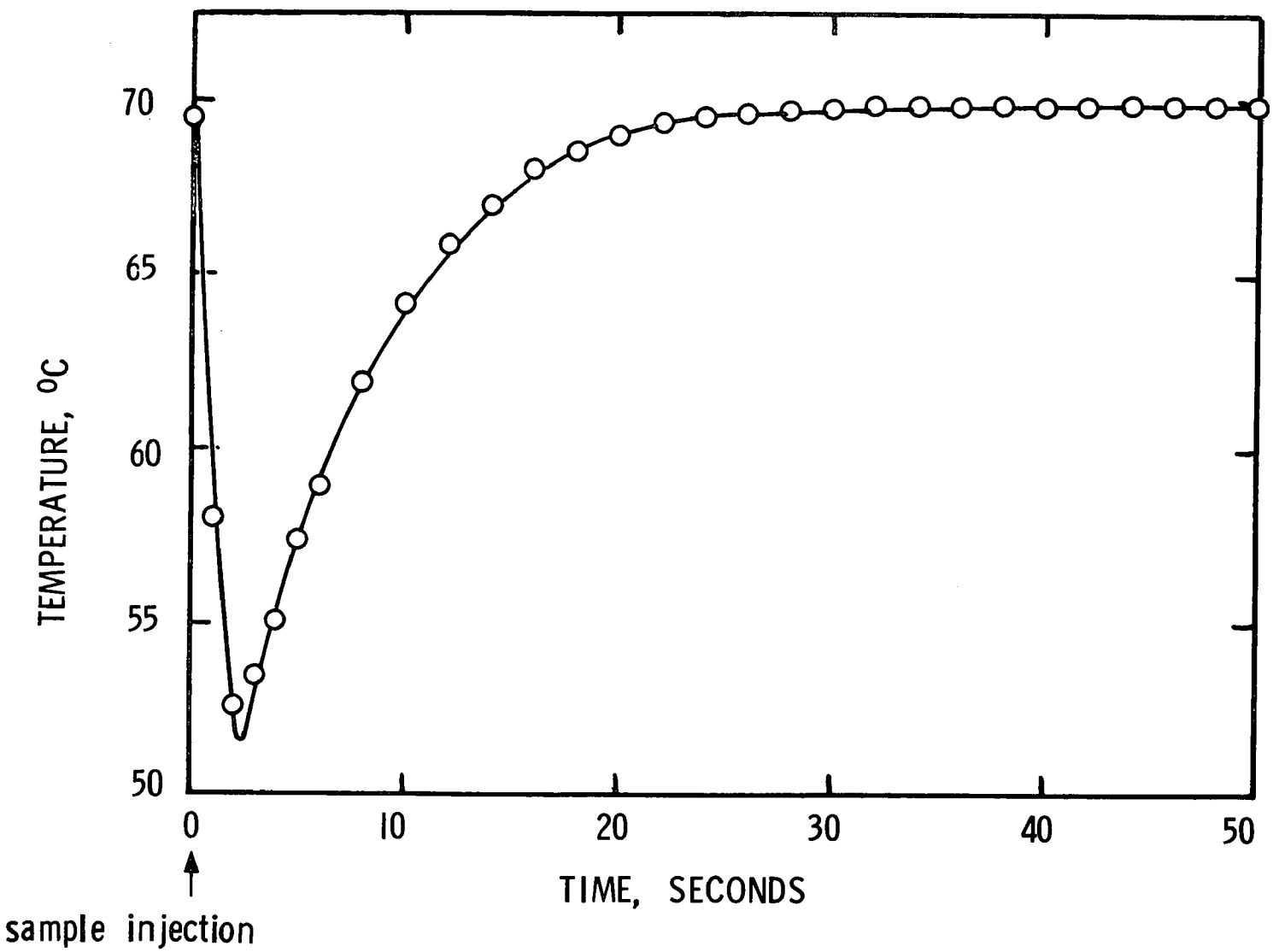


Figure 7. Profile for "Fast-rise" Chamber Heat-up

Results

Inactivation Rates of Coliform Bacteria

Figure 8 shows the radiation inactivation at 20° C of coliforms by ^{137}Cs gamma rays. Different symbols indicate different batches of sludge and/or different "runs." Since controls were not perfectly consistent on two of the four runs, normalization to the first inactivation point would have been justifiable and the data fit better; however, the slope is the important parameter in this curve. It is seen that the "D-value" (the absorbed dose required to decrease the bacterial count by one log, or 90 percent) is approximately 20 krads/log for coliform bacteria in sludge.

Data from a complete "run", i.e., heat, radiation, and thermoradiation are shown for one temperature (50° C) in Fig. 9. These are typical curves. It can be seen that at this temperature, heat alone contributes very little inactivation over this time scale. The inactivation by combination treatment exhibits some synergism (approximately one log), but the effect is considerably less than that observed in some earlier studies, such as those of *E. coli* in broth.[15] The observed synergism in sludge is consistent, however, and is seen from 40° to 65° C.

Figure 10 shows heat inactivation curves for coliforms at temperatures from 40 to 65° C. Only at 55° C and above does the heat play an appreciable role in inactivation on this time scale. The results at 60° C and at 65° C are inconsistent, possibly due to the heat sensitivity of these bacteria and the heatup profile for the system. However, radiation results do not indicate this kind of batch-to-batch variation.

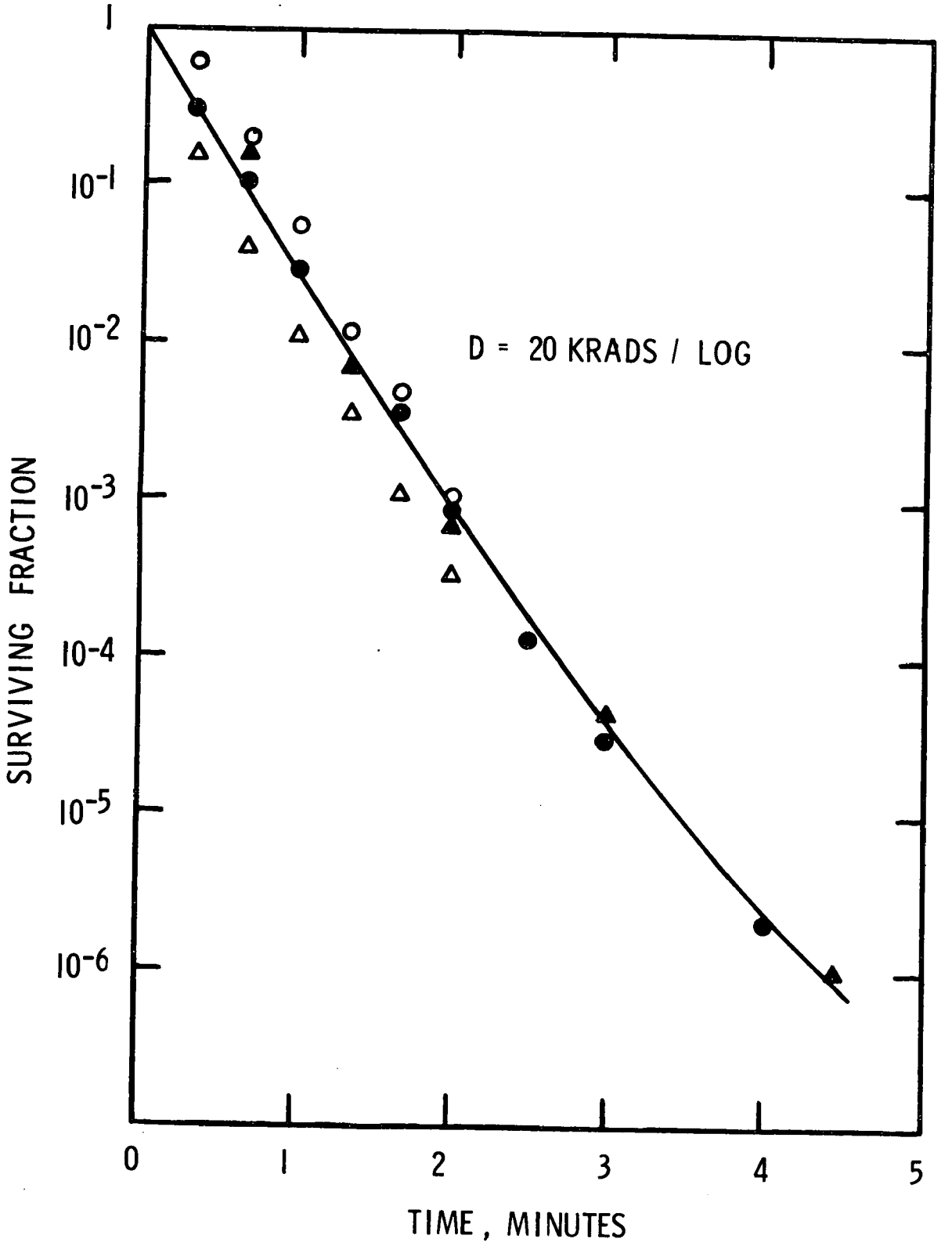


Figure 8. Radiation Inactivation of Coliforms at 20° C and 30 krads/minute

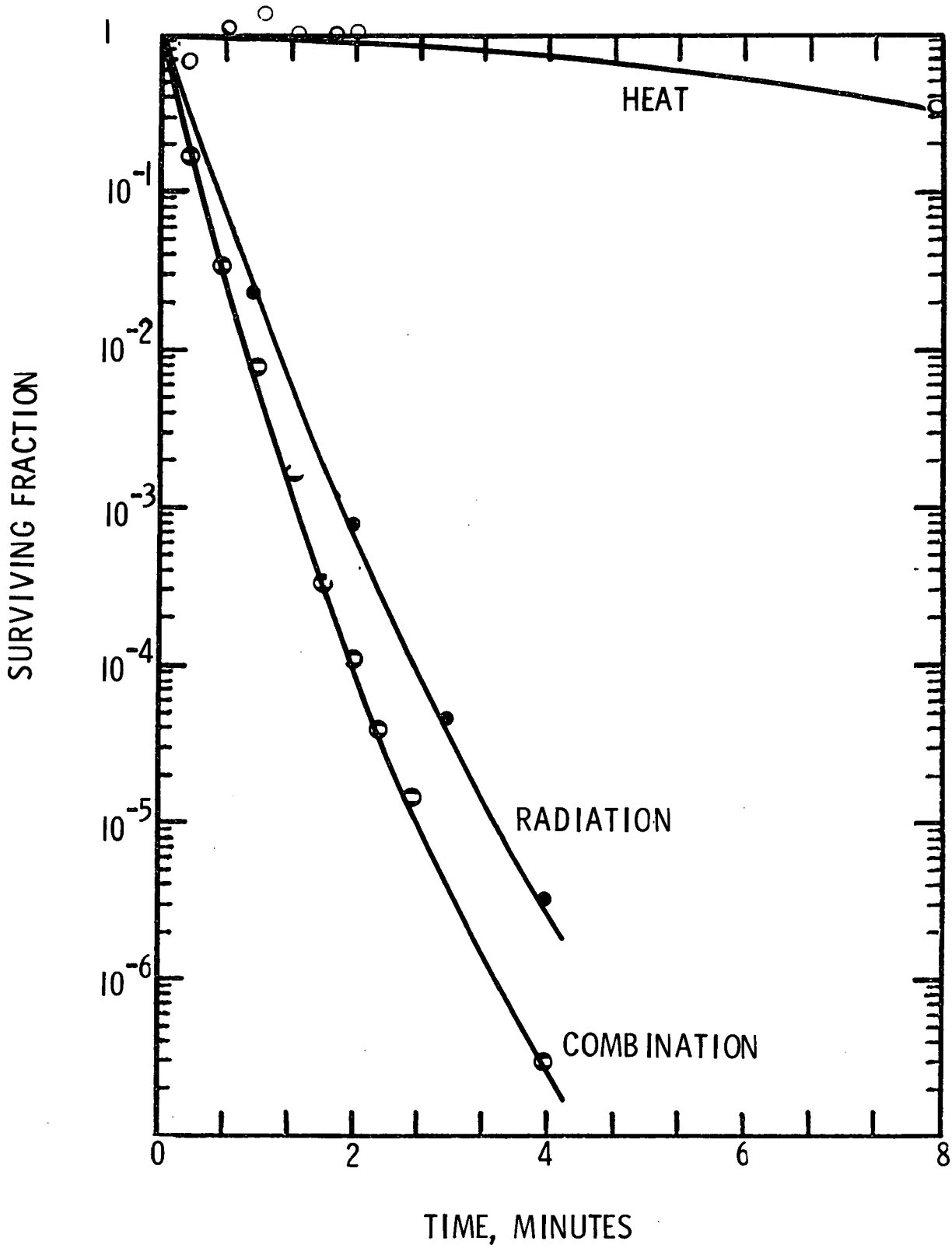


Figure 9. Inactivation of Coliforms at 50° C and 30 krads/minute

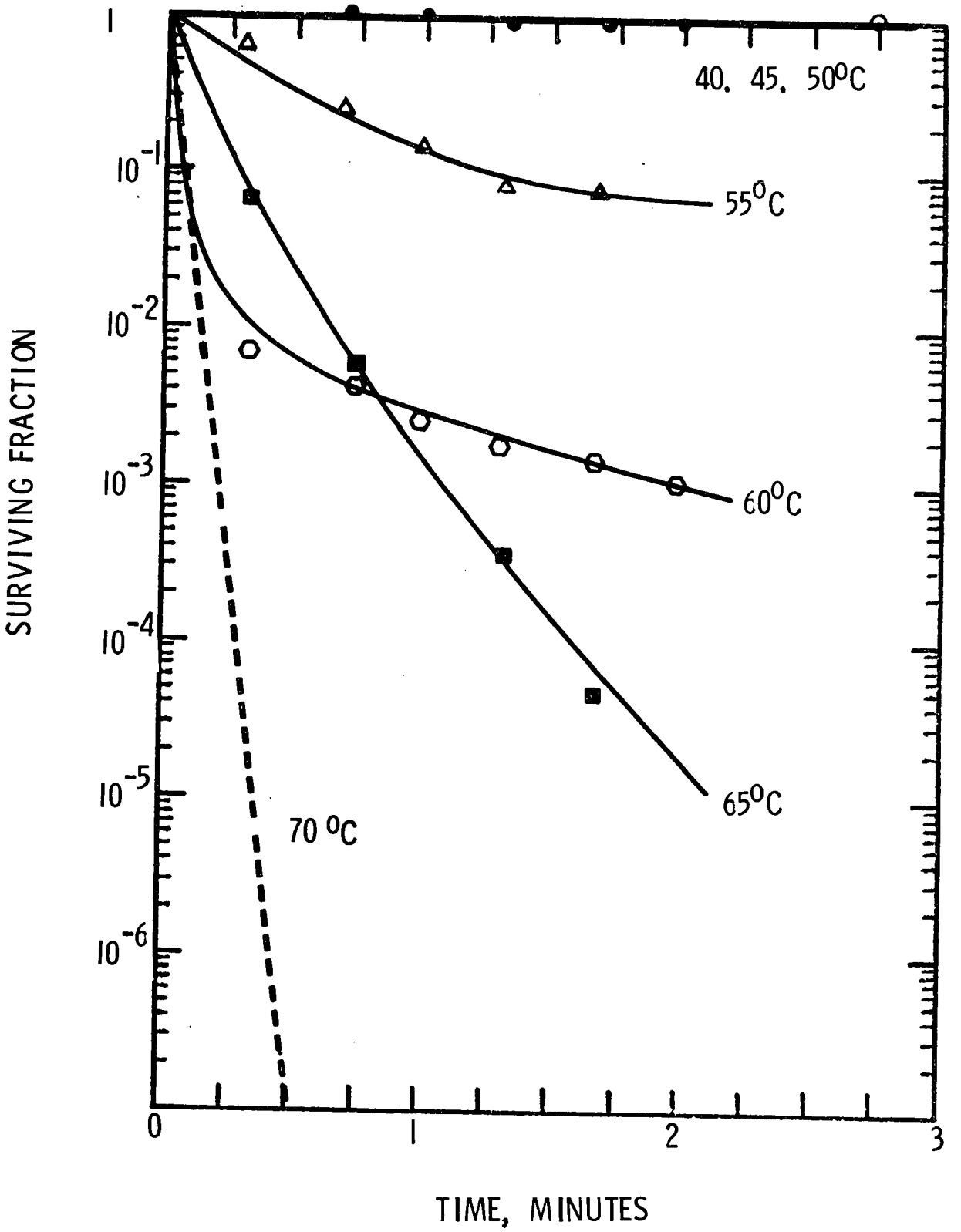


Figure 10. Heat Inactivation of Coliforms

Figure 11 shows a plot similar to the 50° C run but at 65° C. The radiation D-value is seen to be approximately 4.5 krads/log at this temperature (versus 20 krads at 20° C). In Fig. 12, the data for all temperatures are plotted as surviving fraction at 1 minute (arbitrary time chosen for demonstration purposes; synergism is in fact somewhat greater at longer times) versus temperature. While thermoradiation enhancement of inactivation is greater at higher temperatures, at lower temperatures there may be some degree of synergistic behavior.

Figure 13 shows the inactivation of coliforms (and of strep bacteria) by 70° C heat. This presents a "minimum" inactivation rate, since there is still a significant heat-up time for the experimental system. This inactivation rate is approximately twice that measured using the remote-sampling system, due to the much slower heat-up profile of the latter.

These data (and others) on coliform bacteria are summarized at the end of this section.

Inactivation Rates of Fecal Streptococcus Bacteria

Radiation inactivation of fecal streptococci in sludge is plotted in Fig. 14. It is observed that the radiation resistance of these microorganisms is approximately six-fold greater (D-value of about 120 krads/log) than that of the coliform group. The heat inactivation curves (Fig. 15) show that the effect of heat alone is minimal below approximately 55° C as with coliforms. The time scale of this plot is about a factor of three longer than that of the coliform group (Fig. 10). The inactivation curve for fecal strep by 70° C heat is shown in Fig. 13.

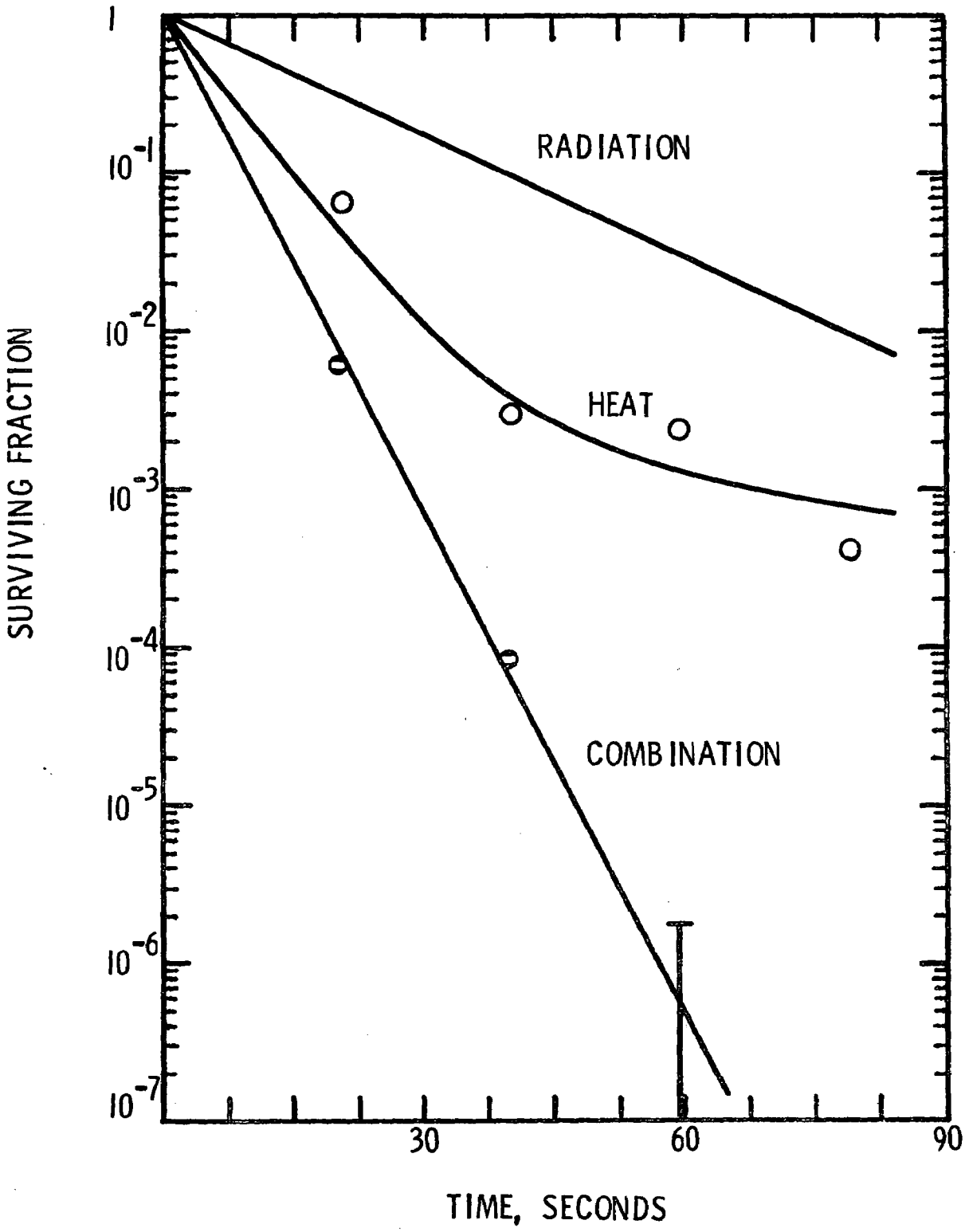


Figure 11. Inactivation of Coliforms at 65° C and 30 krads/minute

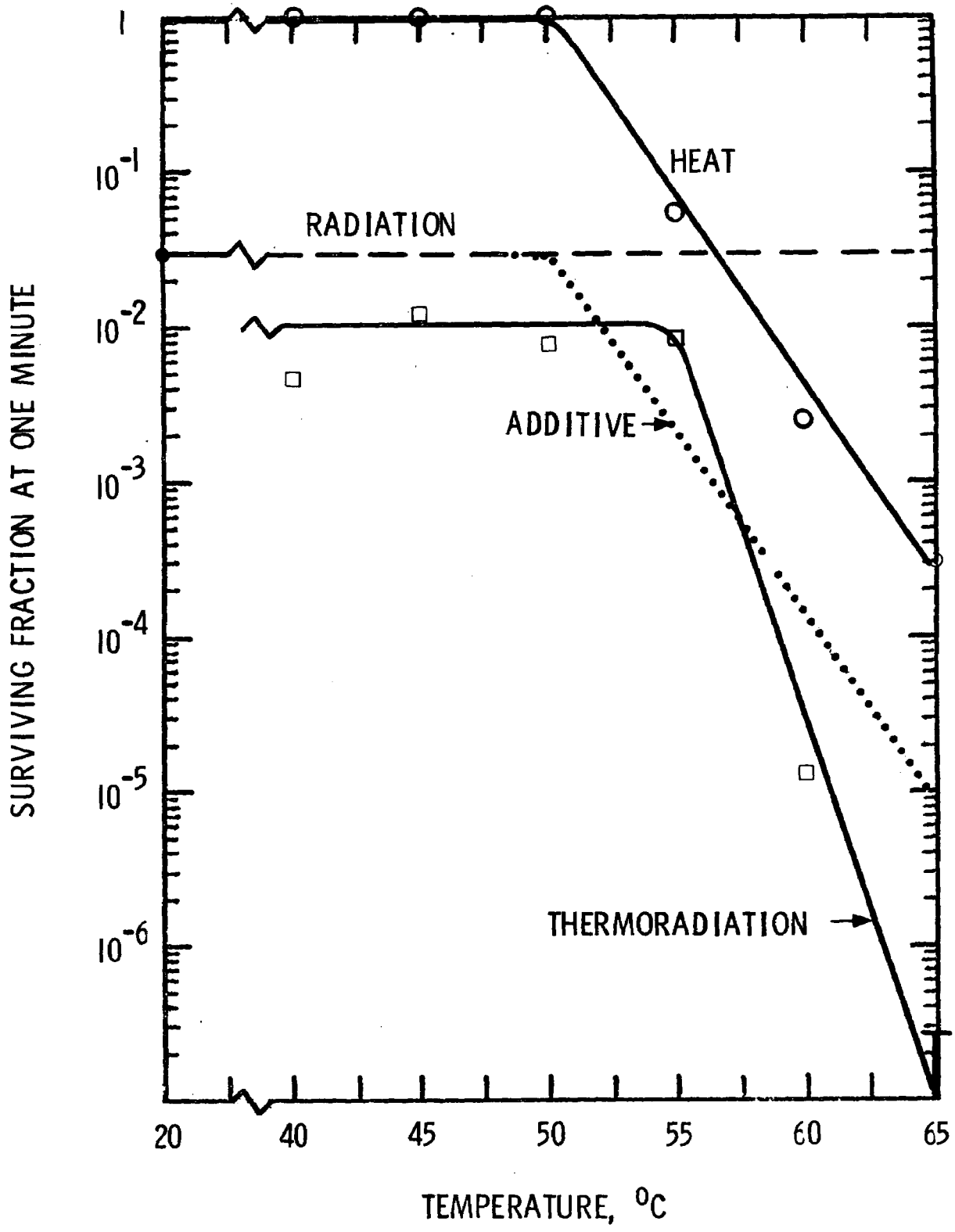


Figure 12. Coliform Survival

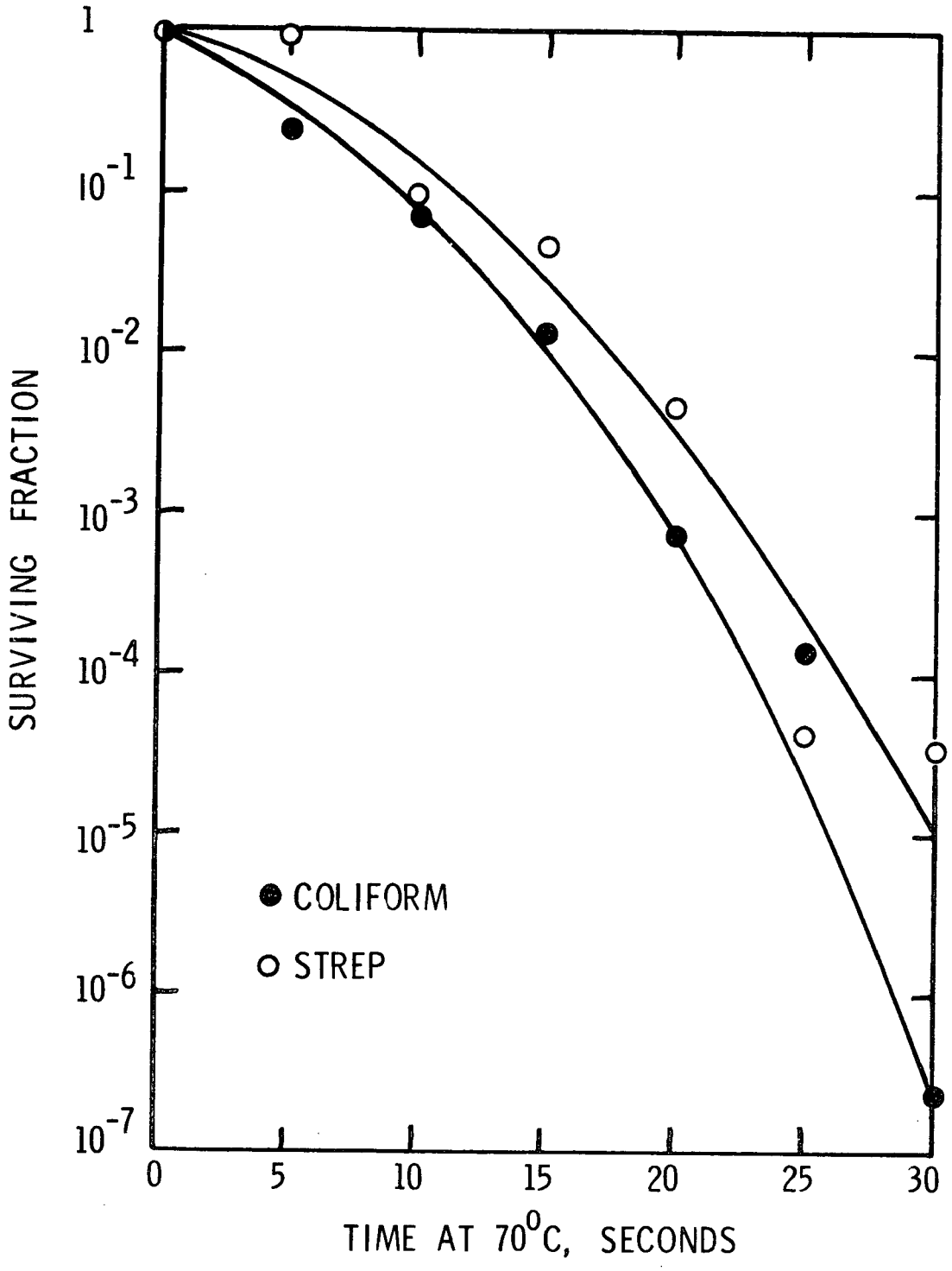


Figure 13. Inactivation of Fecal Strep and Coliform Bacteria by Heat at 70° C

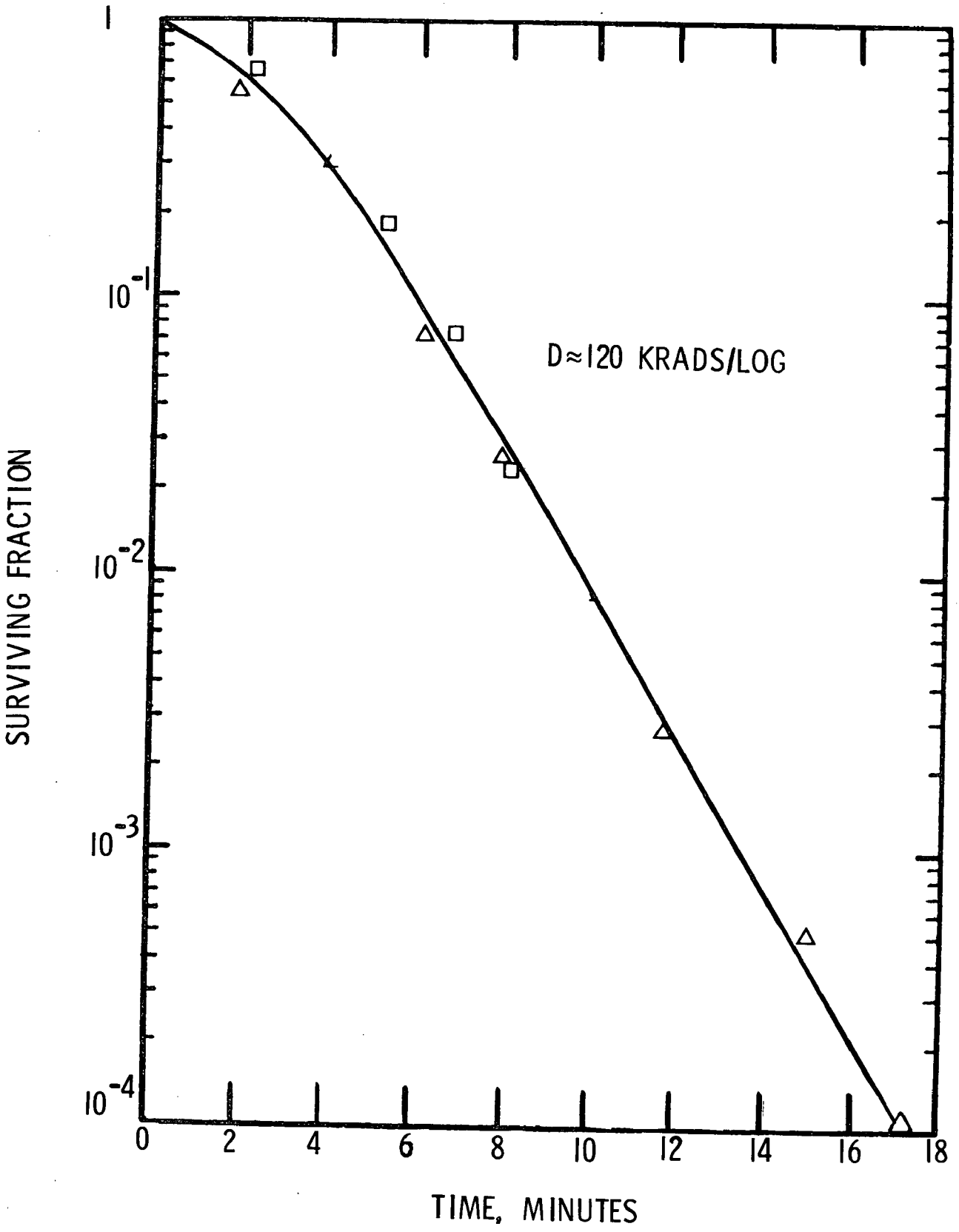


Figure 14. Radiation Inactivation of Fecal Strep at 20° C and 30 krads/minute

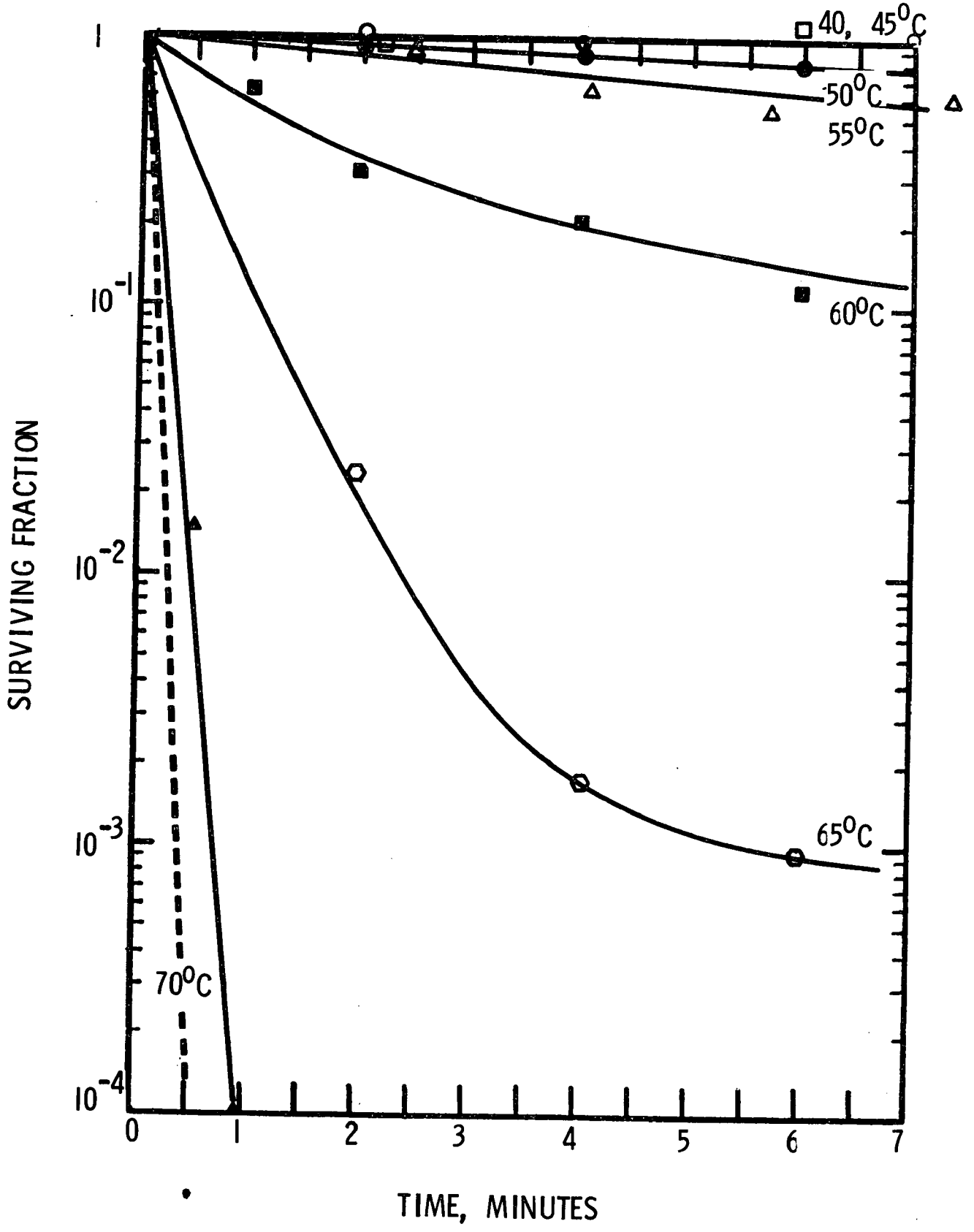


Figure 15. Heat Inactivation of Fecal Strep

These and other data for fecal streptococcus bacteria are summarized at the end of this section. Again, the synergism observed with combined treatment (Figs. 16 and 17 show results for 45° C and for 60° C, respectively) is small but consistent. This is demonstrated in Fig. 18 where the sum of the heat curves and the radiation curve is shown as a dotted line. The vertical difference between the dotted curve and the combined treatment curve is a measure of the "better than additive" effect. It should be reiterated that the "four minute" choice is arbitrary and the synergism is somewhat greater at longer times (for example, refer to Fig. 17 at 9 minutes).

Dose Rate Effects on Inactivation Rates

Since part of the studies included in this program involves use of an "outer-ring" irradiation chamber, it was important to determine whether any dose rate effects would appear in the inactivation curves. The dose rate was varied over two orders of magnitude (using cobalt-60, from 1.2×10^{-2} krads/sec up to 1.3 krads/sec) with no apparent change in the inactivation curves (Figs. 19 and 20). This range certainly includes any feasible dose rate considered for application purposes. It is also clear from the slopes of the curves in Figs. 19 and 20 that, as expected, there is essentially no difference in the effects of cobalt-60 as compared to those of cesium-137 (solid lines on these figures), within experimental error.

Sludge "Protective" Effects on Inactivation Rates

Sterilized sludge was inoculated to approximately 10^6 fecal streptococcus bacteria per milliliter in an attempt to increase the sensitivity of measurement of inactivation rates (normal counts in primary digester sludge are $\sim 10^3$ to 10^4).

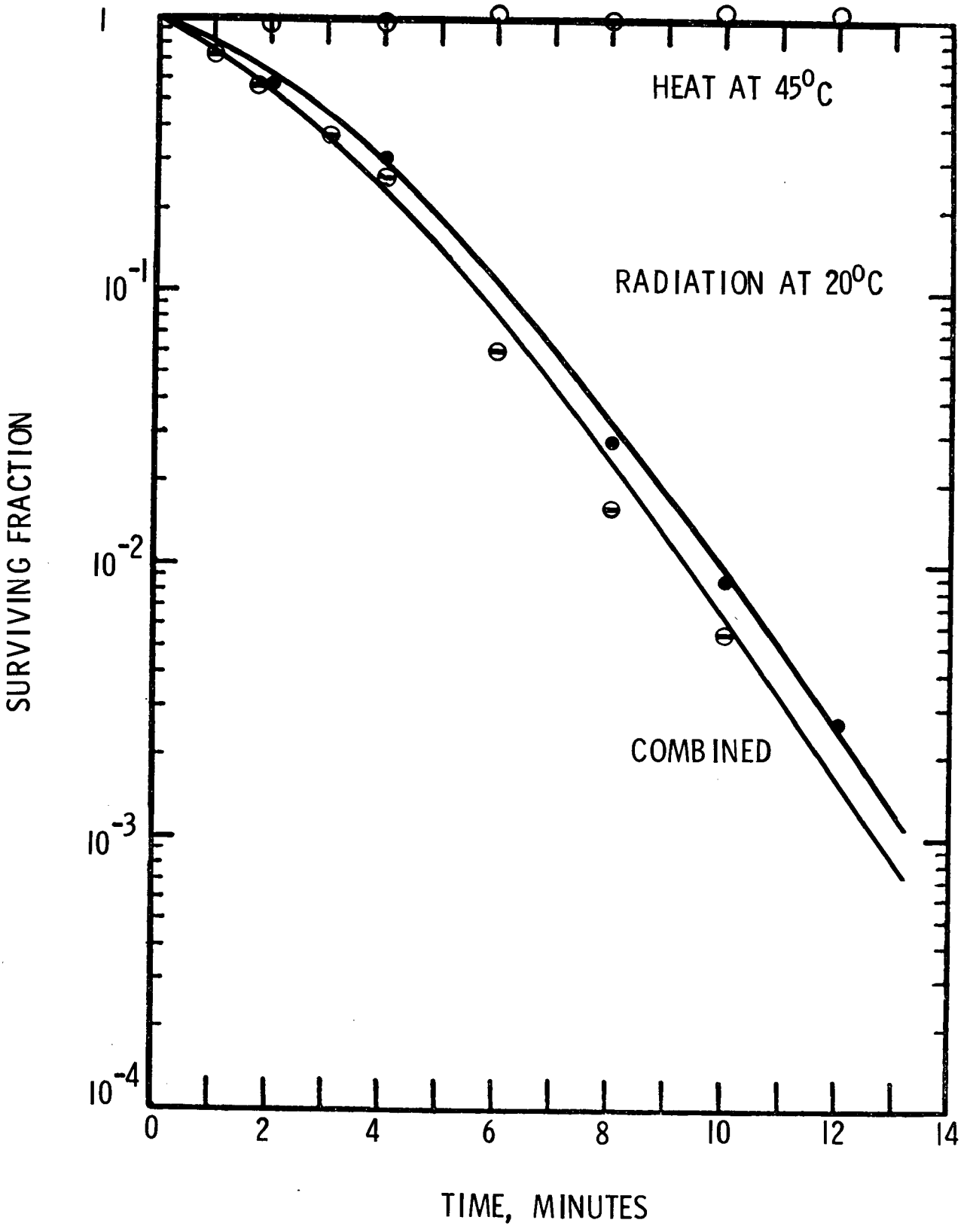


Figure 16. Heat and Radiation Inactivation of Fecal Strep

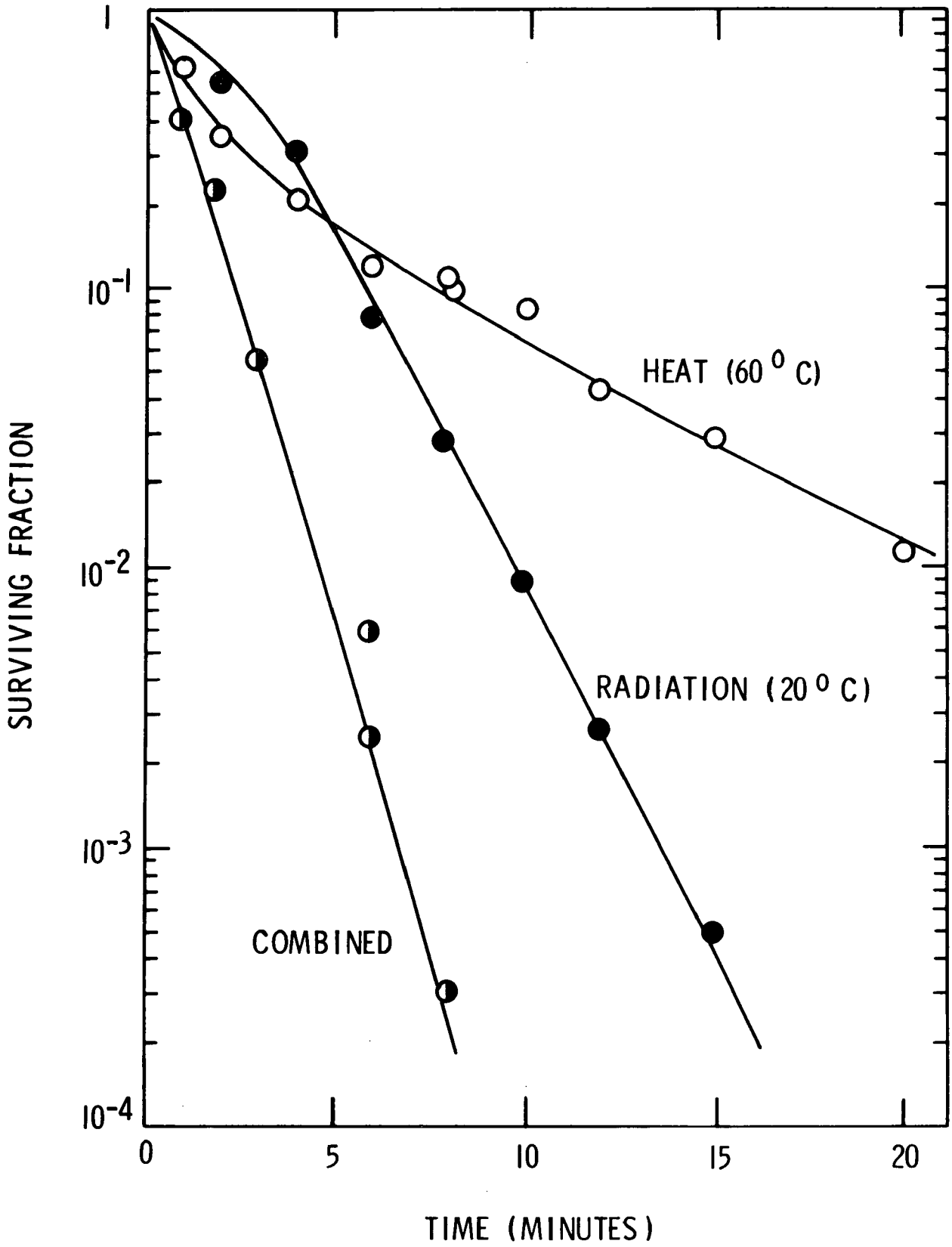


Figure 17. Fecal Strep Inactivation at 60° C and 30 krads/minute

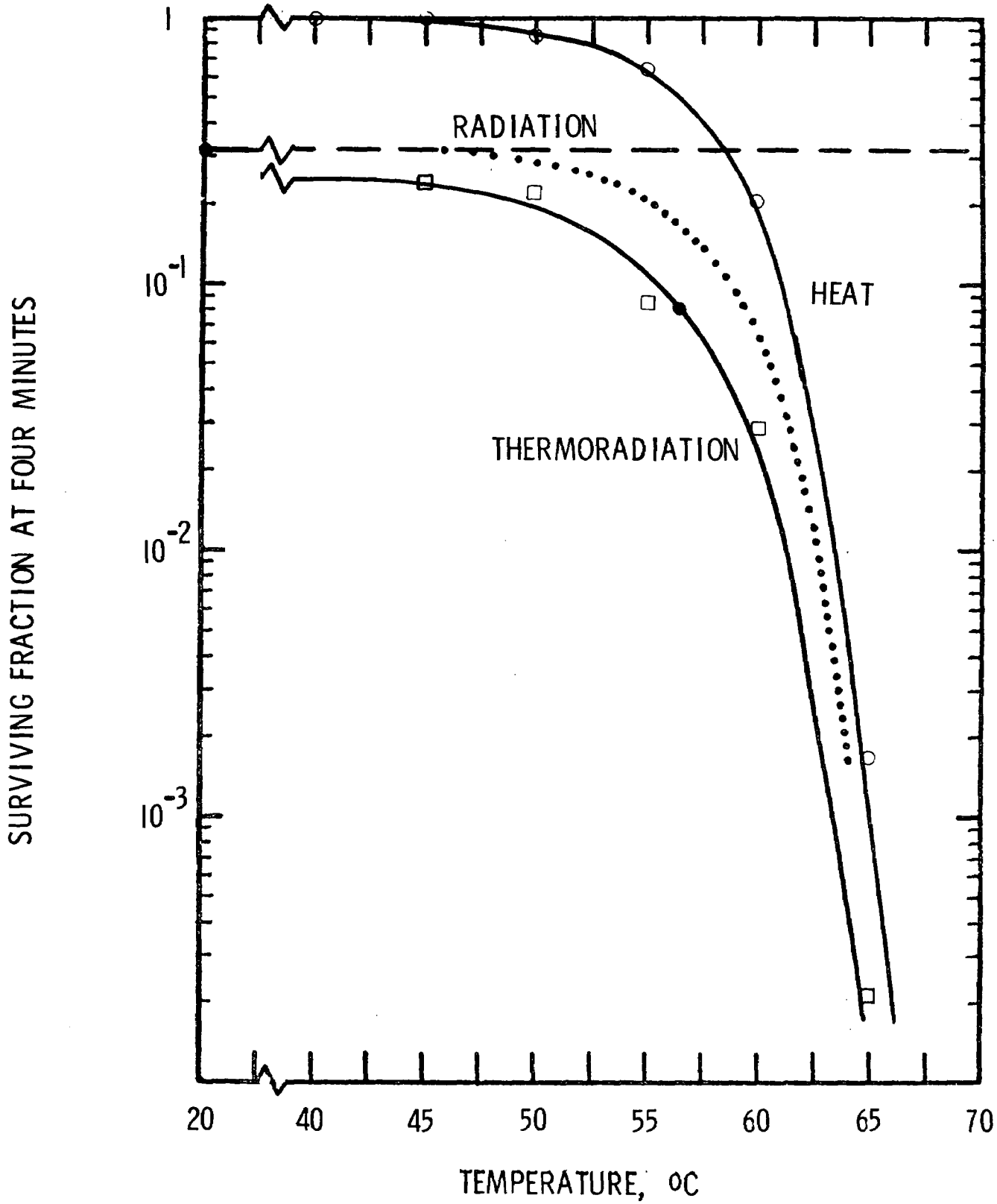


Figure 18. Fecal Strep Survival

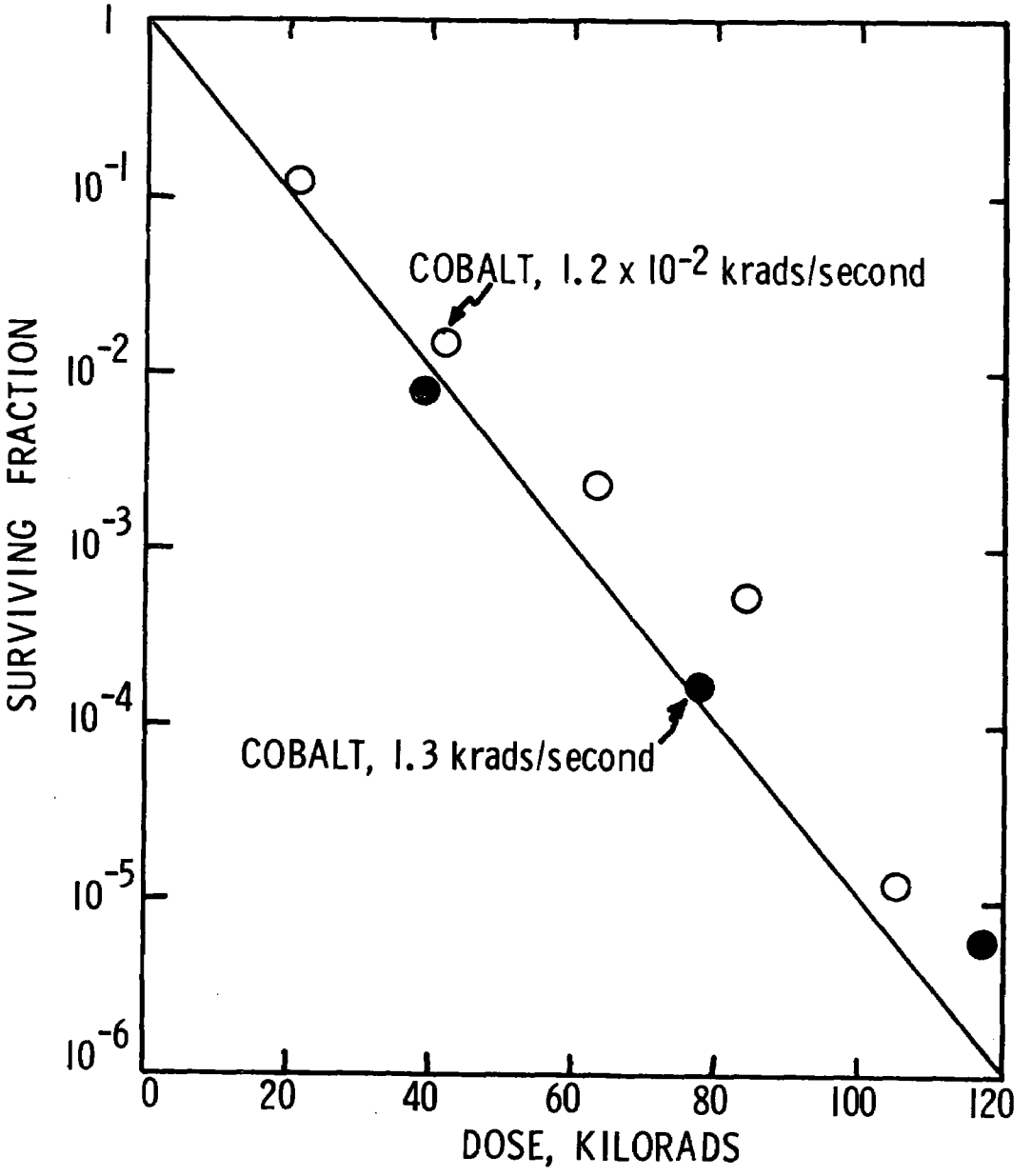


Figure 19. Inactivation of Coliform Bacteria at Different Dose Rates

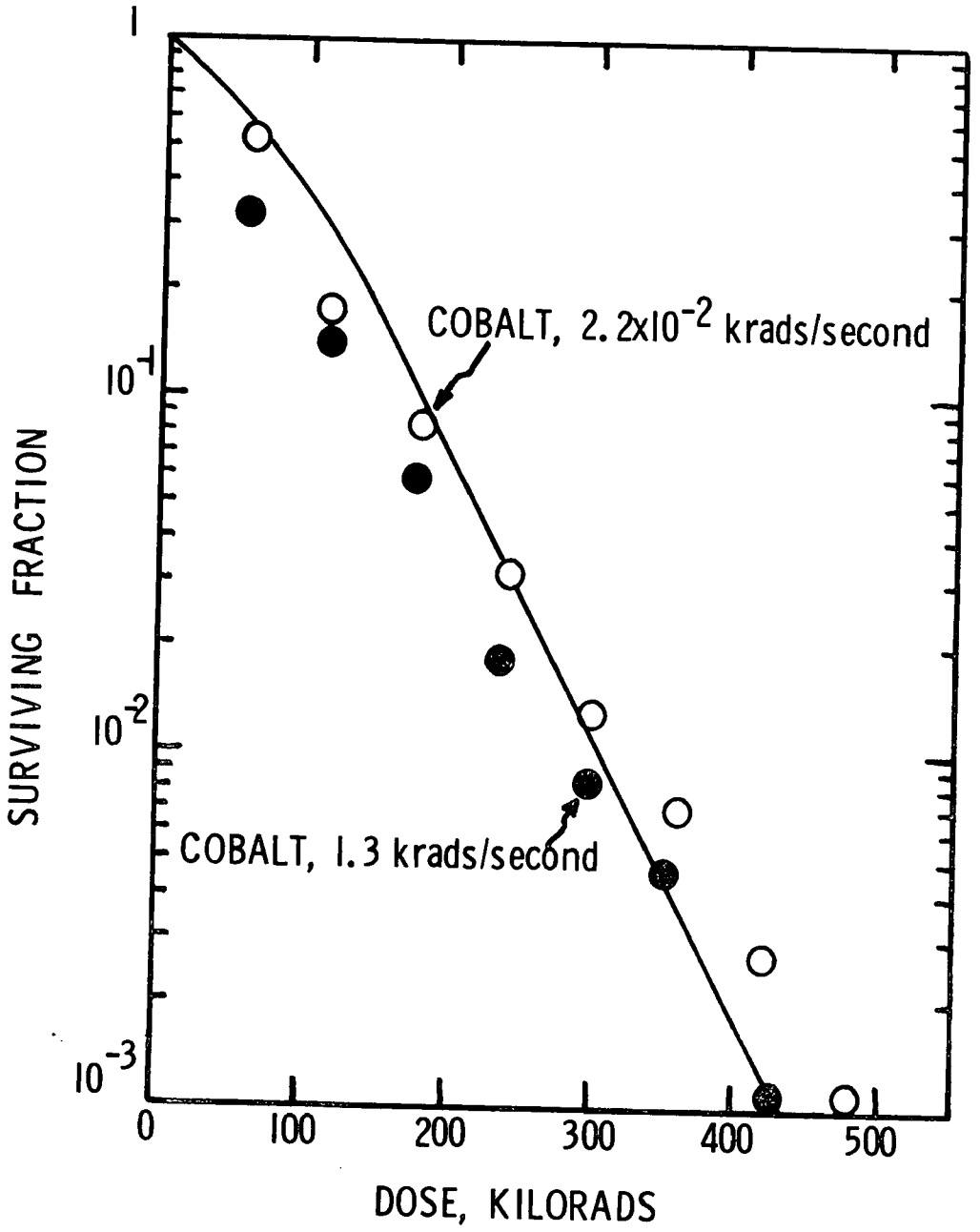


Figure 20. Inactivation of Fecal Strep Bacteria at Different Dose Rates

The purpose was two-fold: (1) To compare inactivation rates in sludge to those in saline, for heat, radiation, and combined treatment, in order to identify any "protective" effects of sludge. (2) To use the increased sensitivity of measurement to determine whether thermoradiation inactivation curves are linear or if they begin to "tail" or flatten with longer treatment times.

Figure 21 shows the results of such an experiment for radiation alone. Apparently there is some protection afforded by the sludge. Other runs confirm this finding. The sensitivity of the experiment is such that the absence of colonies on a large number of plates allows us to state that no "tailing" occurs with radiation inactivation over at least 6 logs of strep.

Only one thermoradiation experiment has been performed at 60° C. Figure 22 shows that there is apparently no significant protective effect. There may be some indication of "tailing" in the sludge samples (the counts at 20 minutes were significant, while no colonies were observed in the saline experiment at 20 minutes of treatment); these limited data, however, are inconclusive.

Bacterial Regrowth Following Thermoradiation Treatment

Following treatment (150 krads at 65° C) of 200 liter quantities of sludge, coliforms and fecal strep bacteria were monitored for regrowth. It is unknown whether contamination of the treated material or a residual bacteria level is responsible for "seeding" the treated sludge. Most likely both contribute. Contamination and/or regrowth may present difficulties in any large scale treatment process; we are presently exploring alternatives to deal with these problems.

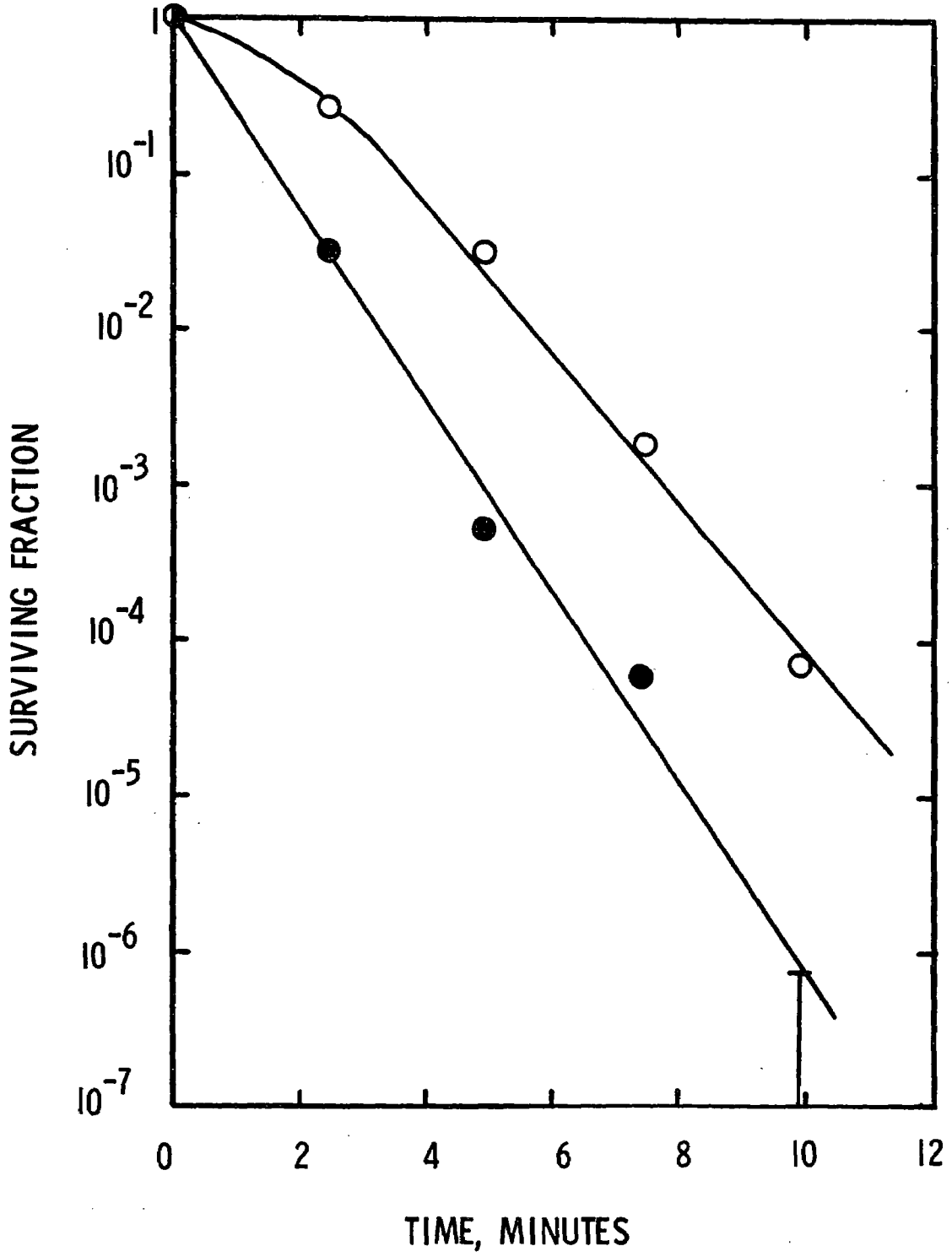


Figure 21. Inactivation Profiles for Radiation Treatment of Fecal Strep in Saline Inoculated with Broth (●) and in Sludge Inoculated with Broth (○). Dose Rate ≈ 70 krads/minute.

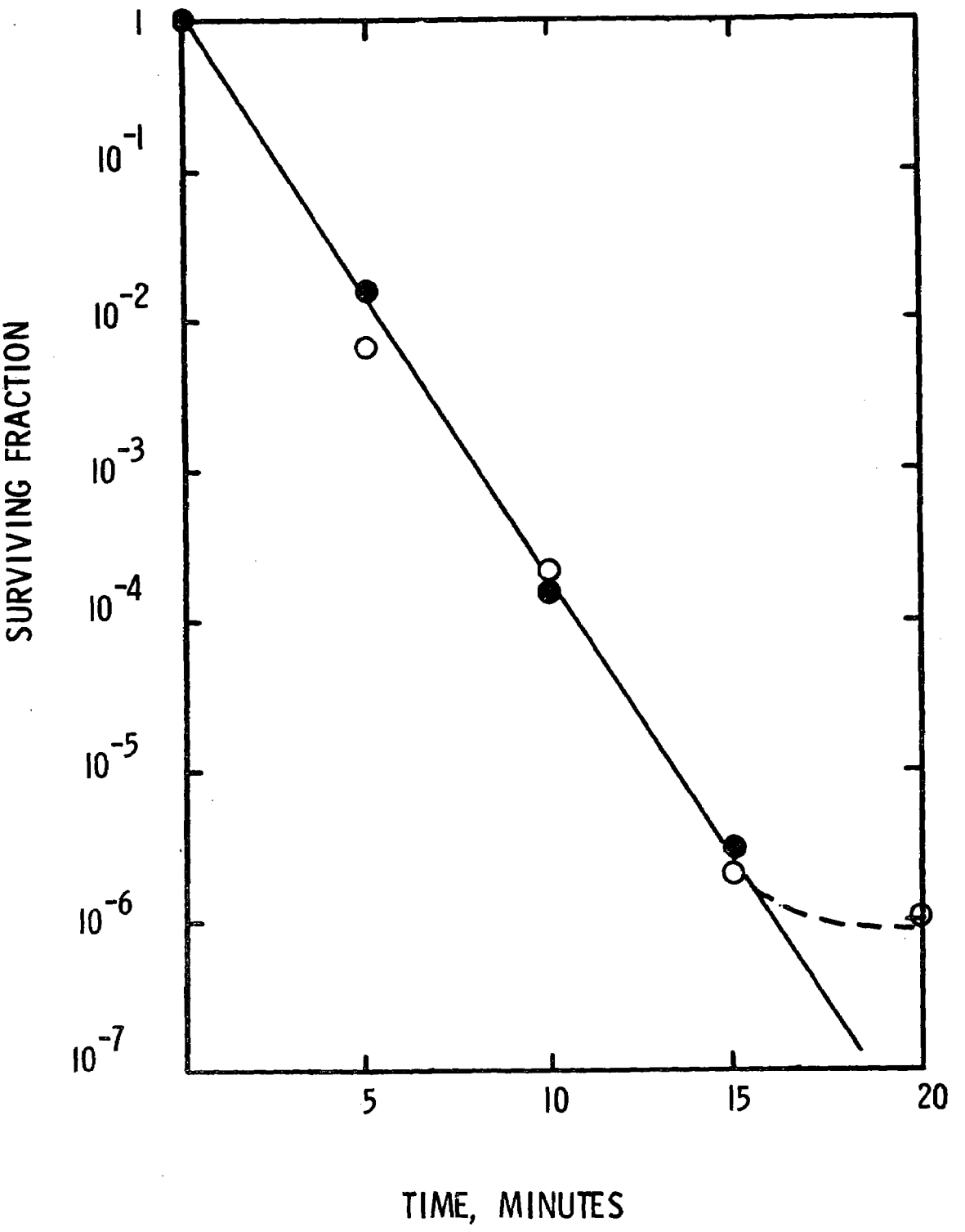


Figure 22. Thermoradiation Inactivation Profile for Fecal Strep in Inoculated Saline (●) and Inoculated Sludge (○). Dose Rate is 15 krads/minute.

Several experiments were performed in an attempt to clarify the regrowth phenomenon in treated sludge. Autoclaved sludge was "seeded" with normal, fresh sludge from a primary digester, such that initial counts were low. The samples were stirred and incubated at 35° C and samples were taken periodically. The results are shown in Figs. 23 and 24 for coliforms and strep, respectively. The open circles represent well-aerated samples (open spinner flasks) and the closed circles represent partially aerated samples (closed flasks with cotton plugs in the two side parts). It is clear that, within a day or two, the levels of both strep and coliform are more than 10 times higher than normal primary sludge levels.

In addition to these experiments, fecal streptococci were grown in KF streptococcal broth to levels of 10^8/ml in order to determine growth curves under various conditions of sludge. Among the samples were (1) sterilized sludge inoculated with broth (initial strep count ~10^6/ml), (2) primary digester sludge inoculated with active broth (initial strep count ~10^6/ml), and (3) primary digester sludge (initial count ~10^4/ml). These samples were stirred at 35° C in open spinner flasks. Rapid growth of fecal strep occurred only in the flasks containing sterilized sludge, indicating that bacterial competition in untreated sludge is such that fecal strep growth is not efficient. Table II shows fecal strep counts versus time under these incubation conditions for the various samples.

Similar growth experiments on Salmonella species from sludge have been unsuccessful and are being repeated.

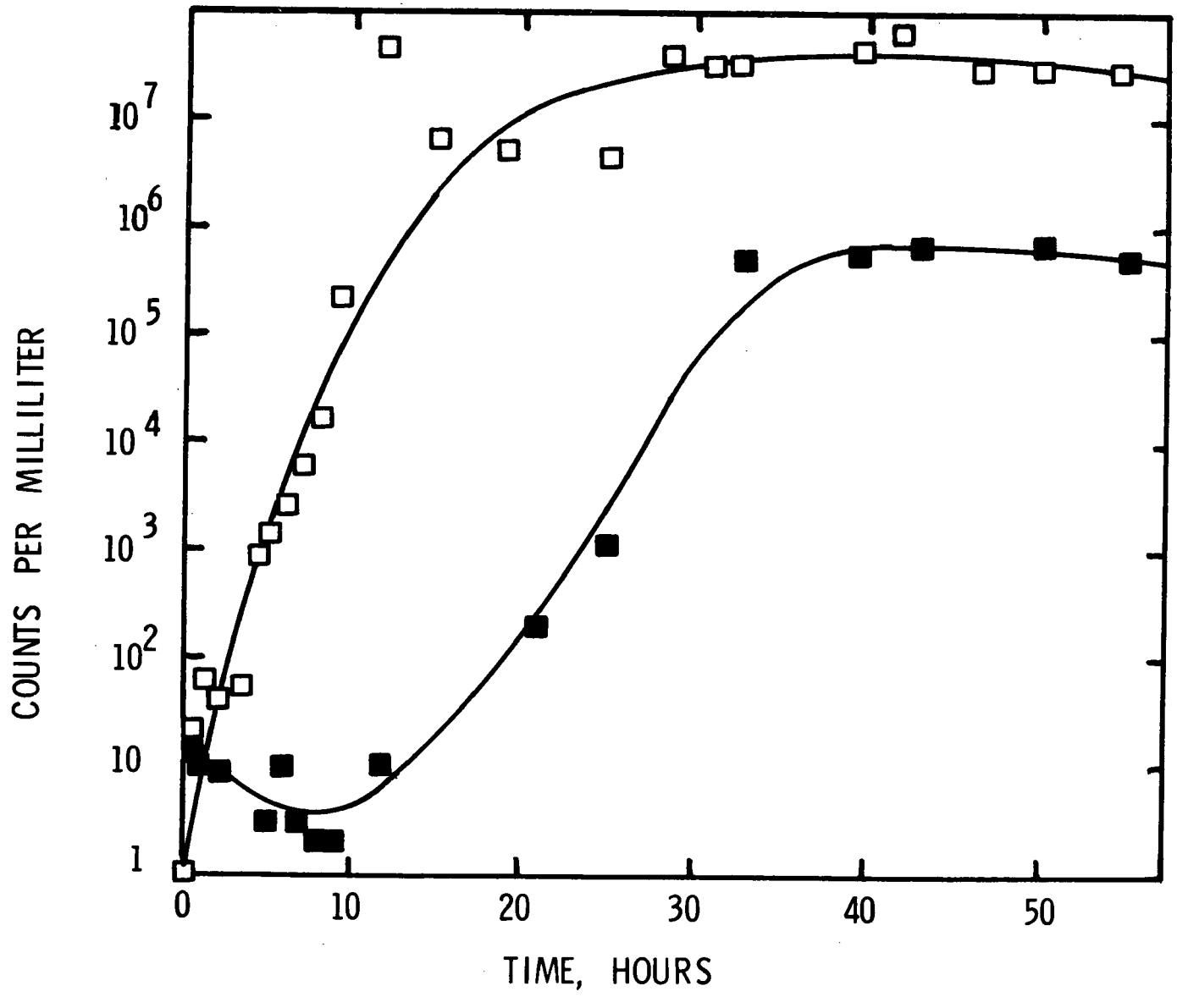


Figure 23. Growth Profile for Sterile Sludge Inoculated with Low Levels of Coliform Bacteria (Untreated Sludge)

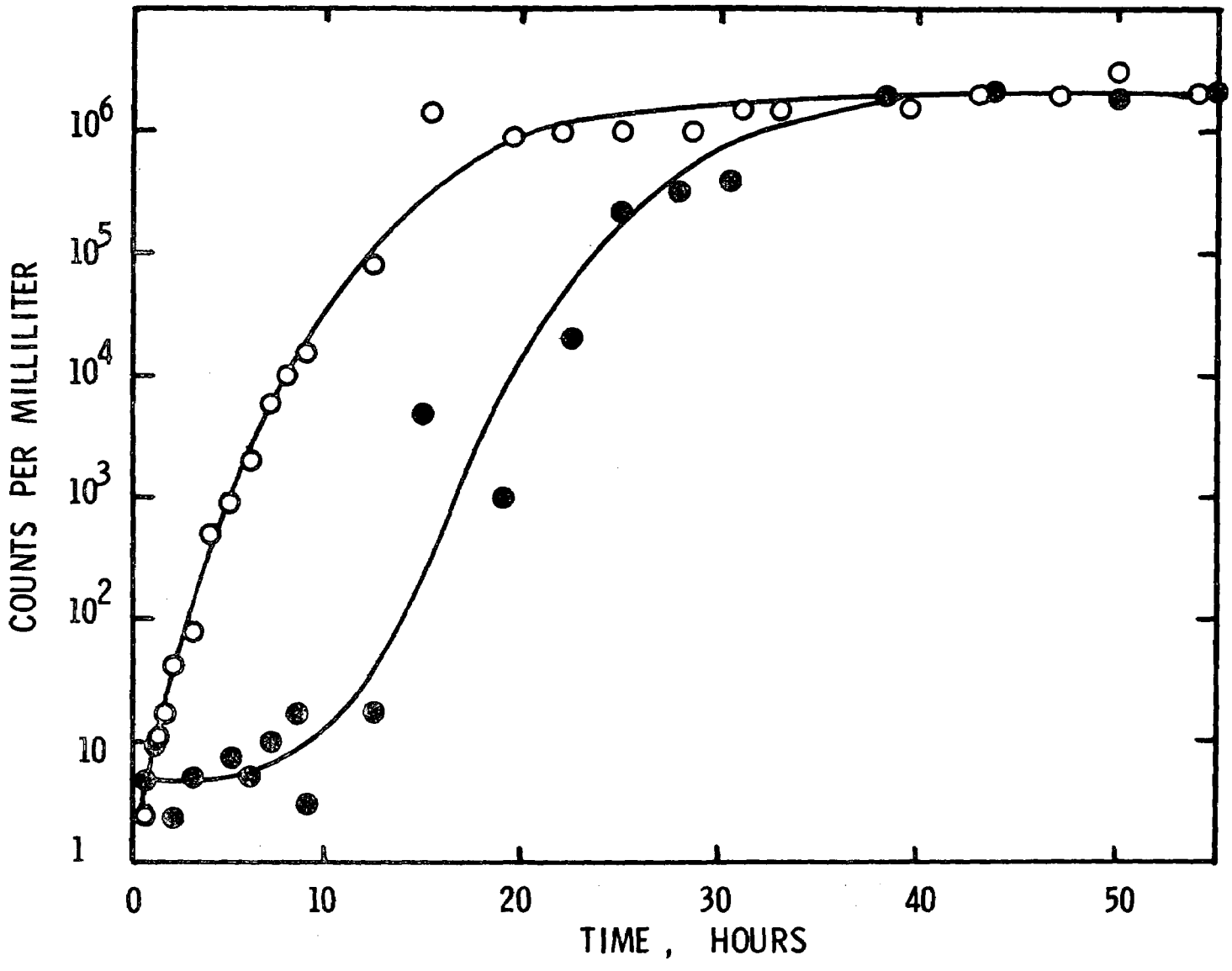


Figure 24. Growth Profile for Sterile Sludge Inoculated with Low Levels of Fecal Strep Bacteria (Untreated Sludge)

TABLE II

Results of "Growth" Experiments in Sludge for Various Initial Conditions. Samples Were Incubated at 35° C.

Sample	Normalized Counts/ml			
	0 hrs.	24 hrs.	48 hrs.	72 hrs.
(a) sterile sludge inoculated with broth	1.0	560.0	990.0	1030.0
(b) untreated sludge inoculated with broth	1.0	0.049	0.0069	----
(c) untreated digester sludge	1.0	0.37	0.12	0.091

Inactivation Rates for Salmonella from Sewage Sludge

Due to the low counts of these pathogenic bacteria in sewage sludge (several tens of bacteria per milliliter), it is virtually impossible to determine inactivation "rates" in the usual way. A somewhat artificial, yet meaningful, system was devised. Salmonella species from sludge were grown in Trypticase Soy Broth to a level of $\sim 10^9$ per milliliter. Small quantities of this broth were added to sterilized sludge, such that the starting count of Salmonella bacteria was routinely $10^5 - 10^6$ per milliliter. Standard inactivation rates were then determined using these samples. Survival of the bacteria after inactivation was measured by colony growth on Hektoen Enteric Agar (HE) which is moderately selective for Salmonella, or on Salmonella-Shigella Agar (SS), which is a highly selective medium. Random colonies were routinely checked biochemically, to insure that there was no interference from other bacteria; in all cases, these colonies tested positive for Salmonella. Figure 25 shows that radiation inactivation rates were not dependent on the type of agar used. Therefore, due to ease in counting and in colony differentiation, HE agar was used in most subsequent experimentation. It should be pointed out that inactivation rates were found to be essentially the same whether a broth sample was added to (a) normal, non-sterilized sludge or to (b) sludge which had been autoclaved for an hour, or to (c) sludge which had been irradiated to approximately 1.4 Megarads (Fig. 26).

There is some question, of course, as to whether data obtained using these "artificial" systems can be used to accurately predict the behavior of the normal Salmonella flora of digested sludge. As indicated, the species were originally taken from normal sludge. However, when fecal strep bacteria were irradiated in a totally analogous system, the inactivation rate was found to be approximately the same as that

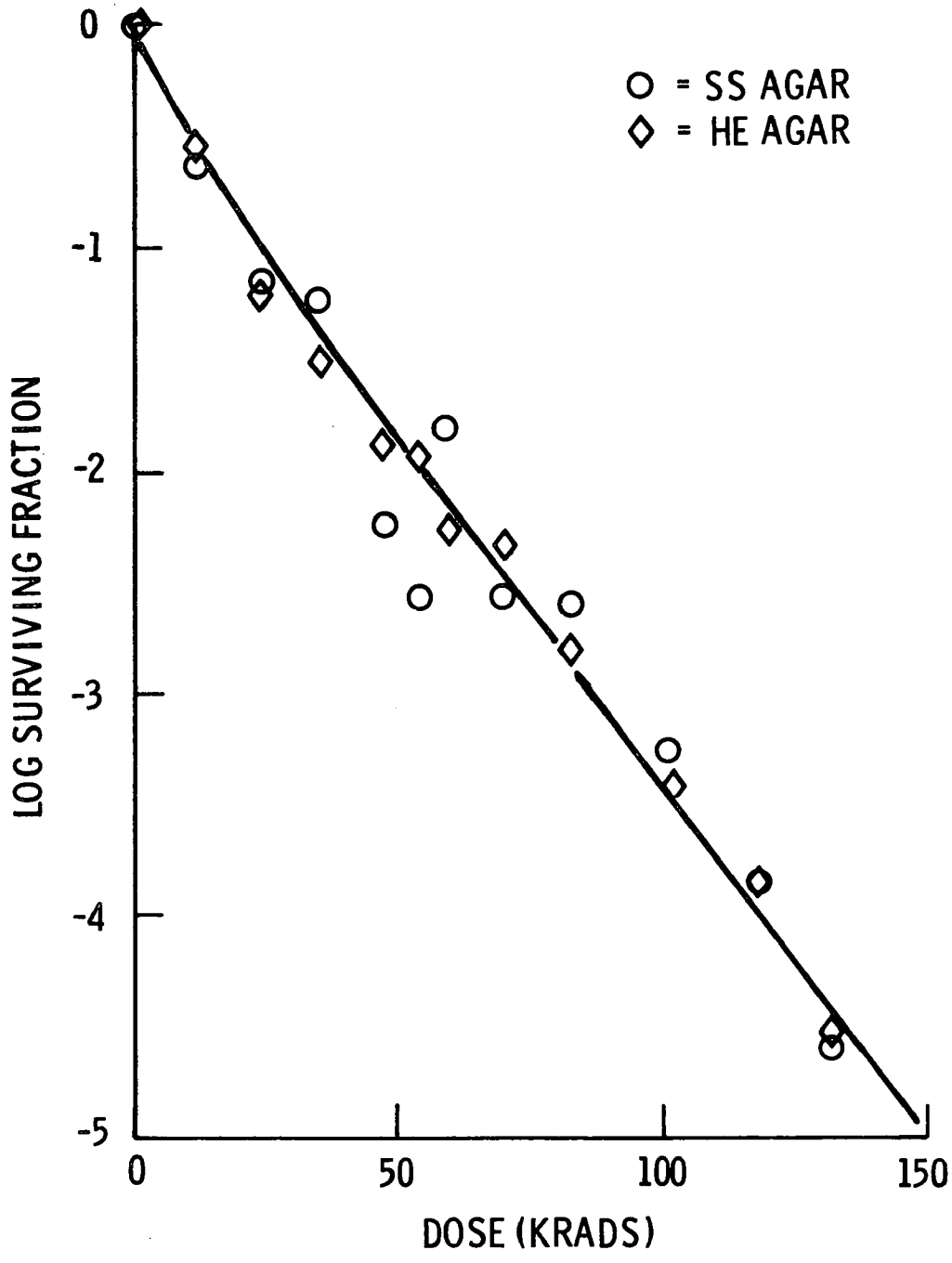


Figure 25. Inactivation Curve for Salmonella Species Using Both Hektoen Enteric (HE) and Salmonella-Shigella (SS) Agars at 23° C.

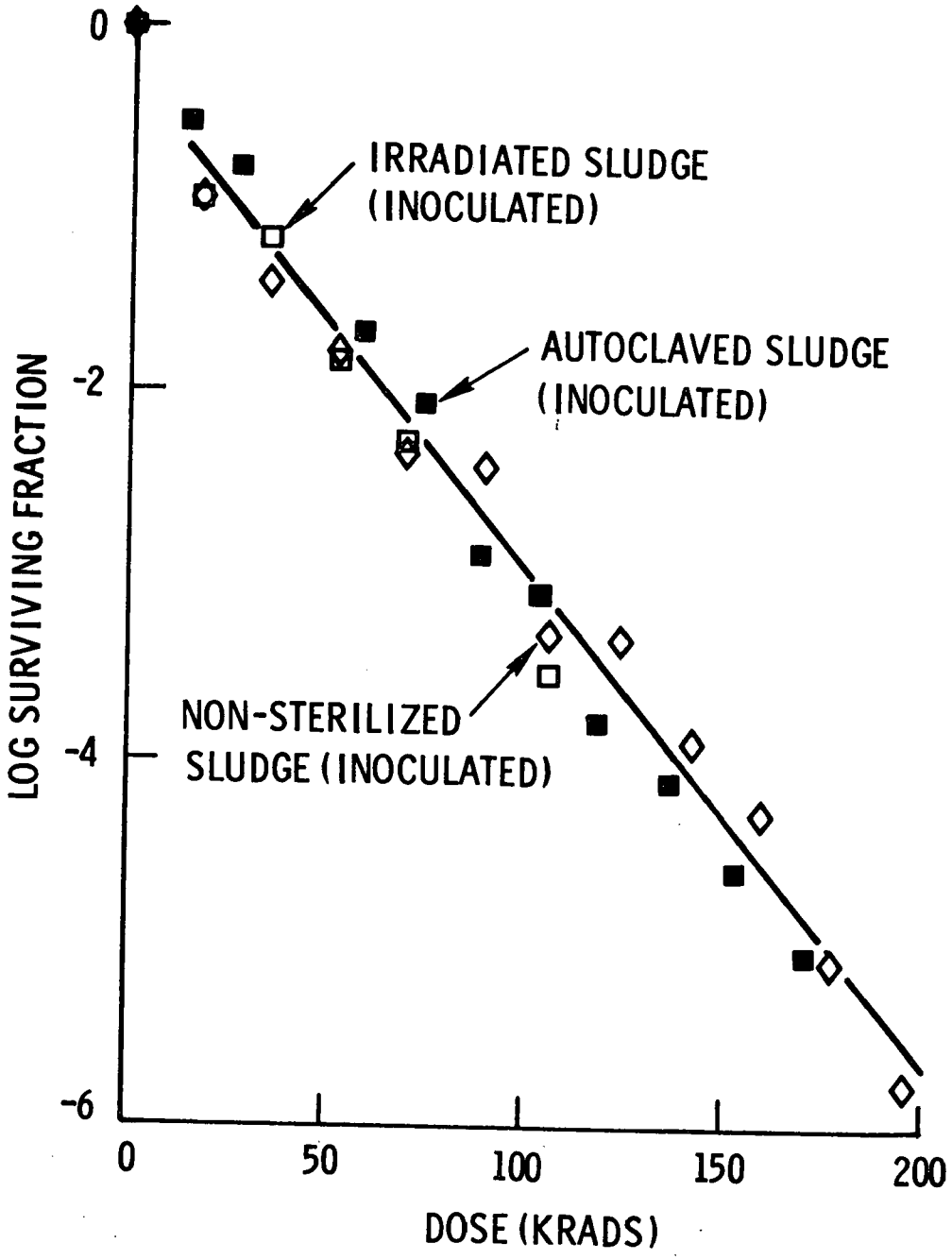


Figure 26. Radiation Inactivation Curves for Salmonella Species in Different Sludges at 23° C.

previously published for fecal strep in sewage sludge.[16] There is an indication that the broth-grown strep may be slightly more heat sensitive, but additional studies will be made to confirm this observation.

Inactivation curves for Salmonella species in sewage sludge were determined at 50° C as described above. The rate of inactivation was found to be approximately 50 percent greater than that at 23° C (compare Fig. 27 with Figs. 25 and 26). Experiments performed several months later gave inactivation rates for Salmonella (same culture) which were considerably higher (organisms appeared to be more sensitive) than the data presented in Fig. 27. Heat inactivation curves have recently been measured for Salmonella in sewage sludge; these data are shown in Fig. 28. It is seen that Salmonella are much more heat sensitive than either coliforms or fecal strep. This may be due in part to the artificiality of the system. Again, further studies are under way with strep and coliforms to determine if they behave similarly under analogous conditions.

Oxygen Effects on Inactivation Rates

Figures 29 and 30 show inactivation curves for coliform bacteria in sewage sludge at 23° C and at 55° C, respectively, for normal runs and for oxygen-saturated samples. For the latter, oxygen was bubbled through the sludge for about 10 minutes prior to irradiation, and continuously during the irradiation. It is seen that inactivation at room temperature by irradiation with oxygenation is at least equivalent to normal (no oxygen) inactivation at the higher temperature. This observation may be important in achieving inactivation at lower treatment temperatures. The effectiveness of oxygenation affords a further enhancement of the rate of inactivation during thermoradiation at 55° C.

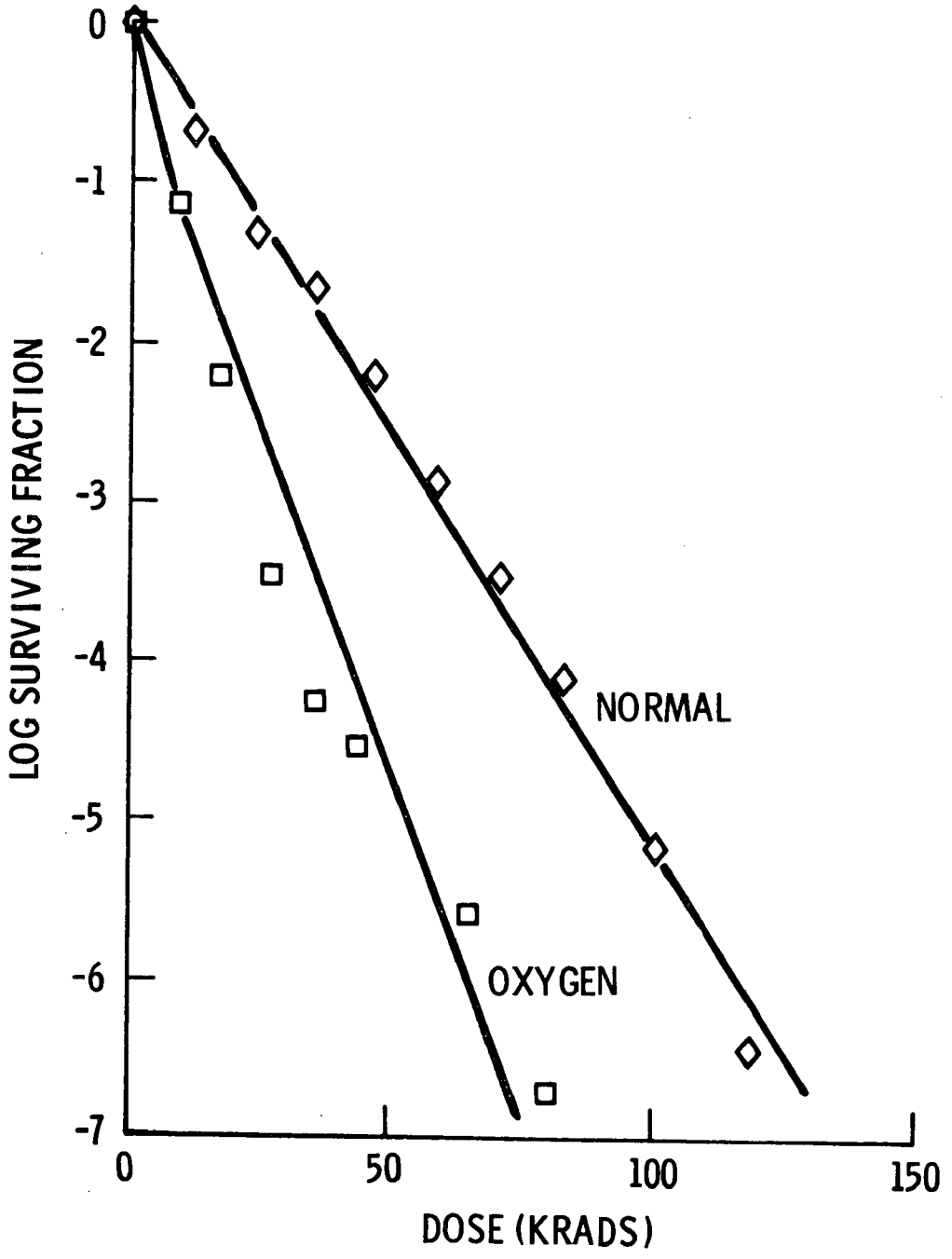


Figure 27. Thermoradiation Inactivation of Salmonella Species, With and Without Oxygenation at 50° C.

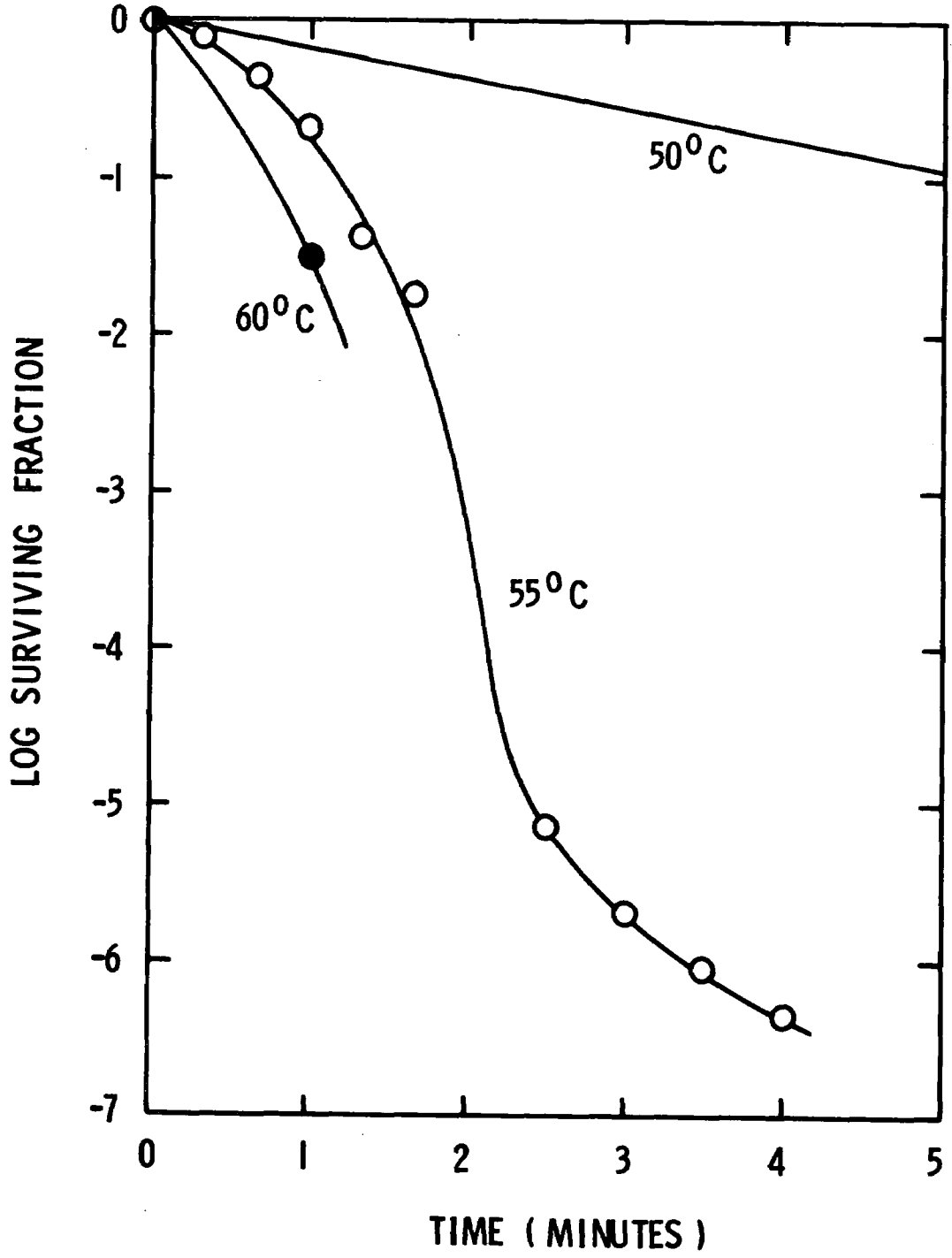


Figure 28. Heat Inactivation for Salmonella

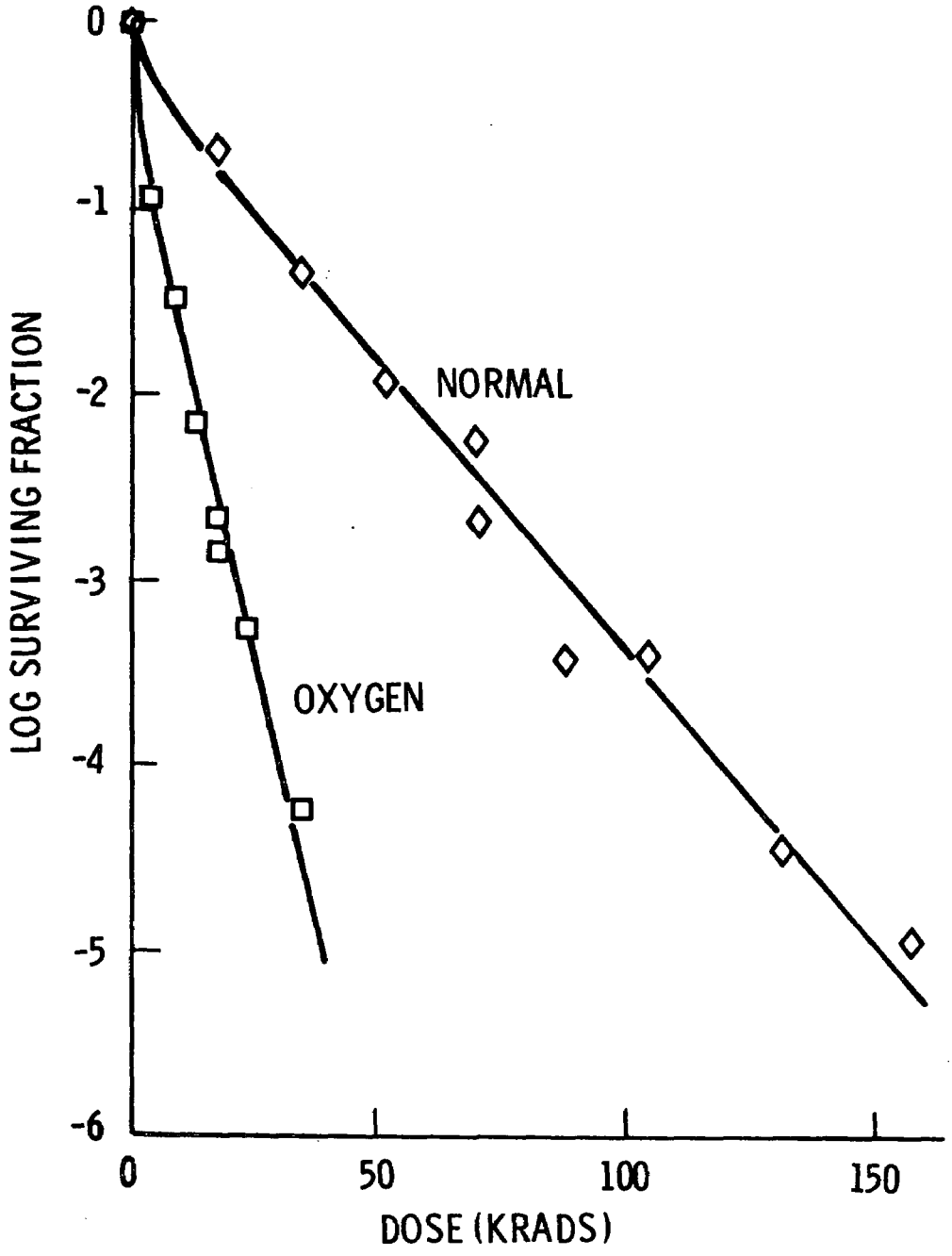


Figure 29. Effect of Oxygenation on Coliform Inactivation Rates at 23° C

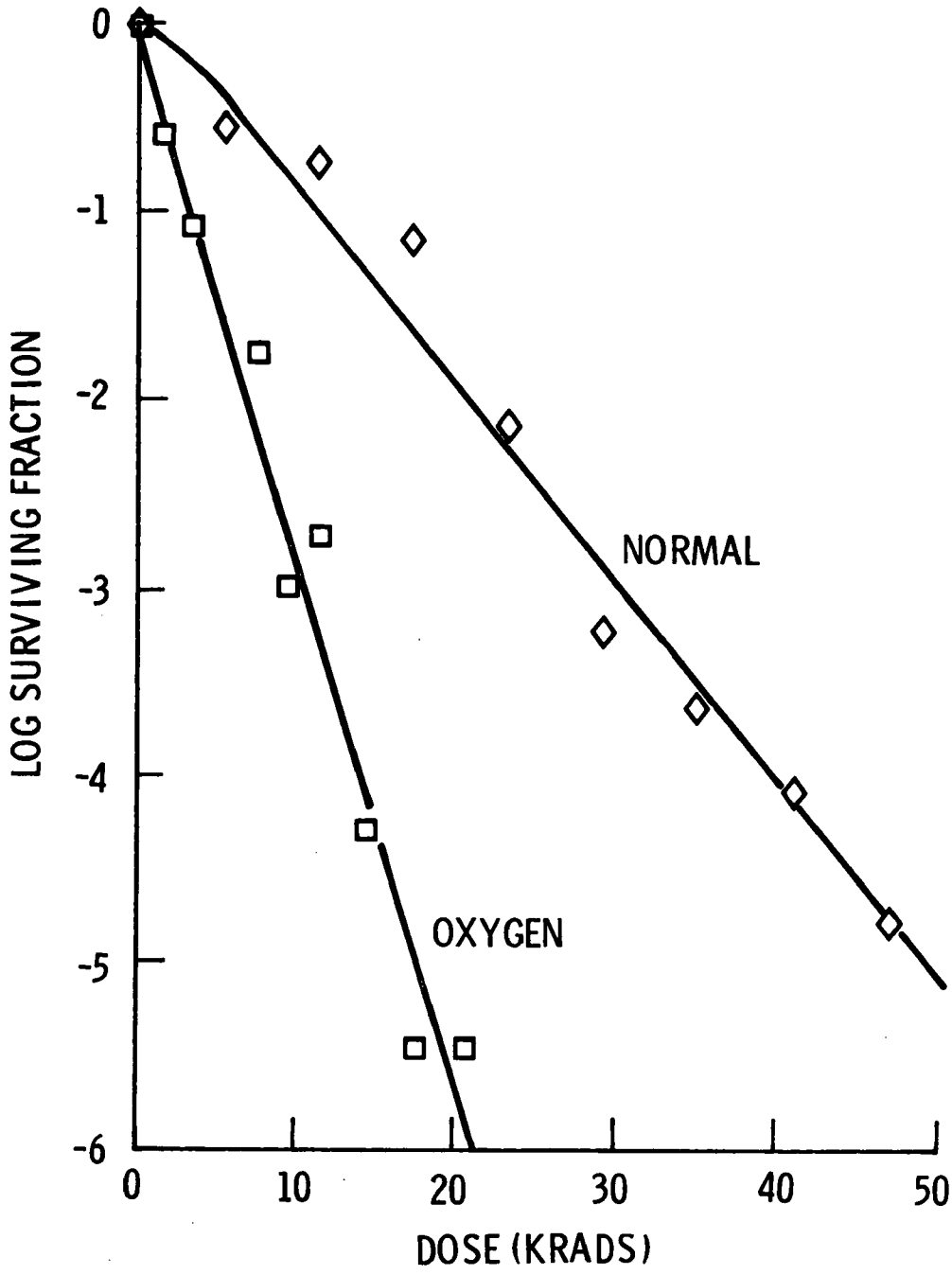


Figure 30. Oxygenation Effect on Thermoradiation Inactivation of Coliforms at 55° C

Oxygenation appears to be somewhat less effective for fecal strep bacteria; but, again, the inactivation at room temperature is as effective with oxygen as is normal thermo-radiation at 60° C.

Figures 27 and 31 show comparison curves for Salmonella. As was observed for coliforms and fecal strep, at the selected temperature, oxygenation is more effective than normal thermo-radiation treatment for Salmonella.

Part of the enhancement in inactivation rates at higher temperatures with oxygen must be due to the increased heat transfer brought about by the bubbling action. This is demonstrated by the observation that bubbling nitrogen enhances the inactivation rate somewhat, even though nitrogen is inert, from a radiation biology standpoint. In room temperature work, nitrogen tends to displace whatever oxygen is normally present in the air-saturated samples, and the inactivation rates generally are slightly less than in normal runs. Oxygen enhancement of radiation damage in biological systems is a well-known phenomenon.

Summary

Inactivation rate data for coliforms, fecal strep, and Salmonella are summarized as D-values for ionizing radiation at a variety of temperatures in Tables III, IV, and V, respectively.

These data indicate that radiation, particularly thermo-radiation, especially in the presence of oxygen, is an efficient means of disinfection of sewage sludge, from a bacteriological standpoint.

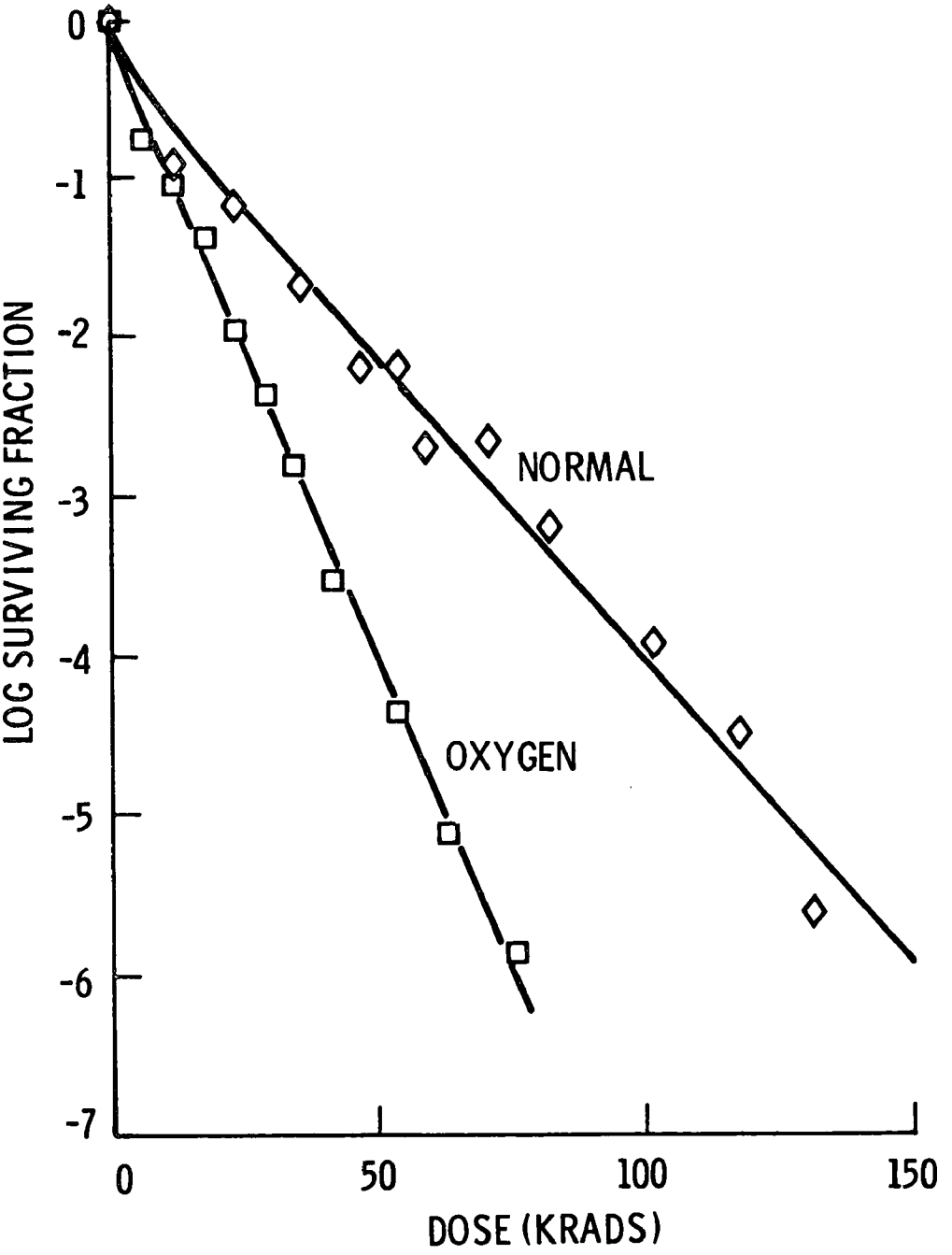


Figure 31. Oxygenation Effect on Salmonella Inactivation Curves (Inoculated Sludge) at 23° C

TABLE III

D-Values (Treatment Per Log Reduction) for Coliform Bacteria at Various Temperatures

Temperature (° C)	D-Value, krads/log	
	Normal	With 0_2
23	25 - 30	8
40	25	
45	20	
50	23	
55	10 - 15	4
60	15	
65	5	

TABLE IV

D-Values for Fecal Strep Bacteria
at Various Temperatures

Temperature (° C)	D-Value, krads/log	
	Normal	With 0_2
23	130 - 135	87
40	129	
45	129	
50	109	
55	86	
60	70 - 97	32
65	46	

TABLE V

D-Values for Salmonella Species
Added to Sewage Sludge

Temperature (° C)	D-Value, krads/log	
	Normal	With 0_2
23	26	13
50	19	11

VIROLOGY

Poliovirus Inactivation in Sludge

This section is divided into two parts. The first part deals with the effects of anaerobically digested sludge on seeded poliovirus at ambient temperatures and below. The second portion is concerned with the effects of both raw and anaerobically digested sludge on the rate of poliovirus inactivation by heat.

Inactivation of Poliovirus in Anaerobically Digested Sludge

Previous studies suggest that some viral inactivation occurs during anaerobic digestion.[17,18] This conclusion is based primarily on results derived during treatment plant operation. It is difficult to evaluate these data because conditions could not be controlled during treatment, the same material could not be sampled before and after treatment, and there was no way of determining the efficiency of virus recovery from either raw or digested sludge. Most of these technical problems can be avoided by studying the effects of milliliter quantities of sludge on highly purified, radioactively-labeled virus. With this procedure, the original number of infectious virus is clearly established and the relationship between inactivation and recovery can be monitored at all times under controlled conditions. Application of this method is made here to a study of poliovirus inactivation in digested sludge.

Recovery of Poliovirus from Seeded Sludge--The effects of digested sludge on poliovirus can be properly evaluated only if it is possible to account for the entire population of infectious virus. Because sludge solids have a tendency to bind virus, thereby creating a potential difficulty in virus recovery, a method designed to release bound particles after treatment with sludge was used throughout this entire study. This method was to sonicate in the presence of the detergent sodium dodecyl sulfate (SDS). Samples were then either directly analyzed for plaque-forming units or first centrifuged (18,000 g, 20 minutes) to remove large particulate matter before assaying for infectious virus. The initial experiments were designed to determine the efficacy of this procedure using the poliovirus type-1 strain CHAT, the strain used for all experiments unless stated otherwise.

After mixing virus with sludge for 15 minutes at room temperature and processing with SDS-sonication, there was typically a 50 percent loss of recoverable infectivity from the complete sample and an additional 17 percent loss from the supernatant fraction, as shown in Table VI. As also noted, the same treatment in phosphate buffered saline (PBS) alone caused no loss of recoverable infectivity. This result indicates that a portion of recoverable infectious virus becomes bound to sludge solids and is removed with the pellet during centrifugation. It also suggests that about 50 percent of the virus is either rapidly inactivated in digested sludge or has its infectivity masked by a component of sludge.

In order to determine whether the initial 50 percent loss of poliovirus infectivity was due solely to trapping by sludge solids, the effect of digested sludge on the recovery of purified poliovirus labeled with ^{3}H-uridine was examined. If trapping were the only factor, then only about 33 percent of the radioactivity should be recoverable in the supernatant

TABLE VI

Recovery of Plaque-forming Units after Mixing Poliovirus Strain CHAT with Digested Sludge for 15 Minutes at Room Temperature

Sample/Treatment	Recovery of PFU
No sludge (PBS)/No treatment	2.4×10^8
No sludge (PBS)/SDS-sonication	2.4×10^8 (100)[a]
No sludge (PBS)/SDS-sonication-centrifugation	2.4×10^8 (100)
Sludge/SDS-sonication	1.2×10^8 (50)
Sludge/SDS-sonication-centrifugation	0.8×10^8 (33)

[a]All numbers in parentheses are percentage recoveries relative to the untreated control.

after sedimentation of sludge solids. For this experiment, labeled virus was mixed with sludge for 15 minutes and processed with SDS-sonication. The samples were then centrifuged and the pellet was suspended in buffer, processed by SDS-sonication and repelleted by centrifugation. After repeating this procedure, the recovery of radioactivity and plaque-forming units in each of the three supernatant fractions was determined. As shown in Table VII, 64 percent rather than 33 percent of the labeled virus was recovered in the initial supernatant fraction. Further extraction of the sludge solids resulted in the release of almost all labeled virus. It is important to note that, if the number of plaque-forming units obtained from total sludge is taken to be 100 percent recovery, then the percentage of radioactive virus recovered during reextraction was parallel to the percent release of viral plaque-forming units. This result strongly suggests that the loss of infectivity upon addition of sludge is due to viral inactivation. Moreover, viruses that remain infectious after a short time in digested sludge are fully recoverable by the techniques used here.

To further substantiate the conclusion that polioviruses that retain their infectivities after mixing with digested sludge are fully recoverable, other strains of the virus were examined using these same procedures. As shown in Table VIII, plaque-forming units of the type-1 strain Mahoney were recovered *in toto* from the complete sludge sample. However, as with strain CHAT, a large portion of these infectious particles were bound to sludge solids because they were removed by centrifugation. A similar result was obtained using the type-2 strain 712. From this it seems clear that poliovirus mixed with digested sludge becomes rapidly bound to solids; but this association, at least initially, does not inhibit the plaque-forming ability of the virus.

TABLE VII

Recovery of Poliovirus in Digested Sludge Supernatant upon Reextraction of Sludge Solids

Sample[a]	Recovery of Radioactivity (CPM)	Recovery of PFU
Total sludge	11,070	1.2×10^8
Sludge supernatant #1	7069 (63.9)[b]	8.0×10^7 (66.7)
Sludge supernatant #2	2161 (19.5)	2.5×10^7 (20.8)
Sludge supernatant #3	1139 (10.3)	8.8×10^6 (7.3)
	(93.7)	(94.8)

a ^{3}H-uridine-labeled poliovirus strain CHAT was mixed with digested sludge for 15 minutes, processed with SDS-sonication, and analyzed as described in the text.

b Numbers in parentheses are percentage recoveries relative to CPM present in total sludge or PFU recovered from total sludge.

TABLE VIII

Recovery of Poliovirus (Strains Mahoney and 712) after 15 Minutes in Digested Sludge

Sample	Recovery of PFU[a]	
	Strain Mahoney	Strain 712
No sludge (PBS)	9.1×10^8	1.8×10^8
Total sludge	9.5×10^8 (104)[b]	1.8×10^8 (100)
Sludge supernatant	3.2×10^8 (35)	6.8×10^7 (38)

[a] Average values from 3 duplicate experiments.

[b] Numbers in parentheses are percentage recoveries relative to sample without sludge.

Loss of Infectivity of Poliovirus in Digested Sludge--Anaerobic digestion of sludge typically occurs at temperatures greater than ambient. For instance, the digesters at the Albuquerque Treatment Plant are kept at about 35° C. At this temperature poliovirus may be subject to rather rapid heat inactivation. To minimize the inactivation due to heat alone, the effect of digested sludge on poliovirus was studied during incubation at temperatures between 28° C and 4° C.

Incubation in the absence of sludge caused the titer of all three strains of poliovirus to decrease about one order of magnitude during 5 days at 28° C, but little or no loss was detectable during 3 days at 20° C or 5 days at 4° C, as shown in Table IX. The presence of sludge at these temperatures caused the apparent rate of viral inactivation to be greatly accelerated. This rate was greater than 1-log per day at 28° C and about 1-log every 5 days at 4° C.

One implication of these results is that anaerobically digested sludge contains a virucidal component whose activity is temperature dependent. However, it is also possible that the observed loss of titer was due to a time- and temperature-dependent masking of viral infectivity by a component of sludge. This possibility can be investigated by determining the status of virus particles following incubation in sludge. Although the properties of viruses in solution can be studied, virus particles physically associated with sludge solids cannot be readily analyzed. Therefore, in order to determine whether the infectivity of poliovirus is irreversibly lost or just masked in sludge it must be shown that virus that remained bound to solids had been subject to the same effects as virus found in the supernatant fraction following centrifugation of solids.

TABLE IX

Recovery of Poliovirus Plaque-forming Units from Digested Sludge as a Function of the Time of Incubation

Incubation Time/Temperature	Strain	Percentage Recovery of PFU	
		- Sludge	+ Sludge
1 day/28° C	CHAT	69	5.8
	MAH	55	7.3
	712	70	1.5
3 days/20° C	CHAT	83	0.65
	MAH	82	0.21
	712	100	0.24
5 days/4° C	CHAT	100	3.8
	MAH	100	3.6
	712	100	3.3
5 days/28° C	CHAT	6.3	0.0003
	MAH	11.0	0.00005
	712	8.4	0.0002

If bound and free virus is subject to the same effects while in sludge, release of bound virus upon reextraction of sludge solids should result in a proportional release of plaque-forming units. To measure the release of poliovirus particles, virus labeled with ^{3}H-uridine was incubated at 20° C for 3 days in digested sludge and processed by SDS-sonication treatment. The sample was then centrifuged to remove solids and the pellet was reextracted two additional times by the same procedure. Finally, the radioactivity and plaque-forming units recoverable in the three supernatant fractions were measured. As shown in Table X, labeled virus was almost completely recovered after three extractions of sludge solids and the percentage release of radioactivity during each extraction step was directly proportional to the release of infectious virus. From these results it is concluded that bound and free virus are subject to the same effects during incubation in sludge. Therefore, radioactive viruses found in the supernatant fraction following the first centrifugation of sludge solids can be considered representative of all virus particles. The nature of these particles was then studied to determine the mechanism by which poliovirus loses its infectivity during incubation in digested sludge.

Sedimentation Coefficient of Poliovirus after Incubation in Digested Sludge--The possibility that a component of sludge masks the infectivity of otherwise infectious poliovirus particles seemed not unlikely in view of the number of papers concerning substances that have this very effect. For example, the methylthiopyrimidine S-7 has been found to bind reversibly to poliovirus and temporarily block its infectivity.[19] For this reason, poliovirus was examined for structural modification occurring during incubation in sludge which would account for the observed loss of infectivity.

TABLE X

Recovery of Poliovirus from Digested Sludge after Incubation for 3 Days at 20° C by Reextraction of Sludge Solids

Sample	Recovery of Radioactivity (CPM)	Recovery of PFU
Total sludge	12,204	1.3×10^6
Sludge supernatant #1	7,669 (62.8)[a]	8.0×10^5 (61.5)
Sludge supernatant #2	2,632 (21.6)	2.4×10^5 (18.5)
Sludge supernatant #3	1,302 (10.7)	1.4×10^5 (10.8)
	(95.1)	(90.8)

[a] Numbers in parentheses are percentage recoveries relative to CPM present in total sludge or PFU recovered from total sludge.

The initial effect on poliovirus during heat inactivation has been reported to be the release of a protein called VP-4.[19,20] This loss causes the sedimentation coefficient of the virus to decrease from 156s to about 130s. Further heat treatment results in additional decomposition and concomitant reduction in the sedimentation value of the viral components. Although it has also been reported that both the protein and RNA of poliovirus can be inactivated without altering the sedimentation value of the particle,[21,22] an attempt was made to identify structural modifications through changes in the sedimentation coefficient of the virus.

To measure changes in viral sedimentation rate, purified poliovirus labeled with ^{14}C-amino acids was incubated in sludge and analyzed by centrifugation in glycerol gradients. As shown in Fig. 32, incubation at 28° C both in the absence and presence of sludge resulted in a considerable decrease in the sedimentation rate of a portion of the labeled virus. However, the percentage of particles detectably affected at this temperature was significantly lower than the percentage loss of infectivity (see Table IV). This is especially evident for the sample containing sludge. Here, survival of plaque-forming units was less than 0.0003 percent while the sedimentation value of only about 90 percent of the particles had been visibly altered.

Loss of titer without a change in sedimentation coefficient of a proportional fraction of virus was even more evident after incubation at 4° C, as also shown in Fig. 32. Although less than 4 percent of the original infectivity was recoverable (see Table IV), there was almost no shift of radioactive particles from the position of infectious virus. These results show that if the loss of infectivity of poliovirus in sludge is due to structural damage of the virion, this damage is not necessarily reflected in the sedimentation

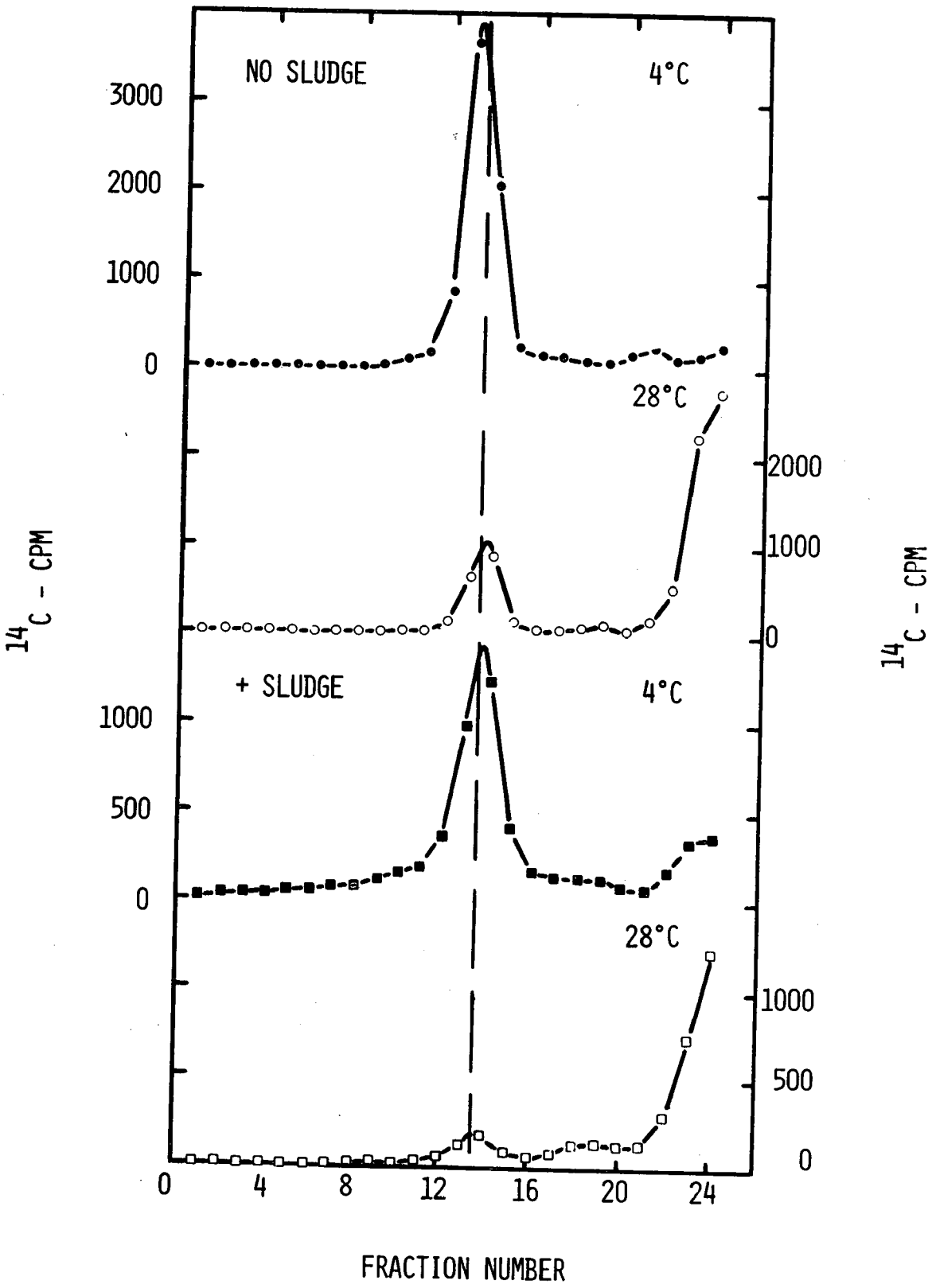


Figure 32. Effect of Sludge on the Sedimentation Coefficient of Poliovirus. (The dashed line designates the position of infectious virus.)

values of the affected particles. Therefore, the individual components of potentially inactivated particles having unaltered sedimentation values were examined for structural modifications.

RNA Component of Potentially Inactivated Poliovirus Particles--The poliovirus particle is composed of a single-stranded RNA genome and a capsid of 4 distinct proteins. Because the genome is itself infectious, loss of viral infectivity due to its damage will result in a proportional loss of infectious RNA. Therefore, viral RNA was the first component to be examined for structural damage.

Because breakdown of poliovirus is so much slower than loss of infectivity in sludge at 4° C, alterations of viral RNA were studied after incubation at this temperature. For this, ^{3}H-uridine-labeled virus was incubated for 10 days in sludge during which time its recoverable infectivity decreased 99.1 percent. The sample was then processed by SDS-sonication treatment in parallel with labeled virus which had been in sludge at room temperature only 15 minutes. Even though some loss of poliovirus infectivity takes place during a short time in sludge (see Table VI), this particular control was included to ensure that any observed effect on viral RNA was not caused by some sludge component present during processing of virus or extraction of RNA. After pelleting sludge solids, the samples were analyzed by density gradient centrifugation. As shown in Fig. 33, the majority of particles in both samples still sedimented at the same rate as infectious virus (see Fig. 32). The peak fractions were combined as shown and their RNA was extracted with phenol. The sedimentation coefficients and infectivities of extracted RNA were then measured.

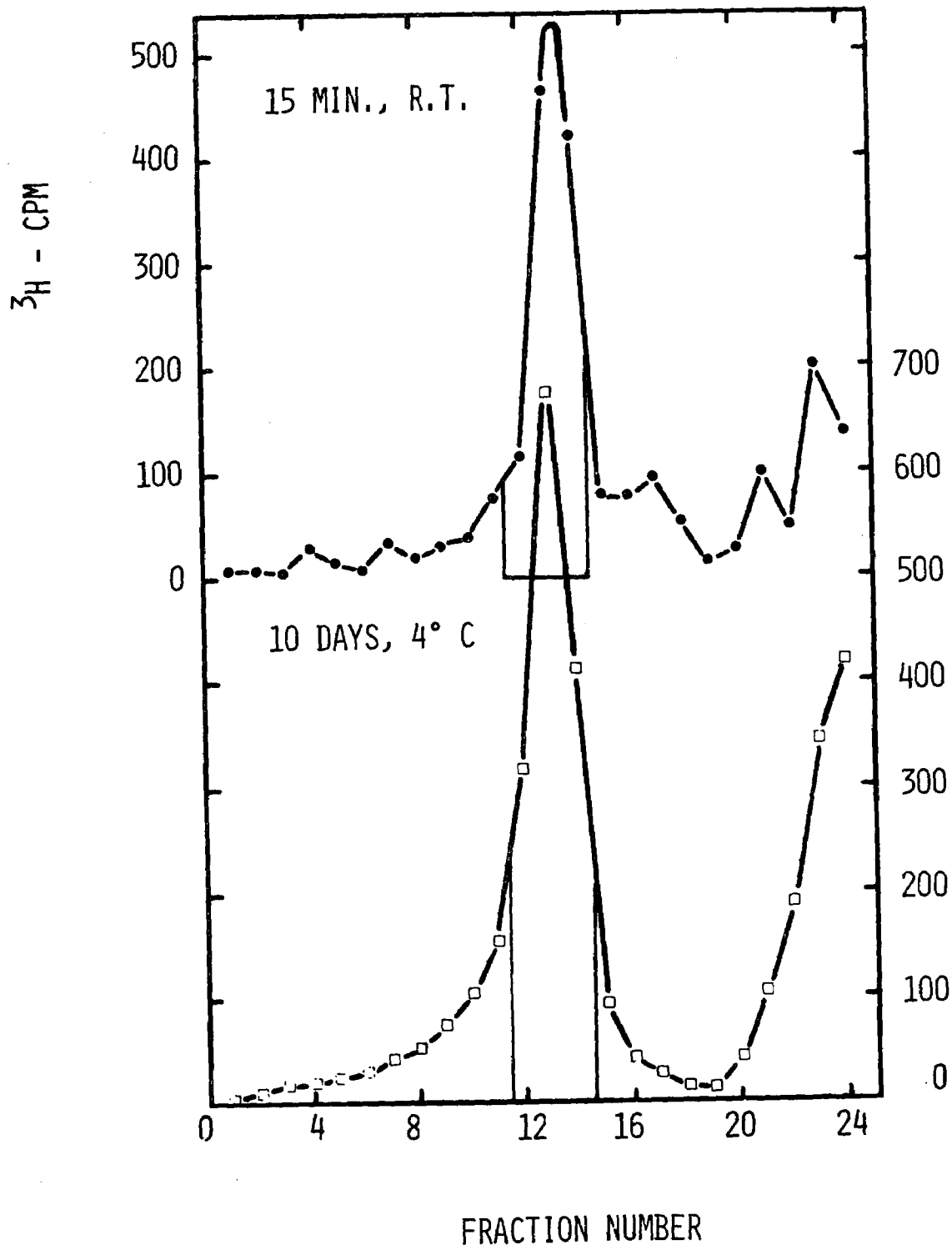


Figure 33. Isolation of Poliovirus Particles by Density Gradient Centrifugation Following Incubation in Sludge.

Changes in sedimentation coefficient were determined by density gradient centrifugation. As shown in Fig. 34, RNA from the 15-minute sample was mostly intact, having a sedimentation value of 35s as determined by co-sedimentation with RNA extracted directly from purified virus. On the other hand, RNA from virus in sludge for 10 days appeared to have been nicked, having an average sedimentation value of less than 25s. If incubation in sludge caused nicking of poliovirus RNA, its specific infectivity should be considerably less than that of RNA from the control sample. As shown in Table XI, this is indeed the case. Furthermore, the ratio of specific infectivities of virus/RNA for the 15-minute sample was essentially identical to that of the sample in sludge for 10 days. From this it is concluded that loss of viral infectivity parallels the decrease in infectious RNA. Thus, the inactivation of poliovirus during 10 days in sludge at 4° C may be due to nicking of the viral genome within the virus particle.

Because an intact, infectious RNA genome is a prerequisite for an infectious poliovirus particle, these data clearly establish one point. Viral plaque-forming units were lost during these experiments as a result of irreversible inactivation.

Proteins of Inactivated Poliovirus--Although the inactivation of poliovirus RNA has been shown to occur in particles whose sedimentation values had not been visibly altered, the cause of inactivation has not been determined. It is possible that ribonuclease present in sludge may somehow penetrate the particle and produce limited digestion of the viral RNA. However, incubation of poliovirus for 10 days at 4° C in 20 μg of ribonuclease per milliliter of PBS at the same pH as sludge (pH 8.0) had no effect on its infectivity. This result suggests that if ribonuclease is

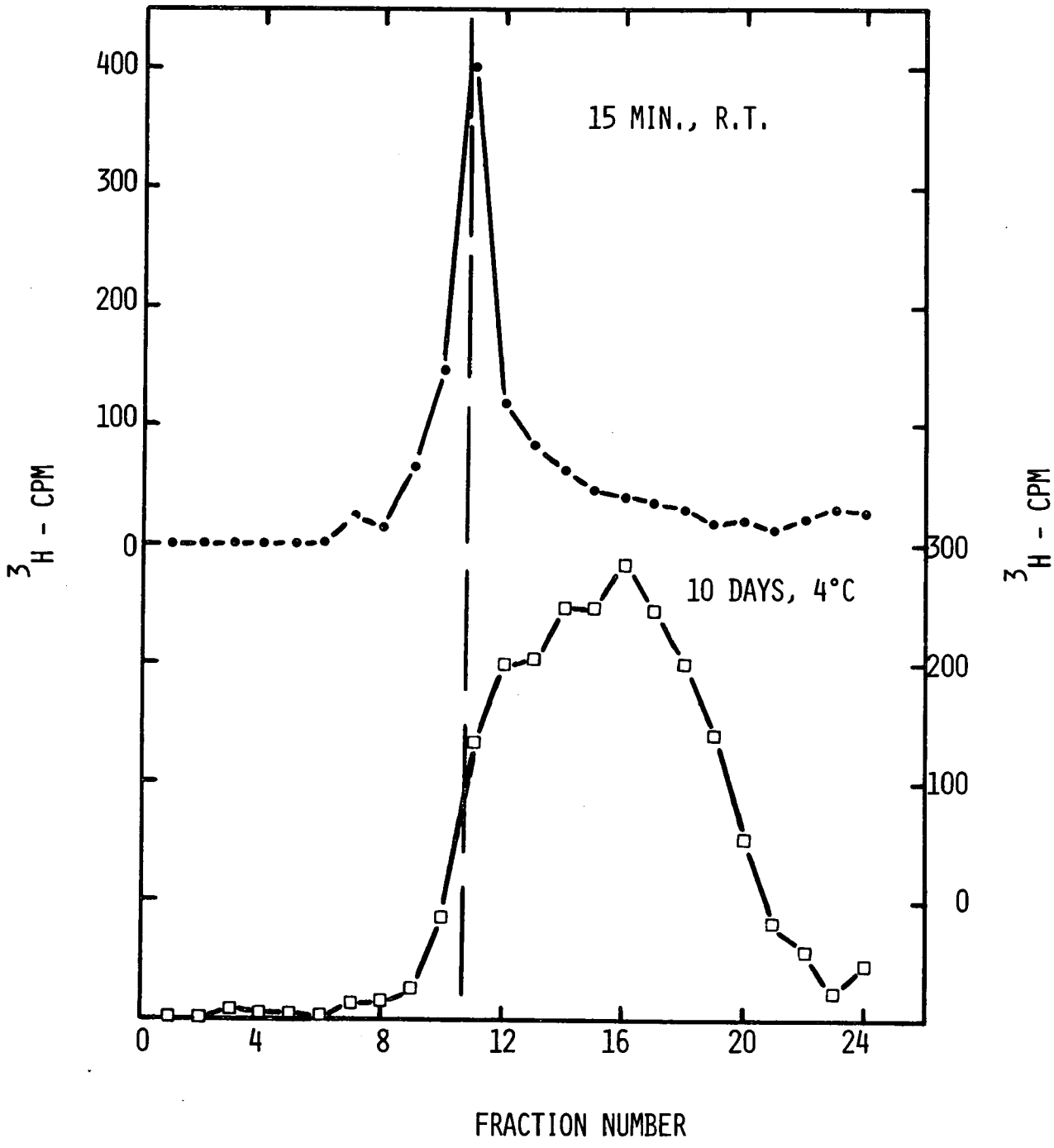


Figure 34. Density Gradient Centrifugation of Poliovirus RNA from Particles Recovered from Sludge. (The dashed line shows the position of labeled RNA extracted from untreated poliovirus and sedimented in an identical gradient.)

TABLE XI

Infectivity of Poliovirus RNA Extracted
from Particles Incubated in Digested Sludge

Sludge Treatment	Specific Infectivity[a]		
	(A) Virus	(B) RNA	Ratio (A/B)
15 min., 20° C	3.5×10^{3}	1.9×10^{0}	1.8×10^{3}
10 days, 4° C	7.7×10^{1}	5.6×10^{-2}	1.4×10^{3}

[a] Plaque-forming units per cpm of ^{3}H-uridine.

involved, some additional alteration of the particle probably occurs which allows its penetration. The most likely alteration is one involving the capsid protein of the virus. It is improbable, however, that this alteration is of major proportion since the sedimentation values of most particles inactivated in sludge at 4° C were not detectably changed. Conceivably, limited breakdown of capsid subunits may allow penetration of the particles.

Possible breakdown of ^{14}C-labeled poliovirus proteins occurring during 10 days in sludge at 4° C was determined by electrophoresis of inactivated, 156s particles in SDS-polyacrylamide gels. The two largest viral proteins (VP-1 and VP-2) appear to have been broken down into smaller peptides during incubation of virus in sludge, as shown in Fig. 35. Based on the recovery of radioactivity in the peak fractions of these gels versus total recovery, at least 75 percent of VP-1 and 25 percent of VP-2 were cleaved. The upper limit of breakdown cannot be determined since labeled peptides may have been released from the particles during treatment or preparation of the samples for analysis. It is of interest to note that VP-4, the first protein to be released during heat inactivation of poliovirus, appears to remain particle-associated. This cannot be definitively stated, however, since some cleavage products of the large viral proteins had molecular weights similar to that of VP-4, but the finding that most particles inactivated at 4° C did not have detectably altered sedimentation values supports this suggestion. Therefore, the mechanism of heat inactivation of poliovirus appears to be distinctly different from the process occurring in digested sludge at low temperatures.

The results presented until now suggest that inactivation of poliovirus in sludge at 4° C may be caused by the combined effect of limited proteolytic digestion of viral particles

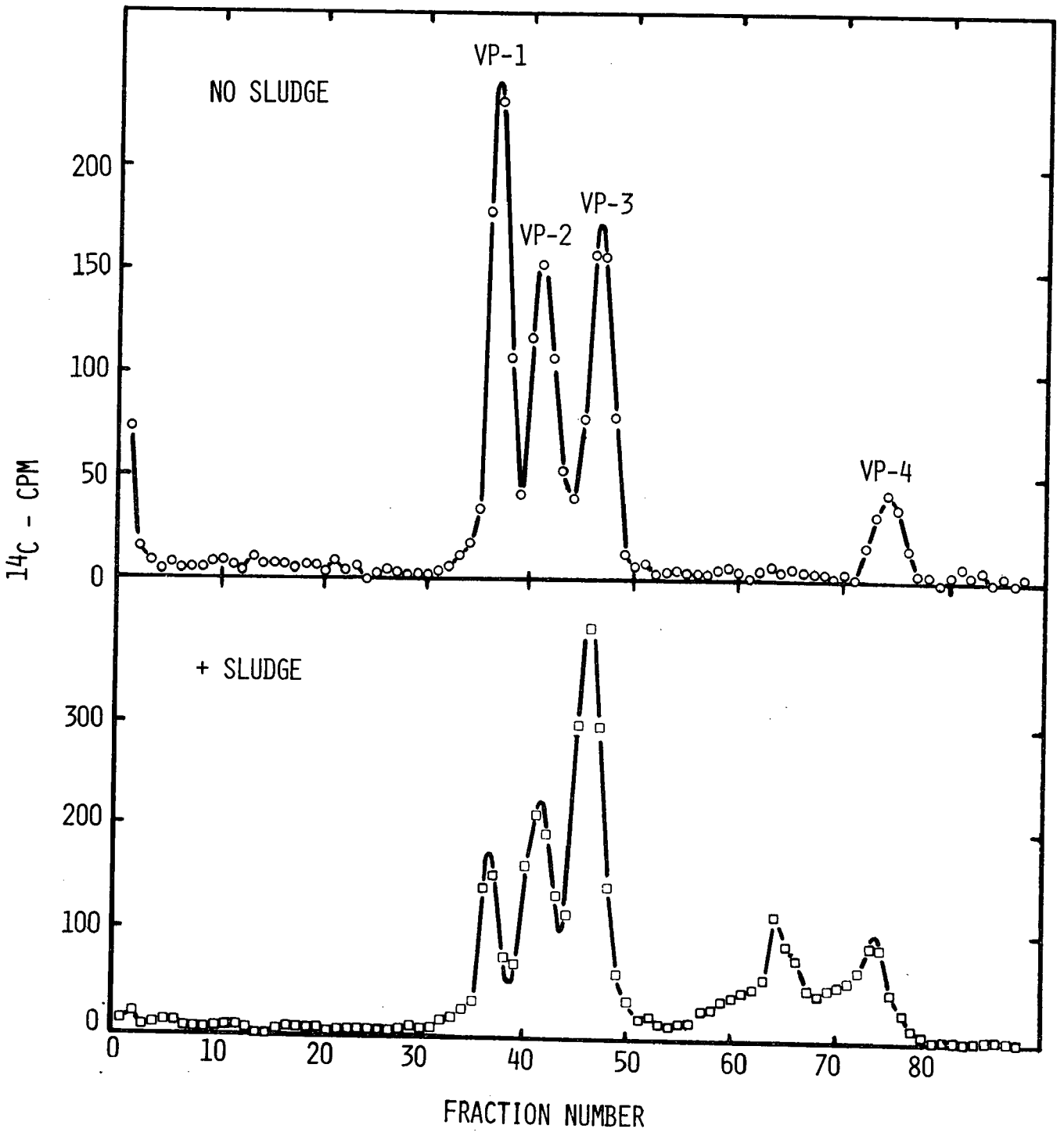


Figure 35. SDS-polyacrylamide Gel Electrophoresis Pattern of Poliovirus Proteins from Particles Recovered from Sludge.

followed by penetration and inactivation of RNA by ribonuclease. Although it has been reported that incubation at 37° C in high concentrations of various proteases does not alter the infectivity of poliovirus,[23] the combined effect of proteolytic enzymes and ribonuclease was studied under conditions simulating those of this sludge experiment. As shown in Table XII, essentially no reduction in the infectivity of virus was found. Therefore, inactivation of poliovirus in sludge appears to be caused by more than the combined effect of their two enzymatic activities.

Absence of Virucidal Component in Raw Sludge--No consideration has been given, up to this point, as to the origin of the virucidal component found in anaerobically digested sludge. This material may be a product of the digestion process or may already be in the sludge prior to digestion. The latter alternative seems unlikely in view of the finding that anaerobically digested sludge from two other cities (Los Angeles and Denver) was found to have a similar amount of virucidal activity to that of digested Albuquerque sludge (results not shown).

In an attempt to determine the point of origin of the virucidal material, raw sludge obtained in route to the digester was measured for this activity. As shown in Table XIII, raw sludge had no detectable effect on poliovirus during 5 days at 20° C. This finding indicates that the component of sludge responsible for inactivating poliovirus originates in the digester.

Location of Sludge Component Responsible for Viral Inactivation: Solids versus Liquid--In an initial step toward the eventual identification of the components responsible for viral inactivation, the liquid and solid portions of digested sludge were separated and individually tested

TABLE XII

Effect of Proteolytic Enzymes and Ribonuclease on Poliovirus Infectivity[a]

Enzyme Treatment	Recovery of PFU
None (PBS)	2.0×10^8
Trypsin[b] + Ribonuclease	2.0×10^8
Chymotrypsin + Ribonuclease	1.8×10^8
Pronase + Ribonuclease	1.8×10^8

[a] Viral plaque-forming units were measured following incubation with the given enzymes for 10 days at 4° C and at the pH found for digested sludge (8.0).

[b] All protease concentrations were 100 µg/ml while that of ribonuclease was 20 µg/ml.

TABLE XIII

Effect of Raw Sludge on Poliovirus Infectivity

Sample	Recovery of PFU
Control	1.5×10^8
PBS (5 days, 20° C)	1.6×10^8
Digested sludge (5 days, 20° C)	4×10^3
Raw sludge (5 days, 20° C)	1.4×10^8

for their ability to inactivate poliovirus. For this experiment, sludge was centrifuged for 20 minutes at 18,000 g and the supernatant decanted. The solids were then resuspended in a volume of PBS equal to that of the removed liquid and the two components were compared with the original sludge in their abilities to cause poliovirus inactivation during 3 days at 28° C. As shown in Table XIV, the material responsible for loss of infectivity was found mainly in the liquid fraction of sludge. Further analysis of this material is in progress.

Heat Inactivation of Poliovirus in Raw and Anaerobically Digested Sludge

A possible method of rapidly ridding sludge of viral pathogens is with heat treatment. However, heating sludge at high temperatures for extended periods of time is not only a costly procedure, but one which may destroy a large portion of its potential value. Therefore, if viruses are to be inactivated in sludge by an elevation of temperature, it is highly desirable to define an effective treatment which requires a minimal amount of heat.

Viral disinfection in wastewater is commonly studied using poliovirus as an indicator. A number of investigations have been made concerning the rate and mechanism of inactivation of this virus at various temperatures in defined media.[19,20,24-28] However, the rate of heat inactivation of poliovirus in sludge has not been measured. Because a variety of substances protect poliovirus against heat inactivation (c.f. 19,29-31), the presence of sludge may cause a considerable reduction in its inactivation rate. Therefore, a study was undertaken to determine the effects of raw and anaerobically digested sludge on the rate of heat inactivation of poliovirus.

TABLE XIV

Inactivation of Poliovirus by Various Fractions of Digested Sludge During 3 Days at 28° C[a]

Sludge Fraction	Recovery of PFU
Control	2.0×10^8
No sludge (PBS)	7.0×10^7 (35)[b]
Total sludge	5.7×10^3 (0.003)
Sludge supernatant	6×10^2 (0.0003)
Sludge solids	1.5×10^7 (7.5)

[a] Virus was incubated in various digested sludge fractions following centrifugation at 18,000 g for 20 minutes.

[b] Percentage recovery relative to control sample which remained frozen during the time of incubation.

Heat Induced Loss of Poliovirus Plaque-Forming Units in Sludge--The effect of raw and anaerobically digested sludge on the rate of loss of poliovirus (strain CHAT) plaque-forming units as a function of temperature was investigated. As shown in Fig. 36, raw sludge is quite protective of the virus at all temperatures studied here. On the other hand, the rate of loss of plaque-forming units in digested sludge relative to that occurring in buffer alone is dependent upon the temperature. At the lowest temperature studied (43° C) digested sludge is somewhat protective, but at the highest temperature used (51° C) digested sludge accelerates loss of titer.

Probably the most significant feature of the data presented in Fig. 36 is the dramatically different effects of raw and digested sludge in these experiments. The apparent explanations for this difference are that raw sludge either contains a protective substance that is lost upon digestion or acquires an activity during digestion that accelerates the rate of heat inactivation of poliovirus. However, a third explanation cannot be overlooked. Digested sludge may contain a component that is stimulated by heat to become strongly bound to poliovirus and, in so doing, mask the plaque-forming ability of otherwise infectious viruses. This latter possibility can be ruled out if it can be shown that the loss of poliovirus titer with heat in digested sludge is due to viral inactivation. Therefore, the physical nature of virus particles following heat treatment in digested sludge was investigated.

Breakdown of Poliovirus During Heat Treatment in Anaerobically Digested Sludge--The nature of poliovirus particles after heat treatment in digested sludge was studied by the use of purified, radioactively-labeled virus. For this, digested sludge was seeded with labeled virus, heated at 43° C for 200 minutes, and prepared for analysis by sonication in 0.1 percent SDS. This treatment was found to cause about

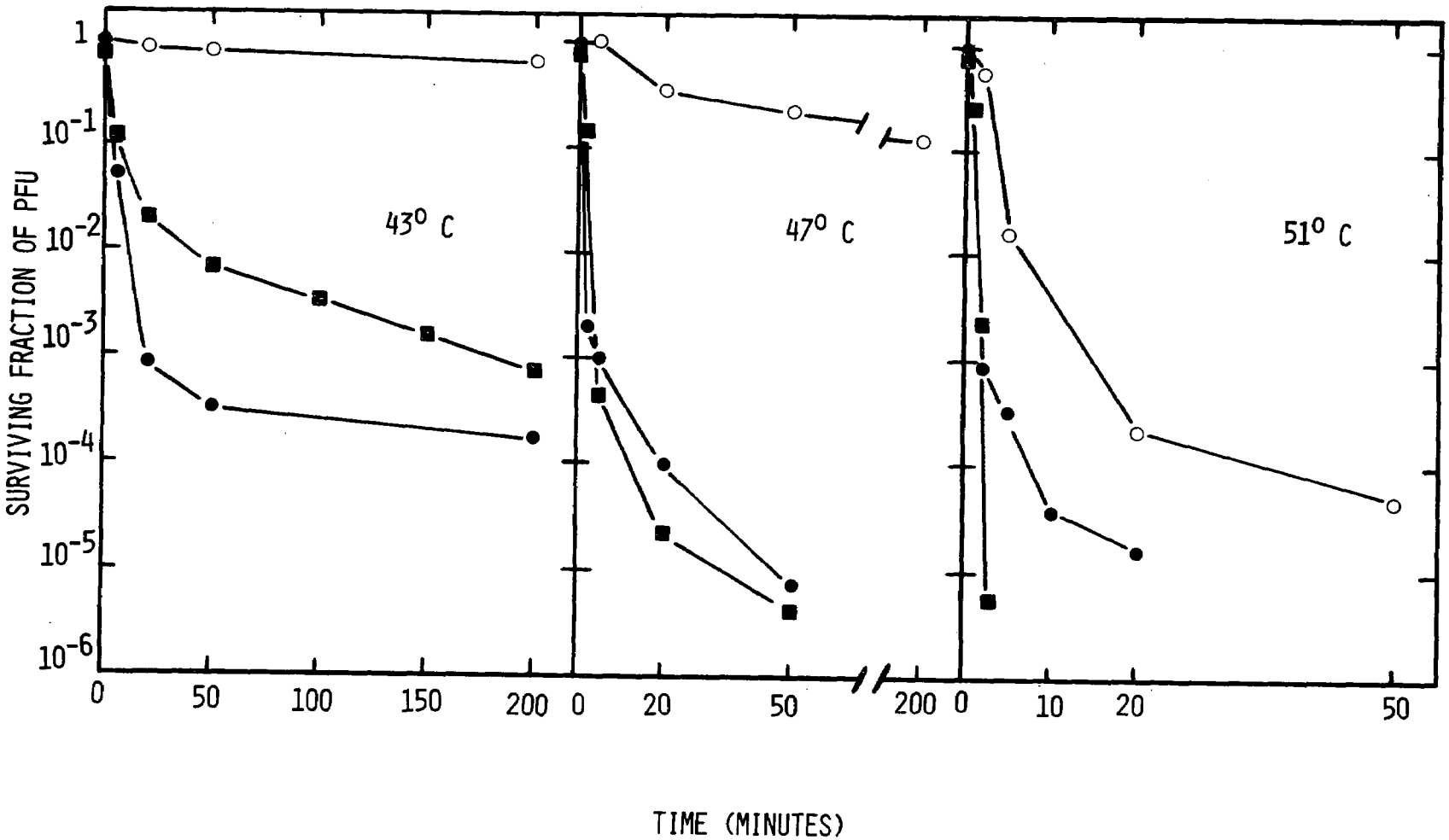


Figure 36. Effect of Sludge on the Rate of Heat Inactivation of Poliovirus. Symbols: Raw Sludge (O); Digested Sludge (■); PBS (●).

a 3-log decrease in recoverable plaque-forming units (see Fig. 36). After heating, sludge solids were removed from the samples by centrifugation at 18,000 g for 20 minutes and virus retained in the supernatant fraction was analyzed.

The initial experiment was designed to determine virus recovery. By measurement of total radioactivity, as shown in Table XV, it was found that some radioactive material (15 percent) is removed with the solids when the virus is labeled with ^{14}C-protein hydrolysate. However, when the particles are labeled with ^{3}H-uridine, radioactivity is recovered in full. The difference between the percentage recovery of viral RNA and viral protein can be explained by examination of the recovery of acid-precipitable radioactivity. As shown in Table XV, very little of the labeled viral RNA remains acid-precipitable following heat treatment of poliovirus particles in digested sludge while almost one-half of the recoverable viral protein is still large enough to be precipitable with acid. This result indicates that poliovirus particles are broken down during heat treatment and that their RNA molecules are hydrolyzed and released into the medium. However, viral proteins are less extensively degraded than viral RNA and a small percentage of these protein molecules apparently remains associated with sludge solids during centrifugation.

The conclusion that breakdown of poliovirus particles occurs in digested sludge during heat treatment was confirmed by a second experiment. Here, the sedimentation coefficient of radioactively-labeled virus was measured. As shown in Fig. 37, labeled particles have much lower sedimentation values after than before heat treatment. Thus, poliovirus is broken down and irreversibly inactivated when heated in digested sludge.

TABLE XV

Recovery of Radioactively-Labeled Poliovirus Strain CHAT in Digested Sludge Supernatant after Heat Treatment

Radioactive Label	Sample	Radioactivity Recovered (CPM)	
		Total	Acid-precipitable
^{14}C-protein hydrolysate	unheated control (PBS)	1,685	1,602
	heated 200 min, 43° C in digested sludge	1,432 (85.0)[a]	703 (43.9)
^{3}H-uridine	unheated control (PBS)	7,145	5,182
	heated 200 min, 43° C in digested sludge	7,287 (102)	606 (11.7)

[a] Numbers in parentheses are percentage recoveries relative to unheated control.

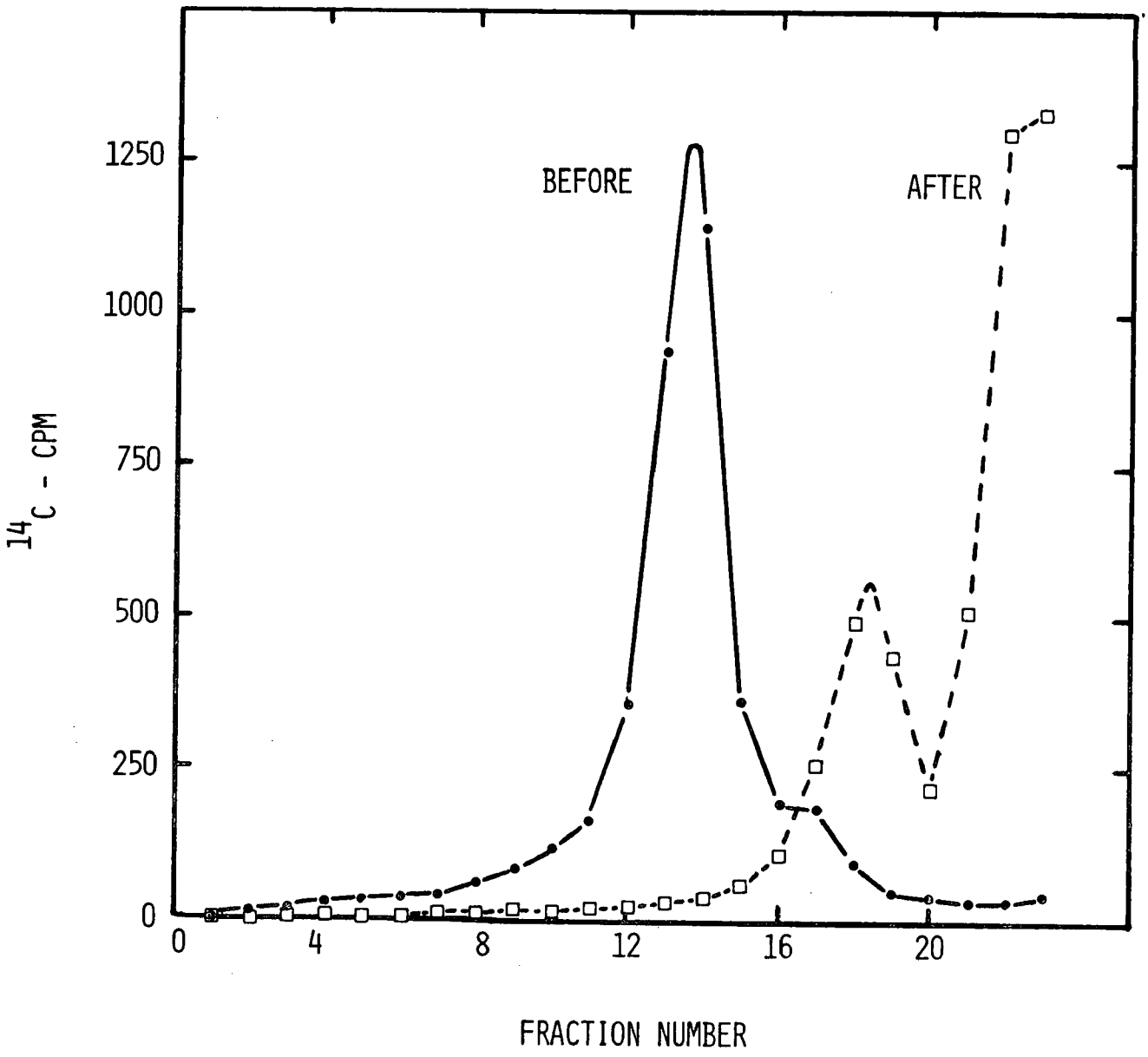


Figure 37. Sedimentation Profiles of Radioactively-Labeled Poliovirus Before and After Heat Treatment in Anaerobically Digested Sludge.

Effects of Different Concentrations of Sludge on Heat Inactivation of Poliovirus--In the experiments presented above, undiluted sludge was seeded with poliovirus before heat treatment. In an attempt to determine the difference between the effects of raw and anaerobically digested sludge seen in these experiments, the rate of heat inactivation of poliovirus was studied after seeding lower concentrations of sludge. The first experiment was designed to quantitate the inactivation of strain CHAT during 200 minutes at 43° C over a wide range of concentrations of digested sludge. The results were quite unexpected. As shown in Fig. 38, extremely small amounts of sludge are highly protective of the virus. However, this protection diminishes as the concentration of sludge is increased. At the highest concentration used, digested sludge is almost as unprotective as PBS.

To examine the effect of sludge concentration in greater detail, heat studies were carried out with all 3 strains of poliovirus in low and high concentrations of both raw and digested sludge. As shown in Table XVI, raw sludge is quite protective at both concentrations but this capability is especially evident in the greatest concentration at the highest temperatures. Digested sludge, on the other hand, is significantly protective only at relatively low temperatures and concentrations.

These results indicate that the protective component of raw sludge is quite active even when present in very low concentrations. Because the same low concentrations of digested and raw sludge are almost equally protective at the lowest temperatures studied, this component of raw sludge appears to be retained after digestion. At higher sludge concentrations and temperatures, the expression of the protective component seems to be limited by another substance found only in digested sludge. Thus, the difference between

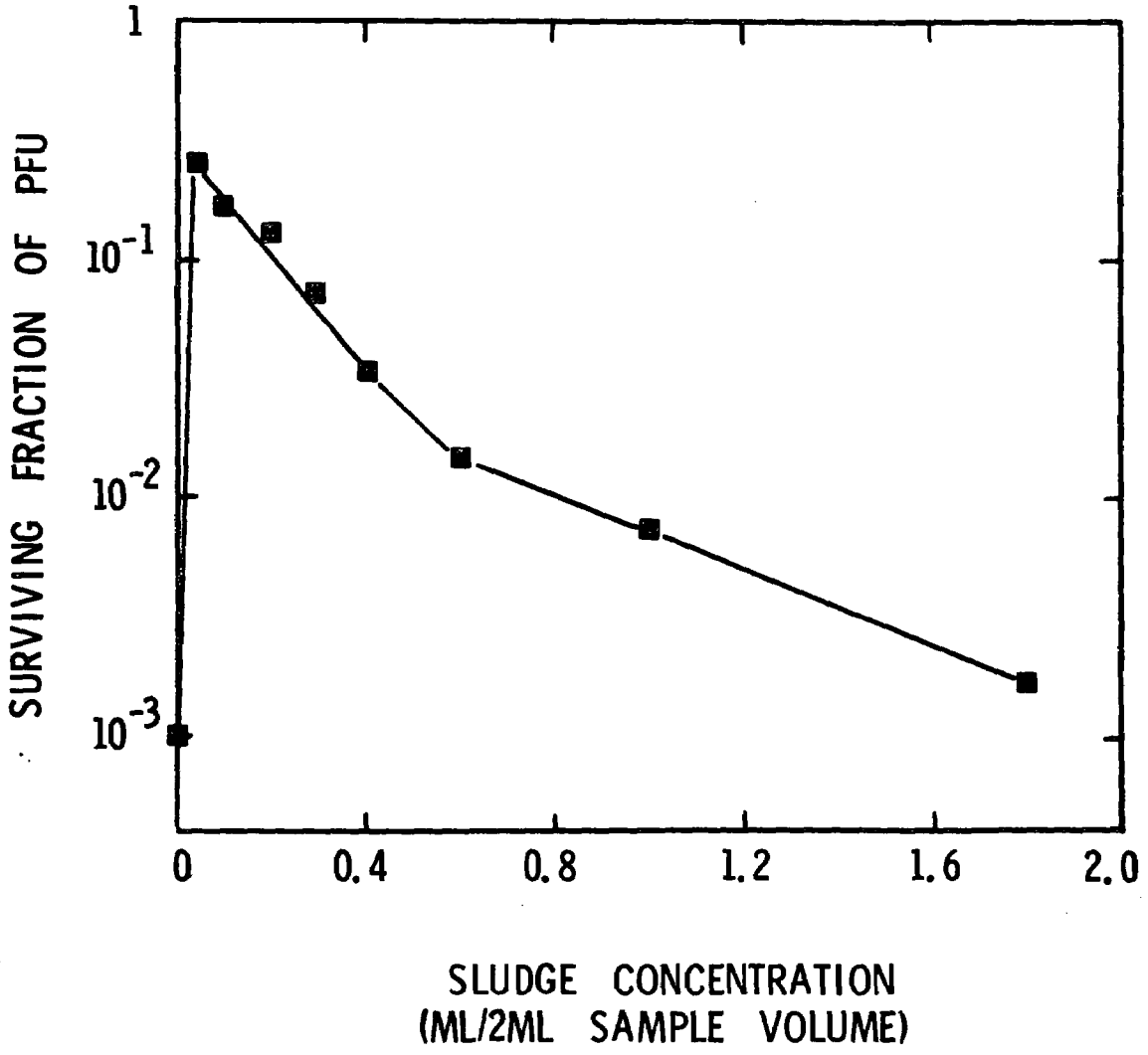


Figure 38. Survival of Poliovirus After Heat Treatment in Various Concentrations of Anaerobically Digested Sludge.

TABLE XVI

Effect of Sludge Concentration on Heat Inactivation on Poliovirus

Sample[a]	Treatment	Percentage (%) Survival of PFU[b]		
		Strain CHAT	Strain MAHONEY	Strain 712
No sludge	39°, 200 min.	3.4	67	77
0.05 ml raw sludge		95	93	83
1.8 ml raw sludge		98	59	62
0.05 ml digested sludge		69	64	83
1.8 ml digested sludge		4.3	4.7	28
No sludge	43°, 200 min	0.088	1.5	0.26
0.05 ml raw sludge		53	74	98
1.8 ml raw sludge		72	67	74
0.05 ml digested sludge		5.8	39	29
1.8 ml digested sludge		0.056	0.093	5.3
No sludge	47°, 20 min.	0.024	0.43	0.066
0.05 ml raw sludge		6.4	60	108
1.8 ml raw sludge		64	60	100
0.05 ml digested sludge		0.013	4.1	5.0
1.8 ml digested sludge		0.0027	0.0036	0.18
No sludge	51°, 5 min.	0.037	0.011	0.023
0.05 ml raw sludge		0.10	0.33	23
1.8 ml raw sludge		2.5	4.1	52
0.05 ml digested sludge		0.034	0.026	0.038
1.8 ml digested sludge		<0.000026	0.00028	0.0036

[a] Each sample contained 0.2 ml poliovirus lysate, the specified volume of sludge and the remainder as PBS in a total volume of 2.0 ml.

[b] Survival was determined relative to unheated control in PBS.

raw and digested sludge in these experiments may be due to a virucidal activity acquired during digestion. The agent responsible for this activity is possibly the same sludge component previously shown to cause poliovirus inactivation at much lower temperatures than those used here (see Part I of this section).

Physical Separation of Sludge Components Affecting Heat Inactivation of Poliovirus--Because anaerobically digested sludge exhibits two activities having opposite effects on the rate of heat inactivation of poliovirus, it should be possible to physically separate the components responsible for these activities. This was attempted by fractionation of sludge into solids and liquid through centrifugation (18,000 g, 20 minutes) and comparison of the virucidal and protective capacity of each to that of unfractionated sludge. As shown in Table XVII, when the solids from a small amount of either digested or raw sludge are resuspended in PBS, their protective capabilities are very similar to that of unfractionated sludge containing an identical concentration of solids. In contrast, an equivalent volume of the liquid fraction from raw or digested sludge was found to be totally unprotective in this experiment. These results show that the solids of both raw and digested sludge contain most of the protective component. They also support the previous conclusion that raw sludge retains its protective capability after anaerobic digestion.

The fraction of digested sludge that contains the virucidal component was then determined. Because this agent is much more readily expressed when present in high concentrations, heat inactivation of poliovirus was studied in fractionated samples from undiluted sludge. As shown in Table XVIII, most of the virucidal activity is removed with the liquid portion of digested sludge. However, even when

TABLE XVII

Survival of Poliovirus Strain CHAT after Heat Treatment in Fractionated Sludge

Sludge Concentration	Sample	Percentage (%) Survival of PFU after 200 Minutes at 43° C	
		Digested Sludge	Raw Sludge
No sludge	PBS	0.03	
Low[a]	total sludge	23	76
	sludge supernatant	0.009	0.02
	resuspended solids (PBS)	28	34
High[b]	total sludge	0.13	99
	sludge supernatant	0.03	33
	resuspended solids (PBS) (first centrifugation)	3.1	104
	resuspended solids (PBS) (second centrifugation)	5.5	N.D.[c]
	resuspended solids (PBS) (third centrifugation)	6.3	N.D.

[a] Each sample contained either 0.05 ml of total sludge, the solids from this volume of sludge, or an equal volume of digested sludge liquid in a total sample volume of 2 ml.

[b] Samples contained 1.8 ml of either total or fractionated sludge per 2 ml sample volume.

[c] Not determined.

TABLE XVIII

Effect of Virucidal Activity on Heat Inactivation of Poliovirus in Raw Sludge

Sample	Percentage (%) Survival of PFU after 5 Minutes at 51° C		
	Strain CHAT	Strain MAHONEY	Strain 712
PBS	0.036	0.034	0.0055
Digested sludge	0.000034	0.00023	0.00035
Raw sludge	1.1	3.7	52
Raw sludge solids resuspended in digested sludge supernatant	0.0018	0.00047	0.22

the solids of digested sludge are washed several times with PBS to remove this component, these solids are still not as protective as those of raw sludge. In addition, a high concentration of digested sludge solids is not as protective as a low concentration. Therefore, it appears that a small amount of virucidal activity is retained with this fraction of digested sludge.

It should be noted that heat inactivation of poliovirus under the conditions studied here is equally effective in PBS and in undiluted supernatant from digested sludge. Because this sludge fraction contains most of the virucidal agent, a greater amount of inactivation was expected in it than in PBS. The apparent explanation for this result is found upon examination of the protective capability of a high concentration of raw sludge supernatant. As shown in Table XVIII, this quantity of raw sludge supernatant is highly protective. Therefore, some protective material is retained in the liquid fraction of raw sludge and is probably also still present in the supernatant of digested sludge. From this it appears that the actual rate of heat inactivation of poliovirus in digested sludge supernatant, as in other fractions of digested sludge, is determined by a competition between protective and virucidal components.

Taken together, these results conclusively demonstrate that anaerobically digested sludge contains both protective and virucidal components and that these components can, for the most part, be physically separated by the fractionation of sludge solids and liquid through centrifugation.

Reversal of Raw Sludge Protection with the Virucidal Agent of Digested Sludge--The solids of both raw and digested sludge are highly protective of poliovirus during heat treatment but inactivation occurs much more rapidly in digested

than in raw sludge because a virucidal agent is acquired during digestion. Because this agent is found primarily in the liquid portion of digested sludge, it may be possible to resuspend the solids of raw sludge in this liquid and reverse the stabilizing effect on poliovirus normally provided by these solids. This is indeed the case for all three strains of poliovirus tested. As shown in Table XVIII, the amount of heat inactivation of poliovirus that occurs during 5 minutes at 51° C in raw sludge is much greater in the presence than in the absence of the virucidal agent. In fact, the amount of inactivation approaches that observed in digested sludge under these conditions. Therefore, once this agent has been identified, its addition to raw sludge should significantly reduce the heat requirements needed to inactivate poliovirus and possibly accelerate the inactivation of other viruses.

PARASITOLOGY

Introduction

This project began in early FY 75. It had as its objective, the determination of a reasonable treatment of combined heat and radiation that would rid sewage sludge of pathogenic parasites in an effort to make sewage sludge usable as a soil conditioner or fertilizer on productive land.

The first stage in the project was the choice of a parasite (and stage in its life cycle) to be studied. Some of the criteria used in making this choice were that the organism should be:

- a human pathogen
- found routinely in sewage sludge
- capable of surviving standard sludge digestion procedures and environmental stress
- capable of study using *in vitro* methods (without animals)
- more resistant to heat and radiation than other known parasites satisfying the above criteria.

The final choice was the unembryonated ovum of *Ascaris lumbricoides*. *Ascaris* ova are human as well as animal pathogens. *Ascaris* is ubiquitous and its ova are found in large quantities in sewage and tend to concentrate in sludge. These ova are resistant to chlorination and heat and may remain viable (and infective) for years in moist soil. In

addition, of the parasite eggs found in sewage, those of Ascaris species are most common. Finally, the embryonation of Ascaris ova can be studied in vitro. In discussions with recognized parasitologists, there was no disagreement with the selection of Ascaris lumbricoides unembryonated ova as a model system for studying the inactivation of sludge parasites.

The second phase of the project involved technique development. While in vitro procedures for embryonating Ascaris ova were available, methods of extraction of eggs from worms, cleaning ova, preparing suspensions for treatment, embryonating, and counting had not previously been developed with the study of "survival" in mind. In addition, there existed no single standard method for embryonation. Some of the criteria for techniques were that they should:

- yield the highest number of ova after removal from adult worms consistent with a low percentage of unfertilized eggs
- minimize the loss of eggs to glassware during preparation of samples to be treated
- yield uniform environmental (heat and radiation) exposure to all ova in a sample
- lend themselves to microscopic examination of ova preparation at each stage
- yield maximum embryonation of untreated samples
- be as safe as possible
- yield needed information with minimal effort

Finally, concentration of the ova in sewage sludge was required if inactivation measurements were to be made in sludge with high (5 percent) solids content.

Experimental

Preparation of Eggs

Ova were obtained from female Ascaris lumbricoides measuring between 27 and 36 cm in length. These were obtained from the Carolina Biological Supply Company. It was determined that extraction of eggs from about 5 cm of the uterus (posterior end) resulted in the maximal number of ova with an acceptably small number of unfertilized eggs (6 to 8 percent). Also, from this portion of the uterus, the in vitro embryonation procedures (see below) yielded about 93 percent embryonation of fertilized ova - complete to the larval stage. The yield of fertilized ova per worm varied from 6.1×10^4 to 5.5×10^5.

Uteri (5 cm) were deposited in 1N NaOH, mashed into small pieces with a glass rod, and stirred for 30 minutes with a magnetic stirrer. These procedures were adopted to aid in minimizing the loss of eggs through their adherence to glassware. The use of a blender to fragment uteri caused serious loss of ova.

The resultant mixture was poured through a 48-mesh (295-μm) screen into a flask to remove uterine fragments. After settling for 40 minutes, most of the NaOH was decanted from the flask. Settling was used, rather than centrifugation, to avoid exposure of ova to additional glassware (to which they adhere). The ova settled readily (15 to 20 minutes) in deionized water and 0.1N H_2SO_4, but required 30 to 40 minutes in NaOH. The ova were washed twice more in the same manner, first with NaOH and then deionized water, after which 0.1N H_2SO_4 was added to the cleaned suspension; this preparation was stored at 4° C.

Other steps taken to minimize the loss of ova included: the use of plastic disposable pipets whenever possible and silicon-coating of most glassware - when not to be used with NaOH which dissolves the silicon.

Sample Preparation

One milliliter of the stock suspension (above) was placed in a 16 x 150 mm screw cap test tube. The suspension was allowed to settle (30 minutes) and 0.5 ml liquid supernatant was removed. Then 4.5 ml deionized water (or 3.5 ml water and 1.0 ml sludge supernatant) was added.

Embryonation

Sample tubes (after exposure to various heat and/or irradiation environments, or as controls) were decanted after 30 minutes settling, and 0.1N H_2SO_4 was again added as the *in vitro* embryonation medium. The ova were incubated for 21 days at 30° C on a slow roller drum. This choice of media was made after considerable study, the results of which are shown in Table XIX. (The numbers shown are lower than those associated with the final procedures due to early inclusion of a high percentage of unfertilized ova.) Generally, H_2SO_4 in air gave the best embryonation results. A hatching technique was also developed, and it was found that all ova which embryonated to the larval stage using the above procedure also hatched using this technique (the converse clearly is true), so that any study of the life cycle beyond embryonation to larval stage seemed unnecessary.

Counting

All counting for total number, number of fertilized eggs, embryonated eggs, hatched larvae, and so forth was done by suspending 0.1 ml of liquid (from appropriate state) in a

TABLE XIX

Percent Embryonation in Selected Media

Concentration	Liquid	Gas	Percent Embryonation	
			Run I	Run II
0.1N	H_2SO_4	In CO_2	53	56
0.1N	H_2SO_4	In Air	51	43*
0.1N	H_2SO_4	In O_2	49	56
2%	HCl	In CO_2	47	24
2%	HCl	In O_2	42	54
2%	HCl	In Air	36	44
1%	Formalin in CO_2		52	39
1%	Formalin in O_2		47	45
1%	Formalin in air		35	44

*0.1N H_2SO_4 - Highest average,
- Least Deviation

McMasters chamber and microscopically counting at 150X on a Leitz microscope. Four counts were made for each sample and means reported.

Microscopic counting is quite tedious. It was originally planned to do most probable number counts and analyses for hatching in this program in order to save time. This proved to be impractical because there was too much variation in initial counts necessitating microscopic examination in any event.

Results

"Survival" (ability to embryonate to the larval stage) curves for heat, radiation, and combination treatments were generated by using the above techniques. Figure 39 shows percent embryonation as a function of heat treatment at several temperatures. These ova were exposed to heat in 4.5 ml DI water and 0.5N H_2SO_4 suspension as described earlier. Roughly, one can conclude that below about 51° C, inactivation is slow; whereas above this temperature, inactivation becomes increasingly rapid. Figure 40 shows radiation and thermoradiation data (at 47° C) compared with the total lack of effect of 47° C alone. In a very few minutes, the combination of heat and radiation inactivates three logs of embryonation synergistically.

Since there was an indication in some experimentation that sludge supernatant afforded some "protection" against the thermoradiation-induced inhibition of embryonation, it became important to determine whether eggs in concentrated sludge (5 percent) would be protected even more against such a sewage sludge treatment process.

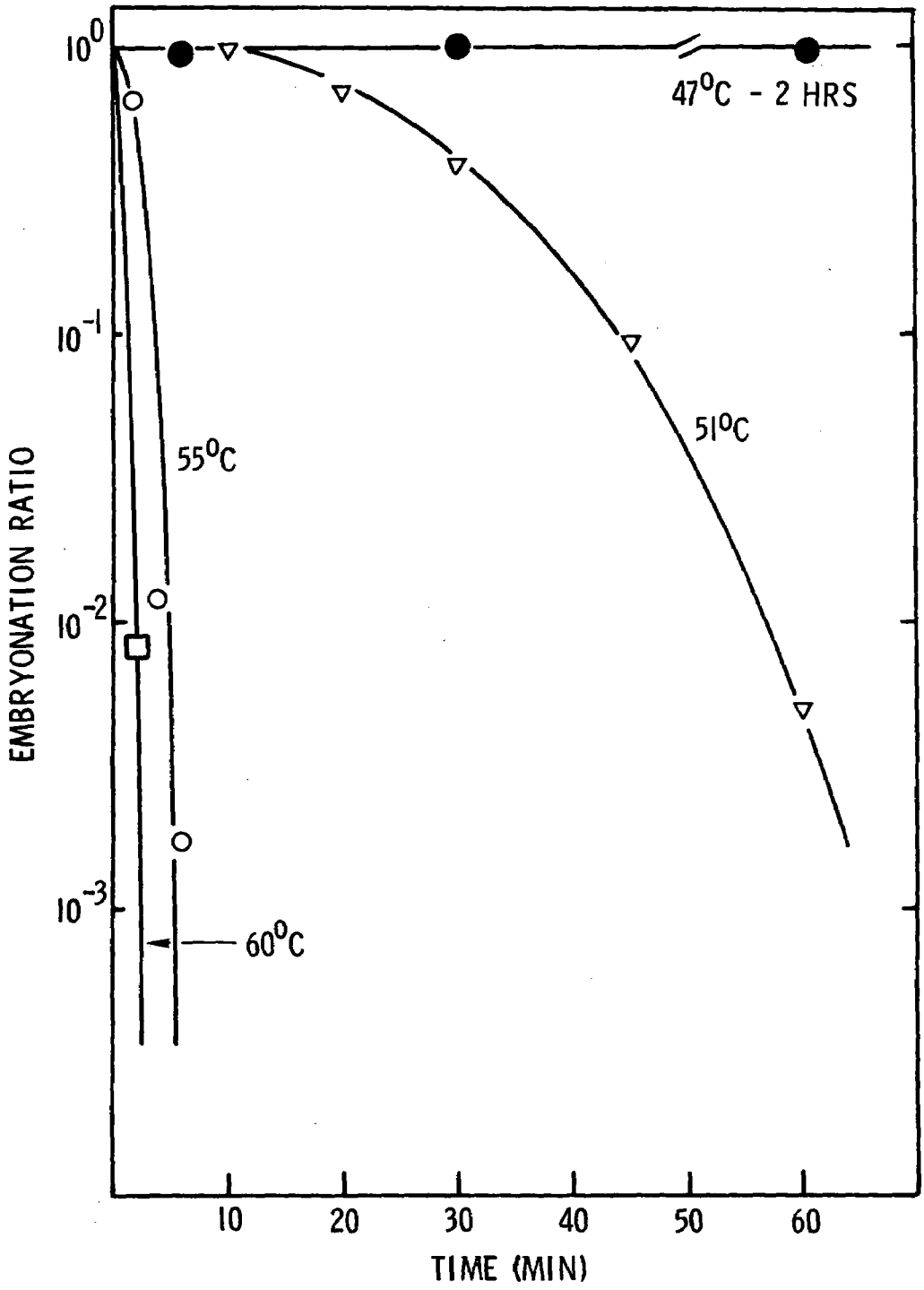


Figure 39. Heat Inactivation of Ascaris lumbricoides Ova

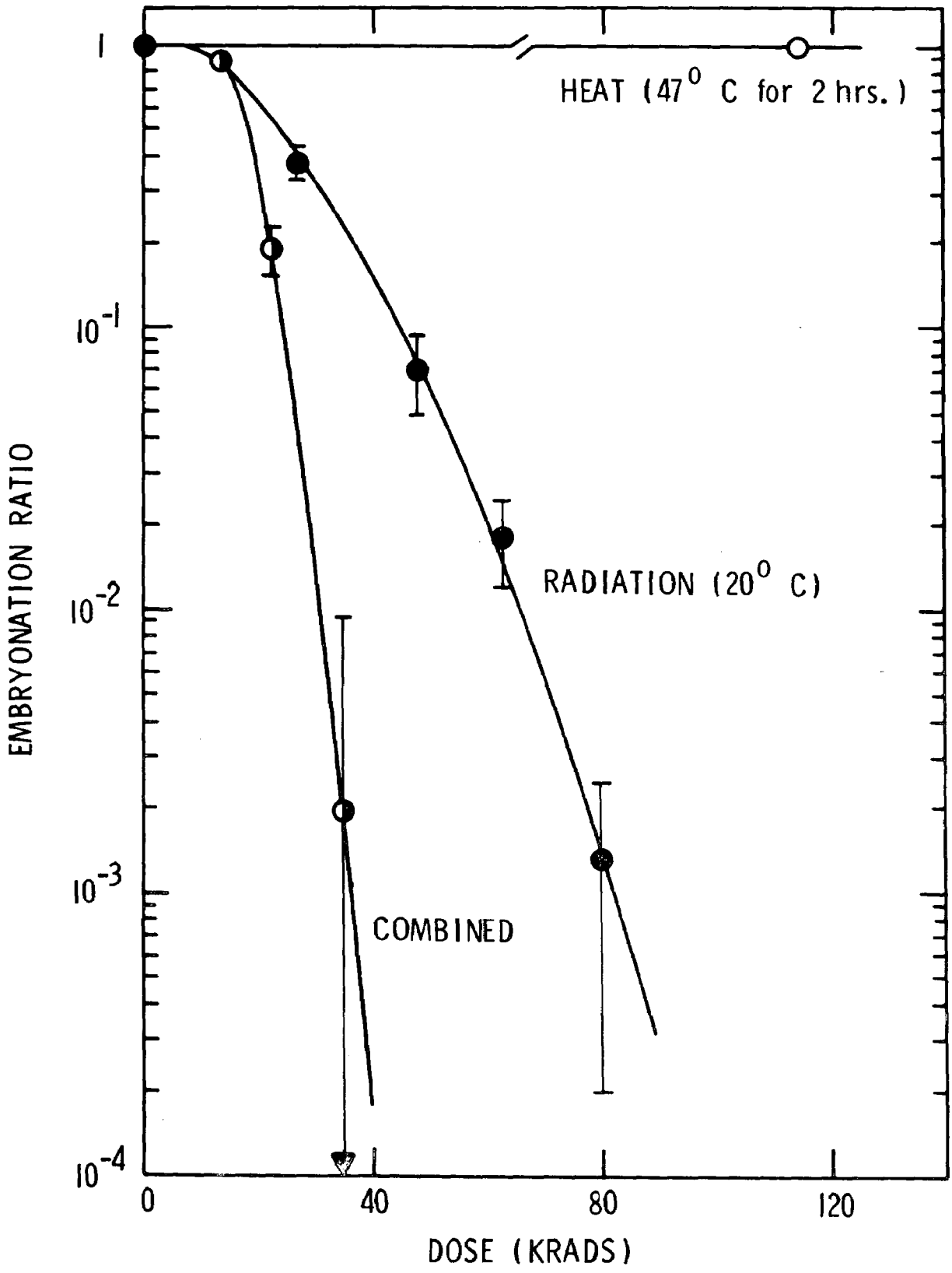


Figure 40. Thermoradiation Inactivation of Ascaris lumbricoides Ova

The techniques have been developed for separating Ascaris lumbricoides ova from seeded sludge in order to visually determine embryonation efficiencies using the formalin-ether technique which has been reported.[32,33] It has been determined in independent experiments that the formalin-ether treatment does not interfere with subsequent embryonation of the eggs.

Figure 41 shows the effects of ionizing radiation on the embryonation of Ascaris lumbricoides ova in sludge and in saline. While there appears to be some protection afforded by the sludge, the difference is less than the normal batch-to-batch variation in previously reported inactivation rate studies.[34] It is clear from these data, however, that relatively "mild" treatment (150 krads) at 23° C will be sufficient to prevent embryonation of three logs of parasite ova; in addition, temperatures >45° C will yield even greater success.[34]

Summary

The inactivation data of Ascaris lumbricoides ova by ionizing radiation for a variety of temperatures and in various irradiation media are presented in Table XX. It is clear that any radiation treatment, even at room temperature, which includes as little as 150 krads of ionizing radiation, will prevent embryonation in at least 99.9 percent of the Ascaris ova in sewage sludge.

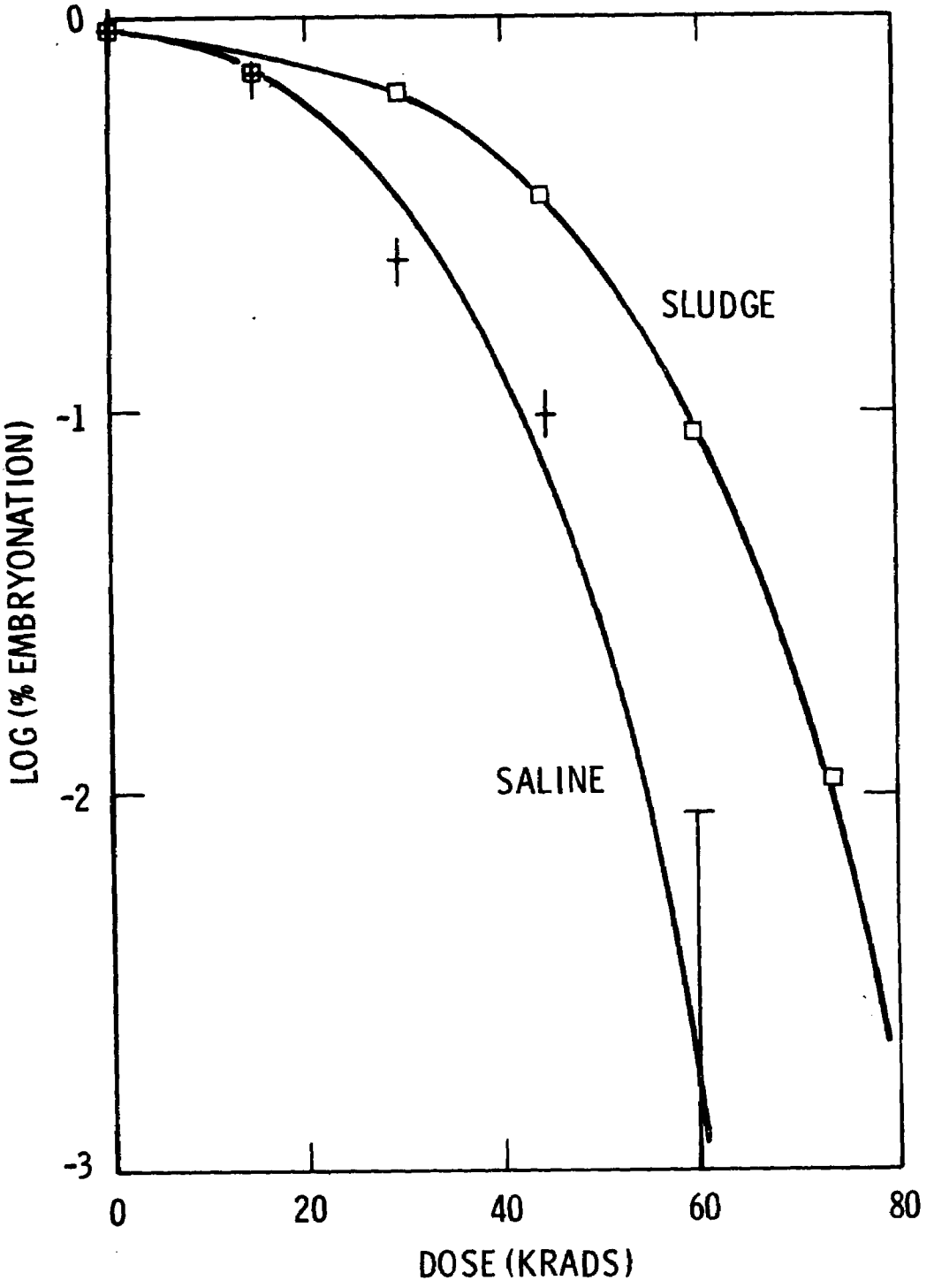


Figure 41. Reduction in Embryonation of Ascaris lumbricoides Ova in Sewage Sludge (5 Percent Solids) and Saline at 23° C.

TABLE XX

Inactivation of Ascaris lumbricoides Ova

Irradiation Temperature (° C)	Inactivation Medium	Dose for 3-Log Reduction[a] (krads)
23	saline	65[b]
23	water	85,140[c]
47	water	40
51	water	40[d]
60	water	10
23	supernatant	140[c]
51	supernatant	70[d]
23	sludge	90[b]

[a] Reduction of embryonation ratio

[b,c,d] Same dates

EFFECTS OF HEAT AND IRRADIATION ON PHYSICAL/ CHEMICAL PROPERTIES OF SEWAGE SLUDGE

Introduction

Much of the cost of wastewater treatment in the United States is directly related to the sludge handling problems (greater than 30 - 40 percent by some estimates).[35] Many large plants presently dewater their sludge (to 20 - 25 percent solids), either by centrifugation or by vacuum filtration. A large fraction of the dewatering cost (approximately 40 percent)[36] is attributed to chemical additives (ferric chloride, lime, alum or polyelectrolytes) which are used for the purpose of facilitating a better separation of the solids. There have been many reports in the last few years on the improvements in dewatering properties brought about by the use of ionizing radiation. If irradiation or thermoradiation treatment can defray all or part of the chemical costs, substantial savings can be realized as an extra benefit (besides sterilization) to such a treatment process. The goal of the research described in this section has been quantification of radiation and thermoradiation induced improvements in settling rates and in filtration. Filterability measurements are somewhat more standardized, and are more meaningful from an applications standpoint.

Experimental

Settlability

Settling rates were measured by monitoring of solid-liquid interface of 1-liter quantities of sludge in a graduated cylinder. Figure 42 depicts the graduated cylinder and the measurements taken. Improvement in settling is given by

$$100 \times \frac{(L_t - L_{oo})\ \text{treated} - (L_t - L_{oo})\ \text{control}}{(L_t - L_{oo})\ \text{control}}$$

or

$$100 \left(\frac{(\Delta L)\ \text{treated}}{(\Delta L)\ \text{control}} - 1 \right)$$

where the levels are as defined in the figure.

Filterability

The vacuum apparatus which was designed and built allows measurement of the volume of filtrate as a function of time for sludges which have been subjected to various treatment conditions. This device is shown in Fig. 43. It consists of a vacuum gauge, 500 millimeter vacuum flask, 12 millimeter graduated cylinder, and a 9 centimeter Buchner funnel.

The following procedures were used in the filterability experiments. A wetted piece of 7 centimeters Whatman Number 1 filter paper was placed in the Buchner funnel and a vacuum was applied. A sludge sample of 50 millimeters was poured into the Buchner funnel. The resulting vacuum was approximately 585 millimeters of mercury. The volume of the

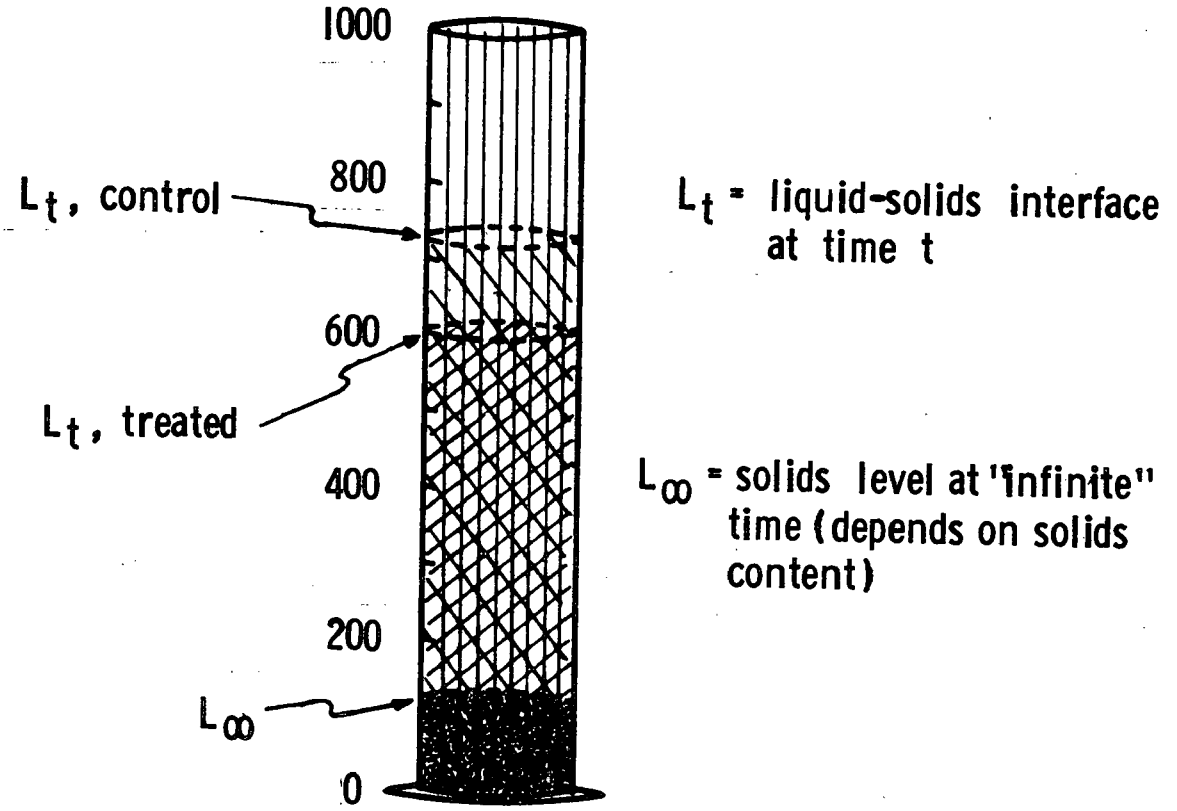


Figure 42. Apparatus for Settlability Measurements

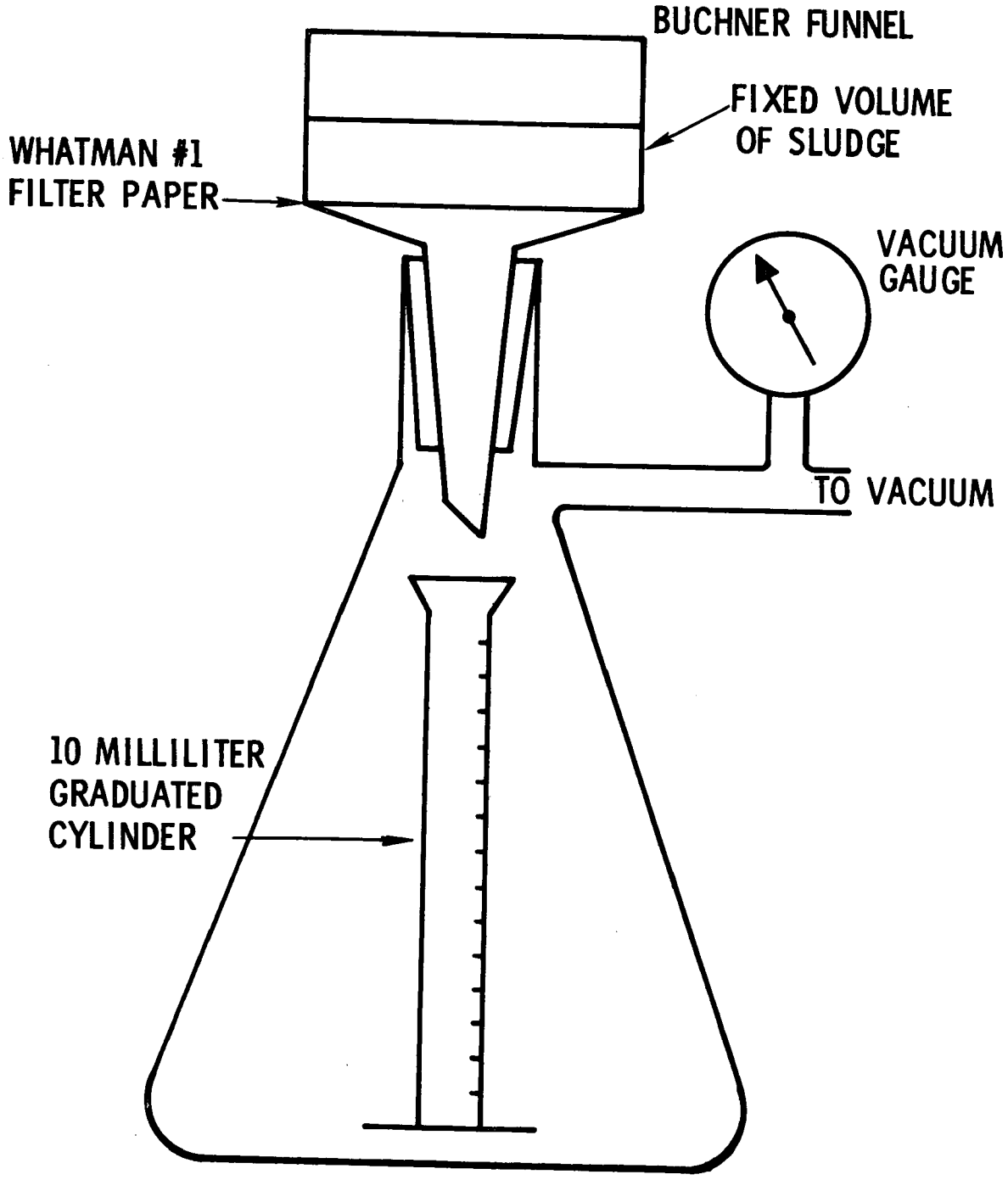


Figure 43. Filtration Apparatus

filtrate collected was recorded at 1/2 minute intervals for the 1 percent solids sludge and at 1 minute intervals for the 3 and 5 percent solids sludge over a 10 minute period.

Results

Settlability

Only diluted (1 percent solids), digested sludge was used. Prompt settlability was found to improve significantly with radiation, to decrease slightly with heat, and to improve significantly (and synergistically) with thermoradiation treatment (Fig. 44). It should be noted that, while these data indicate that at longer times the improvement is only a few percent, this sludge was diluted to afford ease of measurements. In typical, undiluted sludge, the improvement may be long-term, or even permanent (days). Such has been reported previously for irradiation alone.[37] If this were the case, an immediate improvement in sludge handling requirements might be realized in a practical process.

Filterability

The data obtained from the filtration experiments were plotted as time/unit volume versus volume. The slope, 'b, of the line is related to the specific resistance of the sludge. Specific resistance is defined as the resistance of a unit weight of cake per unit area at a given pressure. Specific resistance is expressed by the following equation:

$$r = \frac{2PA^2b}{\mu W}$$

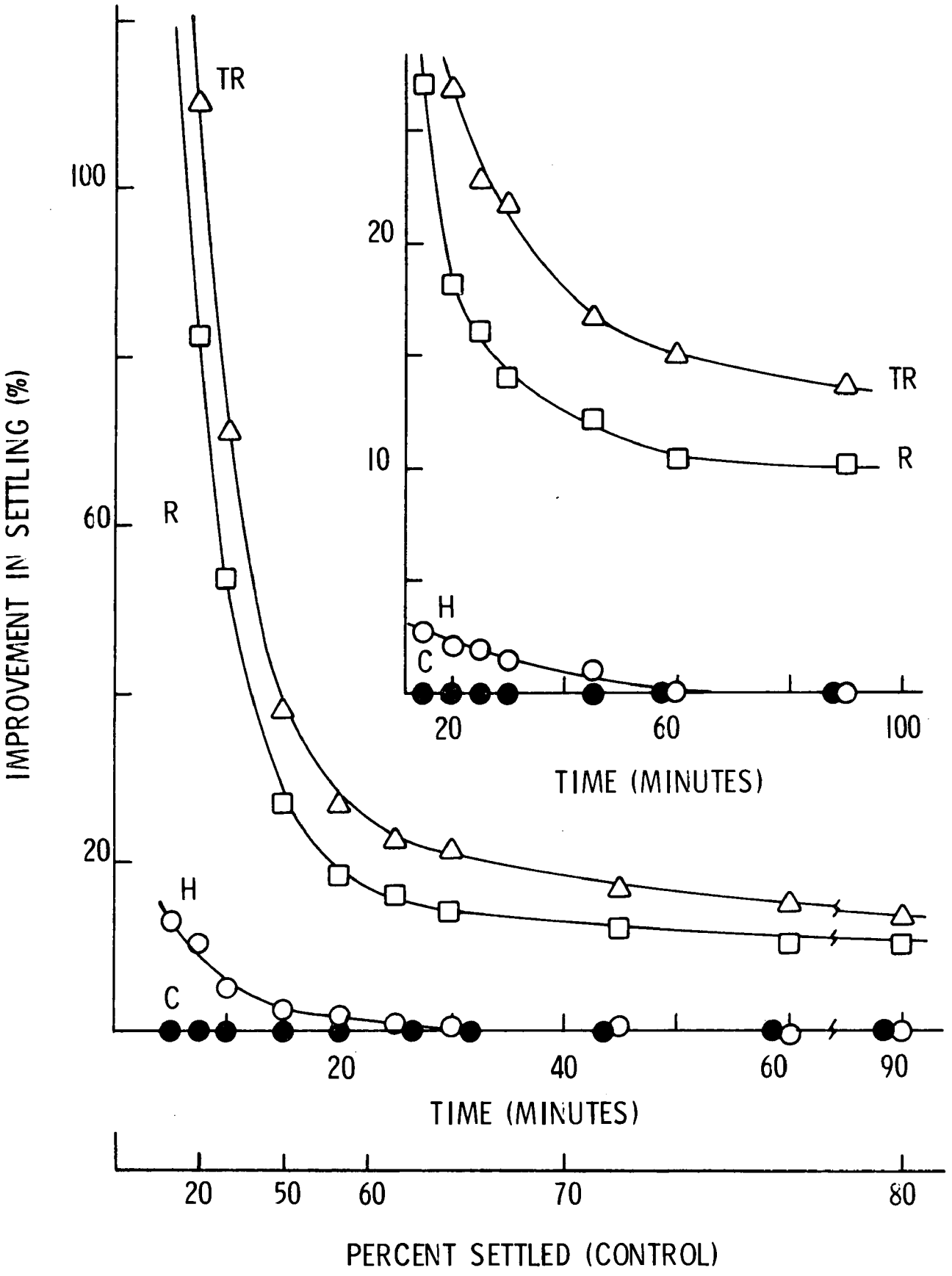


Figure 44. Settlability Profiles for Heat, Radiation and Thermoradiation

where

r = Specific resistance, m/kg
P = Pressure difference, N/m^2
A = Area of filtering surface, m^2
b = Slope of the T/V vs. V plot, s/m^6
μ = Viscosity of filtrate, Ns/m^2
W = Weight of dry sludge cake solids per unit volume filtrate, kg/m^3

Using a series of specific resistances obtained from the slopes of the lines of the time/unit volume versus volume plots (Fig. 45, Plot A), a plot of normalized specific resistance versus temperature or dose results (Fig. 45, Plot B). These plots illustrate the change in specific resistance as a function of treatment conditions. A decrease in specific resistance means an increase in filterability.

Primary Digester Sewage Sludge

The results of the thermal treatment to 1, 3, and 5 percent solids sludge samples are shown in Fig. 46. In all cases, heat alone increases the specific resistance of the sludge, which results in increased difficulty in dewatering. The detrimental effect increases in intensity as the percentage of solids increases. The 5 percent solids samples exhibited approximately a 300 percent increase in specific resistance while the lower percent solids samples exhibited approximately a 25 percent increase in specific resistance.

The effects of radiation and thermoradiation on 5 percent solids are shown in Fig. 47. Radiation alone decreases the specific resistance greater than 5 fold at maximum treatment

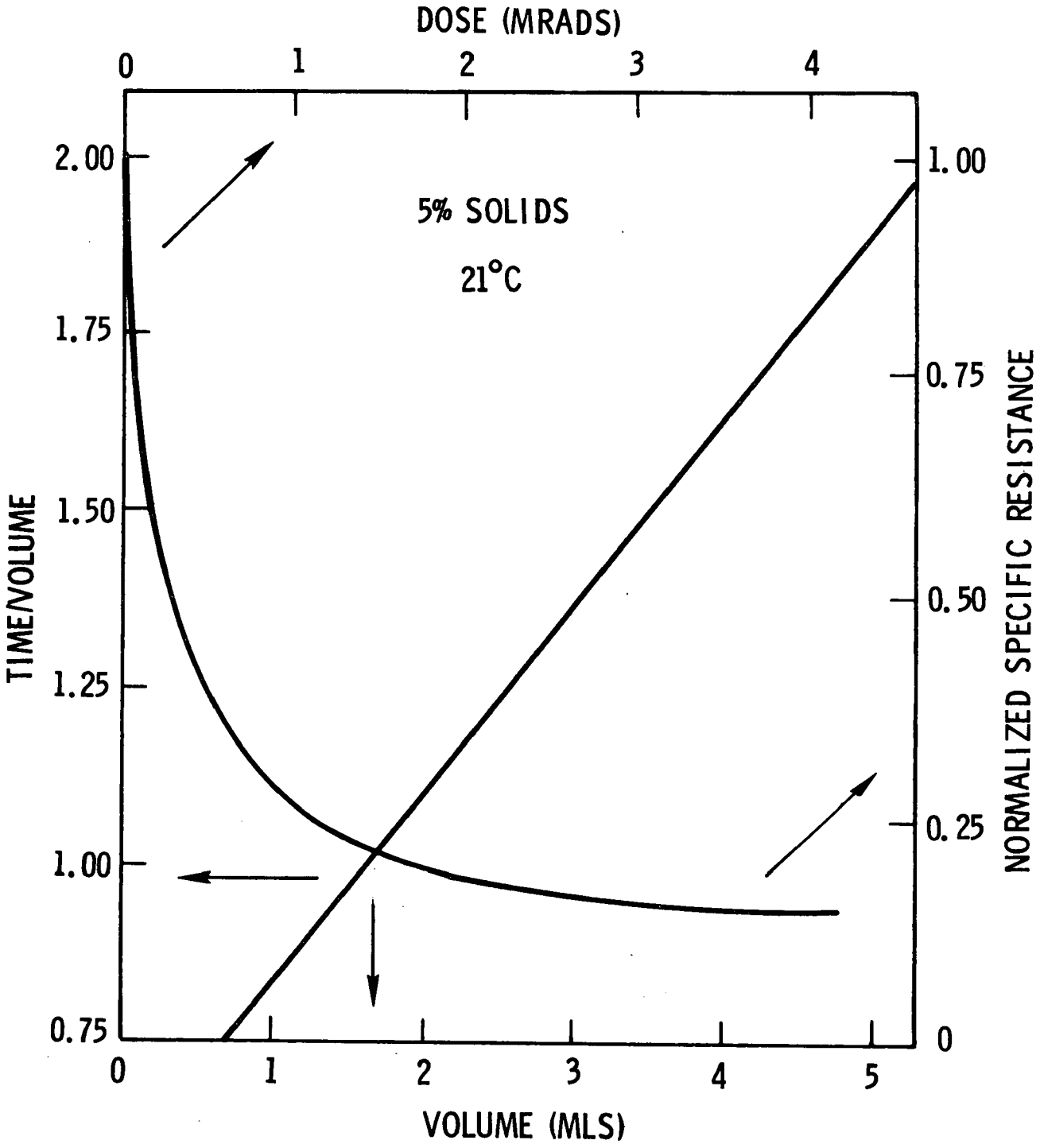


Figure 45. Radiation Effects on Filterability
A. Typical Time/Unit Volume versus Volume Plot
B. Normalized Specific Resistance versus Dose

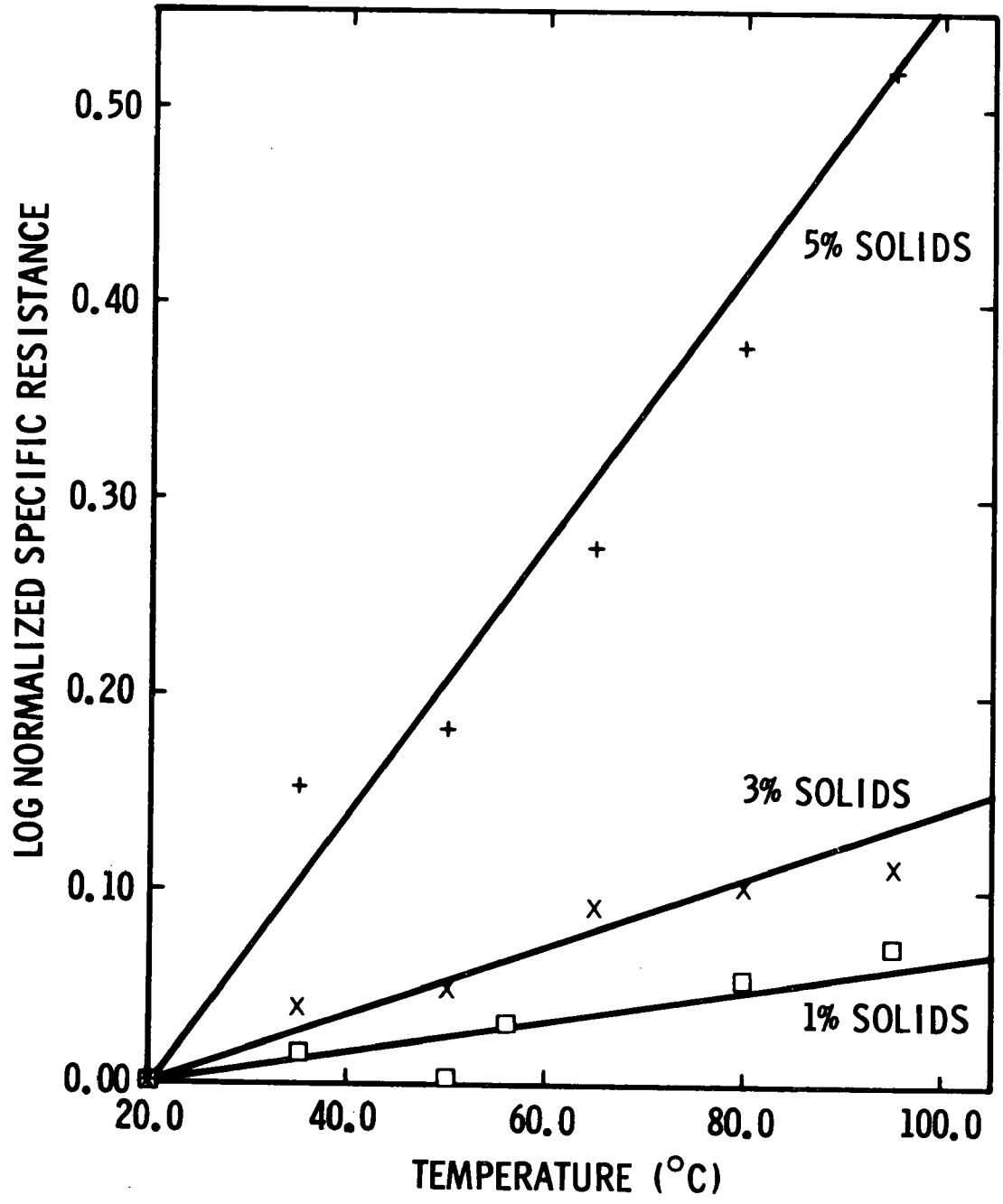


Figure 46. Thermal Effects on the Specific Resistance of 1, 3, and 5 Percent Solids--Digested.

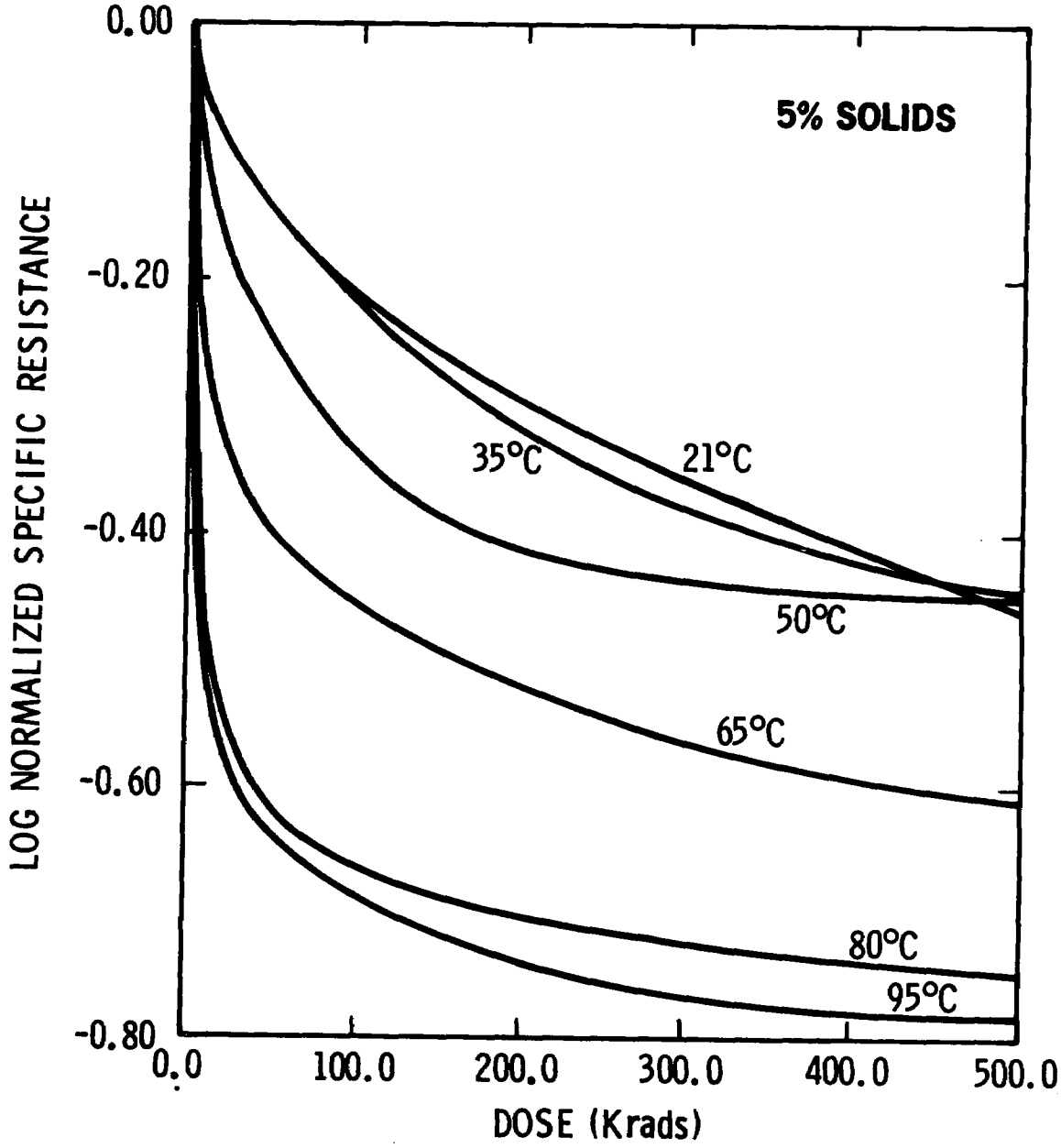


Figure 47. Radiation and Thermoradiation Effects on the Specific Resistance of 5 Percent Solids--Digested.

conditions, while thermoradiation shows a 2 - 8 fold decrease in specific resistance. Similar results were obtained for 1 and 3 percent solids.

For radiation the improvement is dose dependent, with increased improvement up to 1000 krads, where a leveling off occurs. The same trend is true for thermoradiation; however, thermoradiation improvement is also dependent on temperature. Thermoradiation at temperatures of 35 - 50° C show little or no improvement over that at room temperature; however, a significant improvement is observed at higher temperatures, as seen in Table XXI.

The filterability of primary digester sewage sludge increases half a log with radiation conditioning and as much as a log with thermoradiation conditioning (depending on temperature used). This seems to indicate that some agglomeration of the solid material is occurring. This does not compare favorably with the increases seen with chemical additives, which typically increase filterability 2 - 3 logs.

It must be noted that for consistency all experimentation was performed on strained and blended primary digester sludge. The process of blending the sludge may have had a dramatic effect on the filterability. For this reason studies on fresh unblended sludge were initiated. Preliminary results show that the blending did, in fact, reduce the filterability of the primary digester sludge. It is apparent that the blending process breaks up the agglomerations. This leads to a decrease in filterability. The increase in filterability of unblended primary digester sludge due to radiation or thermoradiation is not, however, comparable to the increases obtained by using chemical additives.

TABLE XXI

Specific Resistance (10^{12} m/kg) of 5 Percent Solids-Digested

Dose (krads)	Temperature (° C)					
	21	35	50	65	80	95
0	1.67	2.37	2.54	3.15	3.99	5.53
100	0.968	0.966	0.955	0.586	3.354	0.352
1000	0.444	0.558	0.560	0.407	0.248	0.177

Undigested Sewage Sludge

Studies on the effects of heat, radiation and thermo-radiation on the filterability of undigested sewage sludge were undertaken. Initial results were very erratic. The results are possibly due to the presence of activated sludge which is being mixed into the undigested sludge at the sewage plant. It is known that activated sludge is extremely difficult to dewater. The experiments will be repeated on unadulterated undigested sludge.

Summary

Significant enhancement of "prompt" improvement in settlability has been measured for both irradiation and thermoradiation treatment. Long-term effects have yet to be demonstrated in our laboratory.

Filterability can be enhanced by a maximum of one log by thermoradiation treatment in anaerobically digested sludge which has been blended and strained. It is unknown whether this improvement can defray some of the chemical costs in dewatering operations, and tests are underway currently to determine this. In addition, raw sludge and non-blended anaerobically digested sludge are being studied in terms of filterability improvement.

Odor modification studies are to be undertaken within the next month. Quantitative data should be forthcoming which will compare irradiation, heat, and thermoradiation treatments of normal digested with normal raw sludge.

COST BENEFIT ANALYSES

Cost benefit analyses for the treatment of sludge are being carried out on a continuing basis as variables in the thermoradiation process become better defined. A treatment combination of 500 krads at 65° C for 5 minutes is used to compute the cost of a rigorous treatment cycle. Based on this treatment, analysis is presented below which should yield approximate treatment costs based on current prices and values. Gamma source efficiencies are presented for two candidate source pins to be used in the thermoradiation treatment process.

For a city of 600,000 population producing 91 g of undigested sludge and 64 g of digested sludge per capita per day, 760 M^3 of 5 percent solids sludge is produced daily. Also, 92.7 kcals/capita-day or an equivalent 644 kcal/second total energy in the form of methane is available as output from the anaerobic digestion process, which is currently used in most sludge treatments. To provide the necessary dose of 500 krad with a 35 percent source use efficiency, 32 MCi of ^{137}Cs would be required at an approximate cost of 3.2 M$ (assuming a cost of 10¢/Ci). To heat the sludge from the 35° C of the digester to 65° C requires 265 kcal/second. An additional 36.7 kcal/second is generated by the radioactive source, almost all of which should be recoverable. Assuming a 50 percent heat recovery efficiency from heated sludge, only 95.9 kcal/second or 14.9 percent of the methane energy is needed in this stringent thermoradiation cycle. Since the radiation source is replenished to a full total activity on

a regular basis, we will assume that the total value of the ^{137}Cs is nominally the same at the end of a 20-year amortization period as at the start, at least assuming rods at approximately two-thirds the original specific activity are still useful. A fairer way to amortize the source value will be derived later and will increase the cost. Consequently, we end up with 0.75 M$ in construction cost amortized over a 20-year period giving 37.5 K$/year. If the capital is amortized at 6 percent the figure is 63.7 K$/year. Operating costs should be no more than 100 K$/year and source decay amounts to 115.2 K$/year. The total comes to 252.7 K$/year, while 14,000 tonne/year of treated sewage sludge is produced. A cost of $18.15/tonne or $20.00/tonne with 6 percent amortization is thereby obtained for the assumed treatment.

The value of the treated sludge for fertilizer and for the main ingredient in a cattle range feed supplement has been discussed as thoroughly as the currently available data permit with Dr. Stanley Smith and Dr. Robert McCaslin of New Mexico State University in Las Cruces, New Mexico. Using best estimates (chemical analysis is now being performed) of the composition of Albuquerque sewage sludge, for example, the treated sludge would be worth approximately $15/tonne for fertilizer and as much as $100/tonne as animal feed. Shipping costs and limited availability would restrict the use of sludge at the cost of $18.15/tonne as a fertilizer to the proximity of its source (less than 25 miles). Under these circumstances, for example, the total sludge output of Albuquerque would have limited impact on New Mexico's fertilizer needs, but could furnish up to 25 percent of the range feed supplement needs of the state.

As with any analysis of this type, gross generalizations are involved. These generalizations are being examined more closely. For example, roughly 50 percent of the $18.15/tonne

cost is ^{137}Cs cost and scales linearly with the cost of ^{137}Cs. Thus, a treatment involving only 250 krads (perhaps more realistic) would decrease the cost of sludge per tonne by about $4.50. More efficient ways of packaging the ^{137}Cs are being undertaken elsewhere at Sandia Laboratories. Since the $.10/Ci value used in the analysis is almost totally one of encapsulation costs, ways are being examined to simplify the encapsulation procedure. The fertilizer and animal feed value of the treated sludge are now being quantified accurately by New Mexico State University.

Encapsulation (Efficiency Computations)

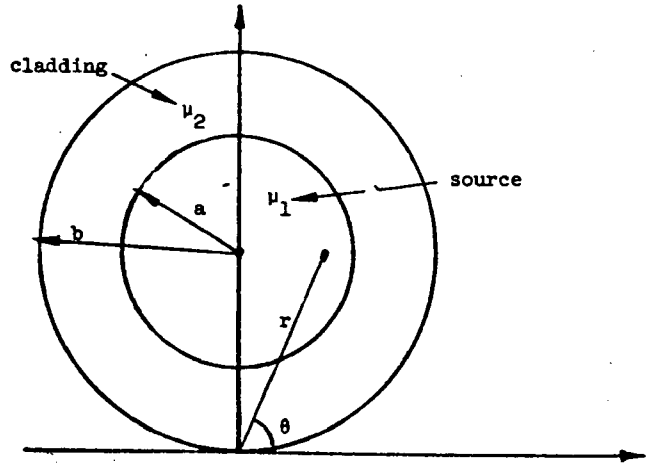
The major cost in building a full-scale sludge irradiation facility is the cost of the source material (^{137}Cs). Since the presently available sources are relatively costly and deliver gamma rays at somewhere around a 35 percent efficiency, a study is being undertaken to quantify analytically the radiation efficiency of various idealized source rod geometries.

All source geometries under consideration at this time consist of fairly long rods (length >10 times the diameter) with cladding. The idealized geometry chosen to represent this long but finite cylinder is an infinite cylinder with cladding. Because only the radiation off the side of the rod will probably be used and because the rods are long, this idealized geometry should quite accurately predict the source efficiencies. Computationally, because the point source kernel rather than the line source kernel would have to be used, the finite cylinder with cladding problem would have been at least an order of magnitude more complicated and time-consuming while providing very little added accuracy.

The results of these computations are being used directly in two cases. In the first case, the possibility of reducing the diameter of the HMD ^{137}Cs capsules from 6.35 cm to approximately 2.54 cm is being discussed. This study will quantify the gain in source efficiency. The second case is the production of ^{137}Cs in a ceramic matrix described above. The rods being produced by this method are lower in specific activity and higher in absorption because the cesium is mixed with ceramic materials. However, because of the extremely low-leach rate of the final product, the amount of cladding needed can be reduced significantly. This study will quantify in terms of source efficiency the various compositions and geometries proposed.

The heart of the study is a computer program to evaluate the photon flux at the surface of the rod. Since the surface is convex, any photons that reach the surface escape and can be used.

The source efficiency is defined to be total number of photons that escape the rod divided by the total number of photons released by the source material. Since all the $\mu_1 b$ and $\mu_2 b$ are less than 1, the buildup factor is properly neglected in these calculations. The geometry used is shown below.



The differential photon flux is given by

$$d\varphi = \frac{Da}{2\pi r} Ki \left\{ (\mu_2 - \mu_1) \left[b \sin\theta - \sqrt{a^2 - b^2 \cos^2\theta} \right] + \mu_1 r \right\} dA$$

where

$$dA = 2\pi r \, dr \, d\theta$$

and

$$Ki(x) = \int_x^\infty dx \, K_o(x)$$

where

$K_o(x)$ - is a modified Bessel function of zeroth order

Da - disintegrations|cm^2 - cm depth

μ_1 - absorption coefficient of the source material

μ_2 - absorption coefficient of the cladding

The final flux at the surface is given by

$$\varphi = 2 \, Da \int_{\frac{\pi}{2} - \sin^{-1}\frac{a}{b}}^{\frac{\pi}{2}} \int_{b \sin\theta - \sqrt{a^2 - b^2 \cos^2\theta}}^{b \sin\theta + \sqrt{a^2 - b^2 \cos^2\theta}} dr$$

$$Ki \left\{ (\mu_2 - \mu_1) \left[b \sin\theta - \sqrt{a^2 - b^2 \cos^2\theta} \right] + \mu_1 r \right\}.$$

Codes have not been written to evaluate the double integral shown and to compute Ki(x). The final equation used is

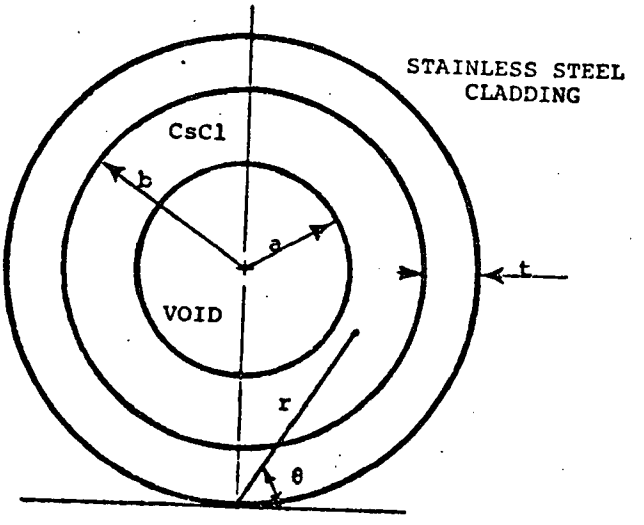
$$\text{Source efficiency} = \frac{2\pi b\varphi}{Da} .$$

The computer codes are now available in punched cards.

Dick Libby of Battelle PNL has recently found that build-up factors increase the computed source efficiencies substantially (in some cases double). To resolve the differences in computed efficiencies, a much more rigorous monte carlo calculation will be performed.

Source Calculations

One approach suggested for increasing the source efficiency of the WESF capsules, large diameter cesium-137 cylindrical sources being manufactured by ARHCO at Richland, WA, was to leave a void in the center of the rod or to fill the center with a material with a low-absorption coefficient. Using an infinite length stainless steel clad cesium chloride rod with a cylindrical void in the center, source efficiency calculations were undertaken. The geometry is shown below.



To compute the photon flux at the surface, it was necessary to evaluate the following integrals:

$$\phi = 2Sa \left\{ \int_{\frac{\pi}{2} - \sin^{-1}\frac{b}{c}}^{\frac{\pi}{2} - \sin^{-1}\frac{a}{c}} d\theta \int_{c\sin\theta - \sqrt{b^2 - c^2\cos^2\theta}}^{c\sin\theta + \sqrt{b^2 - c^2\cos^2\theta}} dr \right.$$

$$Ki\left\{\mu_3\left(c\sin\theta - \sqrt{b^2 - c^2\cos^2\theta}\right) + \mu_2\left(r - c\sin\theta + \sqrt{b^2 - c^2\cos^2\theta}\right)\right\}$$

$$+ \int_{\frac{\pi}{2} - \sin^{-1}\frac{a}{c}}^{\frac{\pi}{2}} d\theta \int_{c\sin\theta - \sqrt{b^2 - c^2\cos^2\theta}}^{c\sin\theta - \sqrt{a^2 - c^2\cos^2\theta}} dr$$

$$Ki\left\{\mu_3\left(c\sin\theta - \sqrt{b^2 - c^2\cos^2\theta}\right) + \mu_2\left(r - c\sin\theta + \sqrt{b^2 - c^2\cos^2\theta}\right)\right\}$$

$$+ \int_{\frac{\pi}{2} - \sin^{-1}\frac{a}{c}}^{\frac{\pi}{2}} d\theta \int_{c\sin\theta + \sqrt{a^2 - c^2\cos^2\theta}}^{c\sin\theta + \sqrt{b^2 - c^2\cos^2\theta}} dr$$

$$Ki\left\{\mu_2\left(\sqrt{b^2 - c^2\cos^2\theta} - \sqrt{a^2 - c^2\cos^2\theta}\right) + \mu_1\left(2\sqrt{a^2 - c^2\cos^2\theta}\right)\right.$$

$$\left.\left. + \mu_2\left(r - c\sin\theta - \sqrt{a^2 - c^2\cos^2\theta}\right)\right\}\right\}$$

Using this formula and defining source efficiency, the following results are obtained where the dimensions are in centimeters and the outer dimensions (b = 3.2 cm and t = 0.508 cm), are similar to those of the WESF cesium capsules.

a	Source Efficiency (%)	a	Source Efficiency (%)
0.2	26.8	1.8	24.1
0.4	26.0	2.0	24.3
0.6	25.4	2.2	24.6
0.8	24.9	2.4	25.0
1.0	24.5	2.6	25.7
1.2	24.2	2.8	26.5
1.4	24.1	3.0	27.5
1.6	24.0		

As can easily be seen from the tabulation, it is of no advantage to place the void in the center. The source efficiency stays low until the thickness of the cesium chloride layer is too small to be practical. This conclusion is in agreement with Dick Libby of Battelle.

The cost/benefit analysis at this point is rather crude in that so many assumptions must be made, such as, the price of cesium-137, whether methane gas from the digester will be available, etc. Further research has yielded answers to many of these questions, and a final cost/benefit report will be available in February 1977. An integral part of this report will be the cost analysis developed by Battelle on competing processes to thermoradiation, such as pasteurization and irradiation. The Battelle results will be available in December 1976.

CONCLUSION

It has been demonstrated in the accumulation of the data included in this report that irradiation, particularly at elevated temperatures, is effective in destroying pathogens and in improving sludge handling properties. Even at room temperature the results are impressive. For example, a dose of 300 krads will induce the following changes in digested sludge:

(1) fecal streptococcus bacteria will be reduced by about 2 logs (99 percent);

(2) coliform bacteria will be reduced by 10 logs;

(3) Salmonella species will be reduced by at least 8 logs;

(4) viable parasite ova will be reduced by 4 logs at the very minimum;

(5) while viruses may be reduced only 1 log (90 percent), by the irradiation, this work has demonstrated the existence of a virucide for poliovirus, produced in the process of digestion;

(6) settlability improves, and filterability is enhanced by a factor of approximately 3.

It must also be noted that increased temperature can substantially enhance the expected inactivations, particularly for viruses and parasite ova, and will lead to a further increase in the filtering and settling properties.

Oxygenation applied during irradiation significantly enhances inactivation rates of bacteria, even at elevated temperatures. While virus inactivation may not be affected, no data are yet available for parasite ova inactivation.

Cost analyses are currently under way to compare this disinfection process with other processes, such as pasteurization or more severe heat treatments.

FUTURE WORK

Experimentation to date has identified several areas in which further study will help determine the value of the proposed treatment. Further basic research in bacteriology, virology, and parasitology is necessary to define these parameters.

Bacteriological studies evaluating the effects of heat on the pathogen Salmonella are intended to correlate with our earlier work on radiation and thermoradiation inactivation. Regrowth experimentation as well as competition experiments affecting the regrowth may lead to the identification of other factors concerned with bacterial inactivation in sludge. The inactivation of biological systems by heat, radiation and/or thermoradiation in undigested sludge is an area in which there is a need for a whole series of experiments, which we feel will benefit the total picture of sludge characterization.

Preliminary effects of oxygenation have been determined for various bacteria. It is intended that this will be combined with heat to give us more data as to the significance of using oxygen in inactivation studies. The viability of Ascaris ova after oxygenation has not yet been determined, but will be correlated with bacteriological data. Aeration, when compared to the oxygenation inactivation data, may well be a feasible treatment from a cost effective viewpoint. Ozonation in conjunction with radiation is another area in which we will look at potential benefits.

Dried sludges which are then treated with irradiation need to be analyzed for pathogen inactivation. In the event that this becomes a more economically feasible treatment, such information would be essential.

The agent of anaerobically digested sludge responsible for inactivating poliovirus must be identified and its activity against other viruses commonly associated with sludge must be determined. Once it has been characterized and its range of activity is known, it will then be important to determine the real potential of the agent as a viral inactivator during treatment plant operation. Because the potential of this agent can be fully realized only if its mode of action is understood at the molecular level, the mechanism by which it inactivates viruses should be studied. Finally, the possibility of extending its use to systems other than sludge will be examined.

Biological modifications of anaerobic sludge digestion by use of special strains of digester bacteria have been considered. Proposals that deal with enhanced methane production and conservation of fixed nitrogen by assimilating denitrification are also being prepared. A lyophilized culture of Chromobacterium violaceium has been obtained and

will be used to study nitrate reduction to nitrite, and nitrite to ammonia.

Odor modifications to be done in the very near future include studies as follows:

(1) odor analyses on control samples of raw and of digested sludge;

(2) analyses on samples heated to 40° C, 50° C, 70° C and 95° C;

(3) analyses on irradiated samples (250, 500, 750 and 1000 krads);

(4) analyses on thermoradiation-treated sludges for a variety of doses and temperatures.

These studies are being performed at Battelle PNL in Richland, Washington.

Filterability studies are planned which will show if the decrease in filterability induced by irradiation or thermoradiation treatment can offset any chemical costs of a standard dewatering process, such as vacuum filtration or centrifugation.

The major aim for the cost benefit analysis is the preparation of a final cost/benefit document, which will be available in February 1977. The final assembly of the document is awaiting cost results on competing processes from studies under way at Battelle, PNL. The Battelle results will be available in December 1976. One major set of monte carlo computer calculations, that are needed to corroborate gamma source efficiency calculations done by

Dick Libby of Battelle, PNL, will be performed this year. In addition, irradiator design calculations will be done to estimate total source use efficiencies for the cost benefit analysis.

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