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RESPIROMETRIC METHODS FOR DETERMINATION OF BIODEGRADABILITY AND BIODEGRADATION KINETICS FOR HAZARDOUS ORGANIC POLLUTANT COMPOUNDS

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ABSTRACT

Electrolytic respirometry involving natural sewage, sludge and soil microbiota is becoming prominent in fate studies of priority pollutant and RCRA toxic organics to generate biodegradation/inhibition kinetic data. A developed multi-level protocol is presented for determination of substrate biodegradability and toxicity, microbial acclimation to toxic substrates and first order kinetic parameters of biodegradation and for estimation of Monod kinetic parameters of toxic organic compounds, in order to correlate the extent and rate of biodegradation with a predictive model based on chemical properties and molecular structure of these compounds. Respirometric biodegradation/inhibition and biokinetic data are provided for representative RCRA alkyl, chloro- and nitro-benzenes, phenols, phthalates, ketones and selected CERCLA leachate toxic organics. Data on the effects of the source of sludge biomass, temperature and concentration of microbial inoculum and toxic substrate on the kinetics of biodegradation are also included.

INTRODUCTION

Electrolytic respirometry is attaining prominence in biodegradation studies and is becoming one of the more suitable experimental methods for measuring the biodegradability and the kinetics of biodegradation of toxic organic compounds by the sewage, sludge and soil microbiota and for determining substrate inhibitory effects to microorganisms in wastewater treatment systems.

Biodegradation of toxic and hazardous organic compounds holds a great promise as an important fate mechanism in wastewater treatment and in soil detoxification. Information about the extent and rate of biodegradation is a prerequisite for informed decision making on the applicability of the biodegradation approach. Unfortunately, relatively little quantitative data are available from which engineering judgement can be made, because of the large effort required to assess biodegradation kinetics.

Current research in our laboratories has shown that it is possible to assess biodegradation kinetic parameters from oxygen uptake data, obtained through the use of electrolytic respirometry. This method greatly reduces the work and expense involved in evaluation of biodegradation kinetics. The ongoing biodegradation studies are concerned with the generation of biokinetic database so that it can be ultimately used to establish a possible correlation between molecular substrate configuration (chemical/physical characteristics) and biomass activity (kinetic parameters) as an index of biodegradation. The experimental respirometric testing is also providing data on the concentration levels of toxic organics inhibitory to microbial activity.

The ongoing biodegradation studies are concerned with the prediction of the biological fate of toxic organic compounds using electrolytic respirometry as an approach to measure the biodegradation of selected organic compounds and generate biokinetic data so that these can be ultimately used to establish a possible correlation between molecular substrate configuration (physical/chemical characteristics) and biomass activity (kinetic parameters) as an index of biodegradation. The experimental respirometry testing is also providing data on the concentration levels of toxic organics inhibitory to microbial activity.

Initially, the inter-laboratory, ring test, Organization of Economic Cooperation and Development (OECD) studies at the EPA laboratory, Cincinnati, Ohio, were undertaken to develop confirmatory respirometric biodegradability testing procedure. Respirometric biodegradability and biokinetic data were provided for the selected non-inhibitory and non-adsorbing compounds, tetrahydrofuran, hexamine, pentaerythritol, 1-napthol, sodium benzene sulphinate, thioglycolic acid and the biodegradable reference compound, aniline.

Subsequently, similar electrolytic respirometry studies were initiated to determine biodegradation kinetic parameters for selected representative toxic compounds of varied classes of organics included in the Priority Pollutant, RCRA and superfund CERCLA lists, and to demonstrate presence of

any inhibitory effects of these organics of specified concentration levels on the sludge biomass and on the metabolism of biogenic compounds.

The objectives of the present study were to utilize the electrolytic respirometry oxygen uptake data to: (1) determine the biodegradability of selected RCRA alkyl, chloro, and nitrobenzenes, phenols, phthalates, and ketones and representative CERCLA leachate toxic organics; (2) generate information on their acclimated times (t_0) and the initiation and termination time values for the declining growth phase (t_1 and t_2); (3) determine their first order kinetic parameters of biodegradation (specific growth rate constants for the exponential growth phase (μ) and for the declining growth phase (μ'); (4) estimate the Monod kinetic parameters (μ_m, K_s and Y) of these compounds without initial growth or growth yield assumptions; (5) demonstrate presence of any inhibitory effects of these compounds on the metabolism of the biodegradable reference compound, aniline; and (6) to correlate the extent and rate of biodegradation of these compounds with a predictive model based on chemical properties and structure of these compounds.

The purpose of this study was to obtain information on biological treatability of the benzene, phenol, phthalate and ketone organics and of the Superfund CERCLA organics bearing wastes in wastewater treatment systems which will support development of an EPA technical guidance document on the discharge of the above organics to POTWs. The study was to generate basic information on the fate of CERCLA leachate organics during on-site treatment and biodegradation and inhibition data for pollutants found in Superfund site wastewater that could be discharged to POTWs. Respirometric biodegradability, biokinetic and inhibition data were generated for the selected RCRA benzene, phenolic, phthalate and ketone compounds.

BACKGROUND

Measurement of Oxygen Consumption

Measurement of oxygen consumption is one of the oldest means of assessing biodegradability. Time consuming manual measurement of oxygen uptake (dilution BOD measurements) was replaced gradually by a more direct and continuous respirometric method for measurement of oxygen consumption in biochemical reactions, for use in routine examination of sewage and in control of sewage treatment process.

A rather comprehensive review of the use of respirometers for the study of sewage and industrial waste and their application to water pollution problems was published by Jenkins in 1960 (1). Montgomery's (2) review of respirometric methods summarized the design and application of respirometers for determination of BOD.

The application of respirometry was gradually directed to research studies to assess the toxicity and biodegradation of specific wastes or compounds, to evaluate factors affecting biological growth and to provide an insight into nitrification reaction. Of the commercial respirometers which have been developed for respirometric studies, the electrolytic respirometers were shown to be most applicable for measurement and

quantitation of biodegradation activity because they automatically produce oxygen as needed, thereby eliminating some of the limitations of other techniques and allowing output data to be collected automatically for direct recording and processing (3-9). A recent detailed review of respirometric techniques and their application to assess biodegradability and toxicity of organic pollutants was published by King and Dutka (10).

Respirometric Biodegradability Testing

Most uses of electrolytic respirometry in biodegradability testing have been for screening purposes to measure the extent of biodegradation as a percentage of the theoretical oxygen demand exerted in some time period (9, 11-17). A more recent study by Painter and King (18) concluded that a procedure based on electrolytic respirometry was reliable for assessing biodegradability, and could serve as an adequate Level I screening test for biodegradability (19).

A considerable amount of studies using electrolytic respirometry to determine the biodegradability of wastes and specific organics is available in published literature and significant data on biodegradation of pollutants based on oxygen uptake have been generated (2, 4, 5, 20-34).

There are many techniques that have been used to evaluate biodegradation kinetics and these were reviewed in detail by Howard et al. (35, 36) and Grady (37). These techniques utilize continuous, fed-batch and batch type reactors for providing data from which kinetic parameters can be evaluated. The use of batch systems in biotechnology and biological wastewater treatment represents a less labor intensive, less expensive and much faster way to model biokinetics.

The kinetic parameters obtained by the above techniques should be intrinsic, that is, dependent only on the nature of the compound and the degrading microbial community and not on reactor system used for data collection. If this condition is satisfied, then the parameters obtained can be used for any reactor configuration and can be used in mathematical models to estimate the fate of toxic organics.

Batch techniques are successful in obtaining intrinsic kinetic parameters by applying non-linear curve fitting techniques to single batch substrate removal curves, provided initial conditions are selected with proper care (Simkins and Alexander (38, 39), Robinson and Tiedje (40), Cech et al. (41), and Braha and Hafner (42). Batch systems can be used with either acclimated or unacclimated biomass for providing kinetic data and require that samples be taken at discrete time intervals during the course of biodegradation [Tabak et al. (43), Larson and Perry (24) and Paris and Rogers (44)].

Measurement of oxygen consumption through electrolytic respirometry is a batch type technique which has been shown to be very promising for automating data collection associated with biodegradation and intrinsic kinetic parameters.

measurements of oxygen consumption in respirometric batch reactors. With the use of computer simulation techniques and non-linear curve fitting methods, intrinsic kinetic parameters were obtained from oxygen consumption data and were shown to be in agreement with those obtained from traditional measurement of substrate removal (DOC, SCOD, ^{14}C) or cell growth.

MATERIALS AND METHODS

Experimental Approach

The electrolytic respirometry approach to determine the biodegradability of the organic test compounds in this study was chosen because of the specific advantages of the respirometric methods over that of manometric procedures in tracking oxygen utilization during the exertion of biochemical oxygen demand (BOD). These advantages are listed in Table 1. General classification of respirometers based on principle of operation and on techniques and applications is presented in Tables 2 and 3.

The electrolytic respirometry studies were conducted using an automated continuous oxygen uptake and BOD measuring Voith Sapromat B-12 (12 unit system) electrolytic respirometer-analyzer. The instrument consists of a temperature controlled waterbath, containing measuring units, a recorder for digital indication and direct plotting of the decomposition velocity curves of organic compounds; and a cooling unit for the conditioning and continuous recirculation of waterbath volume. The recorder shows the digital indication of oxygen uptake and constructs a graph for these values of each measuring unit. The cooling unit constantly recirculates water to maintain constant temperature in the waterbath. Each measuring unit as shown in Figure 1 is comprised of a reaction vessel with a carbon dioxide absorber mounted in a glass joint flask stopper, an oxygen generator and a pressure indicator. This measuring unit is interconnected by hoses, forming an air sealed system, so that the atmospheric pressure fluctuations do not adversely affect the results.

The activity of the microorganisms in the sample creates a vacuum which is recorded by the pressure indicator, which triggers the oxygen generator. The pressure conditions are balanced by electrolytic oxygen generation. The quantity of the sample, the amperage for the electrolysis and the speed of the synchronous motor are so adjusted that, with a sample of 250 mL, the digital counter indicates the oxygen uptake directly in mg/L. The CO_2 generated is absorbed by soda lime. The nitrogen/oxygen ratio in the gas phase above the sample is maintained throughout the experiment and there is no depletion of oxygen. The recorder-plotter concomitantly constructs an oxygen uptake graph for the selected values. The oxygen generators of the individual measuring units are electrolytic cells which supply the required amount of oxygen by electrolytic dissociation of a copper sulfate solution combined with sulfuric acid.

The nutrient solution used in these studies was an OECD synthetic medium (19, 56) consisting of measured amounts per liter of deionized distilled water of (1) mineral salts solution; (2) trace salts solution, and (3) a solution (150 mg/L) of yeast extract as a substitute for vitamin solution.

The microbial inoculum was an activated sludge from The Little Miami wastewater treatment plant in Cincinnati, Ohio, receiving municipal wastewater. The activated sludge sample was aerated for 24 hours before use to bring it to an endogenous phase. The sludge biomass was added to the medium at a concentration of 30 mg/L total solids. Total volumes of the synthetic medium in the 500 mL capacity reactor vessels were brought up to a final volume of 250 mL.

The test and control compound concentrations in the media were 100 mg/L. Aniline was used as the biodegradable reference compound, at a concentration of 100 mg/L.

The typical experimental system consisted of duplicate flasks for the reference substance, aniline, and the test compounds, a single flask for the physical/chemical test (compound control), a single flask for toxicity control (test compound plus aniline at 100 mg/L each) and an inoculum control. The contents of the reaction vessels were preliminarily stirred for an hour to ensure endogenous respiration state at the initiation of oxygen uptake measurements. Then the test compounds and aniline were added to it. The reaction vessels were then incubated at 25°C in the dark (enclosed in the temperature controlled waterbath) and stirred continuously throughout the run. The microbiota of the activated sludge used as an inoculum were not pre-acclimated to the substrates. The incubation period of the experimental run was between 28 to 50 days. A more comprehensive description of the procedural steps of the respirometric tests is presented elsewhere (33, 46).

For fully automatic data acquisition, frequent recording and storage of large numbers of oxygen uptake data, the Sapromat B-12 recorders are interfaced to an IBM-AT computer via Metrabyte interface system. The use of Laboratory Handbook software package allows the collection of data at 15 minute intervals.

The oxygen utilization by the biomass based on the oxygen uptake velocity (BOD) curves, consisting of the exponential and declining phases of microbial growth was the basis for measurement of substrate utilization and growth rate. Figure 2 provides a generalized plot of the substrate concentration, biological solids concentration (biomass), and oxygen utilization during exertion of BOD, versus time in a respirometric biodegradability treating vessel system. A typical relationship between the BOD and substrate degradation curves as well as between biomass and substrate concentrations and growth yield is illustrated in Figure 3.

Possible curves of oxygen uptake attributed to the organic test substrate that can be generated in a respirometric run and which are dependent on the acclimation time, the extent and rate of bio-oxidation of the substrate, the presence of cometabolite(s) and the presence of biocatalytic additive in the nutrient solution are demonstrated in Figure 4.

Factors affecting respirometric BOD determination have been taken into consideration in the study, and are listed in Table 4.

Chemical Analysis

Indirect analysis of culture samples from respirometric vessels during the study included determination of chemical oxygen demand as soluble and total COD and of dissolved oxidizable carbon (DOC) with the use of a Beckman Model 915 B system.

Specific substrate analysis of culture samples for the residual parent compound and the possible intermediate and final products of metabolism was performed with the use of a Gas Chromatograph Varian 3700, equipped with FID and EC detectors and with different detector columns (depending on the substrate to be analyzed) and the Finnigan automated GC/EI-CI Mass Spectrophotometer system. Samples were analyzed as dichloromethane extracts of cultures (continuous liquid-liquid extraction) or by direct aqueous injection.

Suspended solids and biomass were measured gravimetrically (solids retained on a 0.45 μm filter) and by optical density determined spectrophotometrically (percent transmission read at 540 nm).

Determination of Substrate Biodegradability from Oxygen Uptake Data

In this study, biodegradation was measured by three approaches: the first, as the ratio of the measured BOD values in mg/L (oxygen uptake values of test compound minus inoculum control - endogenous oxygen uptake values) to the theoretical oxygen demand (ThOD) of substrate as a percent; the second as a percentage of the test compound as measured by dissolved organic carbon (DOC) changes [OECD Guidelines for Testing of Chemicals (Method DGXI 283/82, Revision 5) (56)]; and the third, as a percentage of the test compound as measured by specific substrate analysis.

Graphical representation of percent biodegradation based on the BOD/ThOD ratio were developed against time for each test compound. The experimental DOC data for the initial samples and samples for reaction flasks collected at the end of experimental run were used to calculate the percent biodegradation based on the percent of DOC removal in the culture system.

Determination of Kinetic Parameters of Biodegradation

Monod equation, relating cell growth to biomass and substrate concentration and the linear law, relating cell growth to substrate removal are the most popular kinetic expressions which can provide adequate description of growth behavior during biodegradation of substrate. The Monod relation states that cell growth is first order with respect to biomass concentration (X) and mixed order with respect to substrate concentration (S) by tbe equation

$$dX/dt = (Su_mX)/(K_s + S) \qquad [I]$$

Cell growth is related to substrate removal by the linear law by the equation

$$dX/dt = -Y(dS/dt \qquad [II]$$

The kinetics of biodegradation were evaluated by quantifying μ_m, K_s and Y kinetic parameters expressed in the equation for the rate of substrate removal, r_s:

$$r_s = -\mu_m X/Y \qquad (1)$$

where X is the concentration of biomass capable of utilizing the organic substrate and Y is the biomass yield coefficient for the compound and in the Monod equation:

$$\mu = \mu_m S_s/K_s + S_s \qquad (2)$$

(if the compound is not inhibitory to its own biodegradation) or by the Haldane equation:

$$\mu = \mu_m S_s/K_s + S_s + (S_s^2/K_I) \qquad (3)$$

if the compound is inhibitory. In these equations, μ_m is the maximum specific growth rate, K_s is the half saturation coefficient, K_I is the inhibition coefficient and S is the concentration of substrate.

A graphical presentation of the Monod substrate utilization equation is illustrated in Figure 5 which provides a relationship between rate of substrate utilization and substrate concentration.

Determination of Rates of Exponential and Declining Growth

The first order kinetic rate constants (specific growth rate parameters) were determined by the linearization of the BOD curves or transforming the typical BOD curve to the linear function of time t, by the relationship of log dO_u/dt to t, which gives straight lines expressing the exponential and declining endogenous phases of the BOD curve as shown in Figures 6 and 7. The slope of the Ln(d oxygen uptake/dt) versus t give specific rate constants of the exponential growth phase (μ values) and the declining growth phase (μ' values) of the BOD curve as described by Dojlido (11), Tabak et al. (45), Oshima et al. (46), and Tabak et al. (54, 55).

Acclimation time values (t_0) and the time values for the initiation and termination of the declining growth phase (t_1 and t_2) for each test compound were determined from linearized expressions of BOD curves.

Estimation of Monod Kinetic Parameters

The estimations of the Monod Kinetic parameters, maximum specific growth rate constant, μ_m, half saturation constant, K_s and growth yield constant, Y were determined directly from experimental oxygen uptake curves without the consideration of initial growth and growth yield assumption [Jobbagy, Grady and Tabak (57) and Tabak et al. (54)].

If the concentrations of the substrate, the products and the biomass are all expressed in BOD units, then the oxygen uptake (O_u at any time in the batch reactor may be calculated from

$$O_u = (S_{so} - S_s) - (X-X_o) - (S_p-S_{po}) \quad (4)$$

where S_{so}, S_{po} and X_o are the concentrations of substrate, products and cells, respectively, at time zero.

To apply equation (4) for the determination of kinetic coefficients, equations must be available which express the concentrations of soluble substrate (S_s), soluble product (S_p) and biomass (X) as functions of time. For batch reactors, those equations are:

$$dS_s/dt = -(\mu_m/Y)S_sX/K_s + S_s) \quad (5)$$

$$dS_p/dt = (Y_p\mu_m/Y)S_sX/(K_s + S_s) \quad (6)$$

$$dX/dt = \mu_mS_sX/(K_s + S_s) - K_sbX/(K_s + S_s) \quad (7)$$

where S_s = soluble substrate concentration; S_p = soluble product concentration; Y_p = product yield; and b = decay coefficient.

To calculate oxygen uptake in a batch reactor, equations (5), (6) and (7) must be solved simultaneously, and the resulting values of S_s, S_p and X over time are substituted into equation (4).

Determination of Y Constant

Y - the true yield parameter or the ratio of growth of biomass to substrate utilization, can be obtained from the experimental oxygen uptake curve at the initiation of the plateau of the curve as shown in Figure 8, with the use of equation:

$$Y = (1-O_{upt}/S_o)-Y_p \quad (8)$$

A vertical line is drawn at the point of intersection of the tangents of the exponential and plateau phases of the curve. The oxygen uptake value obtained at the point of intersection of the vertical line (drawn through intersection of tangents) and oxygen uptake curve is the O_{upt} value - [cumulative oxygen uptake value at the initiation of plateau].

The O_{upt} value is then plugged into the equation (8), $Y = (S_o-O_{upt}/S_o)-Y_p$, where S_o is initial concentration of substrate and Y_p is soluble product concentration formed divided by initial substrate concentration, for Y determination. In this study, product yield (Y_p) was negligible.

Determination of μ_m Constant

The initial estimate of μ_m is obtained by the technique of Gaudy et al. (47, 48). If Y_p is assumed to be zero, equation (6) is eliminated and if b (decay constant) is assumed to be zero, equation (7) is simplified to

$$dX/dt = (\mu_mS_sX)/(K_s + S_s) \quad (9)$$

Combining equation (5) and (9) and integrating from S_{so} to S_s and from X_o to X gives:

$$X = X_o + Y(S_{so} - S_s) \tag{10}$$

If the assumption that $S >> K_s$, the term $S_s/K_s + S_s$ in equations (5) and (9) or [I] approaches one, and these systems can be simplified through the use of equation (10) to give equation

$$dX/dt = \mu_m X \tag{11}$$

Integrating equation (11) and combining with equation [II] and than substituting in equation (4) with P and P_o both equal to zero gives

$$Ln[X_o + O_u/(1/Y)-1) = Ln(X_o) + \mu_{m(est)} \tag{12}$$

The plot of $Ln[(X_o + O_u)/(1/Y) - 1)]$ versus time will give a straight line with slope μ_m. The accuracy of μ_m(est) will depend upon the size of K_s, but this value is good enough as an estimate.

μ_m - the maximum specific growth rate can be determined from experimental oxygen uptake curve plot in the following manner:

(1) Values of the change of O_u with time (dO_u/dt) or slopes are determined along the entire experimental oxygen uptake curve as shown in Figure 9.

(2) These dO_u/dt (slope) values are then plotted against the cumulative O_u values for each time interval, as shown in Figure 10.

(3) The slope of the developed linearized form of oxygen uptake curve is the estimated μ_m value.

Determination of K_s Constant

K_s - the half saturation constant or the substrate concentration at which the specific growth rate is 1/2 the maximum specific growth rate can be obtained from the experimental oxygen uptake curve in the following manner:

(1) Value of O_{ut} can be calculated from the plot of (dO_u/dt) versus O_u provided the K_s value is 1 or less (insignificant in comparison to S_o value) and the plot contains a linear section with the slope μ_m, as shown in Figure 11.

(2) Other (dO_u/dt) versus O_u plot in which the slope deviates from μ_m because of larger K_s values (more significant in comparison to S_o) is illustrated in Figure 12.

(3) The value of dO_u/dt is determined at the intercept of the straight line developed from the plot of dO_u/dt versus O_u (Figure 11) which contains a linear section with slope μ_m.

(4) Beginning with the value of 1/2 the intercept value, another straight line (b) is constructed with the slope 1/2 that of the slope of original line (a) whose slope is μ_m.

(5) At the point where line (b) intercepts the declining experimental curve of the plot, a vertical line from that point of interception can provide the value of O_{ut} on the x axis.

(6) This O_{ut} value is then used in the determination of K_s with the use of the equation

$$S_t = S_o - (O_{ut}/(1-Y_p-Y) = K_s$$

where S_o = initial substrate concentration and S_t = substrate concentration at time t.

(7) When the O_{ut}, Y, Y_p, and S_o values are plugged into the equation, the value of S_t can be calculated - which is the value of K_s (in systems where K_s value is 1 or less).

Thus the oxygen uptake value O_{ut} associated with 1/2 of the estimate of u_m is used in $K_s = S_o - O_{ut}/1(1 - Y)$ to get the estimate of K_s. The major impact of K_s is upon the shape of the oxygen uptake curve in the region of the plateau. Comparison of the experimental curves to a family of standardized curves as an initial estimate provides an initial estimate of K_s that is sufficient for non-linear curve fitting techniques for quantitation of the kinetic parameters.

Quantitation of Monod Kinetic Parameters

The methodology for quantitation of the Monod kinetic parameters requires the use of the above specific methods for estimating them initially and subsequently followed by computer simulation methods coupled with non-linear curve fitting techniques and is based on the use of measured values of initial growth and growth yield. The method requires the use of the kinetic equation relating growth rate of biomass in presence of substrate, the substrate utilization rate, product formation rate and rate of oxygen consumption from O_2 uptake (BOD) curves to calculate and use the theoretical oxygen consumption data to quantitate the biokinetic parameters.

The determination of the kinetic parameters associated with biodegradation requires a series of steps. The initial substrate (S_{so}) and biomass (X_o) concentration must be carefully measured in COD units. The ratio of the two values must lie in a certain range in order to allow independent evaluation of μ_m, K_s and Y [Simkins and Alexander (38, 39)]. Grady's studies (49, 50) have shown that a S_{so}/X_o ratio of around 20 works well. The value of Y_p may be estimated by determining the residual stable SCOD concentration after substrate depletion (plateau area). It is numerically equal to the residual SCOD divided by the initial SCOD. The value of the decay coefficient, b, may be determined by fitting to the oxygen consumption curve after the plateau when the only activity contributing to oxygen consumption is endogenous metabolism and cell decay. Once X_o, S_{so}, Y_p and b are known, μ_m, K_s and Y may be determined by non-linear curve fitting techniques [Grady (49, 50)].

The technique involves the calculation of a theoretical oxygen consumption using oxygen uptake equation (4) and equations for substrate, product and biomass concentrations (5) - (7) with assumed Monod parameters. The residual sum of squared errors (RSSE) associated with the difference in calculated and experimental oxygen uptake values is used to obtain new estimates. The above procedure is repeated until a minimum RSSE is found.

The Grid Search technique was selected as a most suitable non-linear curve fitting technique for application in the determination of the kinetic parameters from oxygen uptake data, because it can allow easy discrimination between local minima and the global minimum RSSE. This technique enables a comparison between the calculated and experimental oxygen uptake data. Value of Y is fixed. For this value of Y, a pair of μ_m and K_s which give RSSE is found on a μ_m:K_s plane. The above procedure is repeated with other values of Y. Values of μ_m, K_s and Y which give minimum RSSE associated with the difference in calculated and experimental oxygen uptake data constitute the best values of the kinetic parameters.

The values of μ_m, K_s and Y developed from grid search technique, which when substituted into equations 5-7, will provide X and S values, which (when substituted into oxygen uptake equation 4) will in turn provide calculated oxygen uptake values at the region of the plateau, closest to the experimental oxygen uptake values, with a minimum RSSE, will constitute the best quantitative kinetic parameter values.

Development of Multi-Level Respirometric Biodegradation Testing Protocol

The oxygen consumption data generated with the use of electrolytic respirometry have been adequately utilized for assessing the biodegradative activity of sludge microbiota, the biodegradability/toxicity of toxic organic compounds, as well as for the determination of the intrinisic kinetic parameters of biodegradation. Methodologies have been developed for quantitating biodegradability and biodegradation kinetics of representative classes of RCRA toxic organics, with the resultant development of a comprehensive multi-level respirometric biodegradation testing protocol based on oxygen consumption data.

The methods of each successive testing level of the respirometric protocol are characterized by increased complexity and a consequent higher cost for performing the tests pertaining to each testing level. Accordingly, the testing levels can be selected as appropriate to the research needs.

Studies to assess the biodegradability and biodegradation rates of toxic organics by sludge microbiota with the use of respirometric oxygen uptake data can involve the use of one of more levels of the protocol, depending on the amount of information needed for assessing the biodegradability of the organic toxic pollutant or the toxicant bearing waste for determination of its fate and rate of biotreatment in the municipal or industrial waste treatment systems. A logic flow diagram of the respirometric biodegradation testing protocol, providing a brief description of each of the testing levels, is shown in Figure 13.

RESULTS AND DISCUSSION

Respirometric biodegradability, biokinetic and Monod kinetic data for selected RCRA alkyl benzenes, phenols, phthalates and ketones are reported in this paper. The electrolytic respirometry oxygen uptake data for the test compounds, the control reference compound aniline, the inhibition and endogenous control systems were generated revealing the lag phase (acclimation phase), the biodegradation (exponential) phase, the different bio-reaction rate slopes (characteristic of the test compound) as well as the plateau region at which the biooxidation rate reaches that of the endogenous rate of microbial activity. Figure 14 illustrates a representative oxygen uptake curve for aniline and the endogenous controls. Figure 15 shows the replicate pentaerythritol oxygen uptake curves and the toxicity control (pentaerythritol plus aniline) curve, Figure 16 illustrates a representative graphical treatment of the percent biodegradation of pentaerythritol with time, which was developed for each test compound (OECD studies).

Based on the biokinetic equations relating growth rate of microbiota in presence of above compounds, the substrate utilization rate, and rate of oxygen uptake (BOD) curves, specific growth rate kinetic parameters (biodegradation rate constants) were derived as slope values of the linearized plots (plots of the log of DO_u/dt) of exponential and declining growth phases of the BOD curve. The acclimation time values (t_0), and time values for the initiation and the termination of the declining growth phases (t_1 and t_2) for the test compounds and aniline were also generated.

The estimations of the Monod kinetic parameters for benzene, phenol, phthalate, and ketone compounds reported here, were determined directly from experimental oxygen uptake curves without the consideration of initial growth and growth yield assumption.

Respirometric Studies with Selected RCRA Alkyl Benzene Compounds

The biodegradation of benzene, toluene, ethyl benzene, m- and p-xylenes, tert-butyl benzene, sec-butyl benzene, butyl benzene, cumene, 1-phenyl benzene and the reference compound, aniline at 100 mg/L concentration by 30 mg/L sludge biomass (as measured by oxygen consumption by sludge microbiota in mg O_2/L) was followed over a period of 20 days. The electrolytic respirometry oxygen uptake and BOD curves were generated and graphical treatment of the percent biodegradation was established for each compound. Figure 17 demonstrates typical oxygen uptake and BOD curves for p-xylene and p-xylene + aniline and Figure 18 illustrates graphically the % biodegradation of p-xylene with time.

The percent biodegradation data based on the BOD/ThOD ratios for benzene, toluene, ethyl benzene, m- and p-xylene and the reference compound, aniline, are summarized in Table 5. All of the above alkyl benzene compounds were shown to be biodegradable substrates at concentration levels of 100 mg/L when exposed to 30 mg/L of activated sludge biomass under the environmental conditions of the respirometric testing procedure, and within the period of 20 days of incubation.

The toxicity test control flask respirometric data revealed no inhibitory effects by these test compounds at the 100 mg/L concentration levels on the bio-oxidation of aniline by sludge microbiota.

Table 6 summarizes the bio-kinetic data for the benzenes studied, showing the specific growth rate constants for the exponential growth phase (μ values) and for the declining growth phase (μ' values) of the linearized form of the BOD curves of these compounds, as well as the t_0, t_1 and t_2 kinetic parameters. Figure 19 shows a typical plot of $Ln(\delta O_u/\delta t)$ vs. time for toluene, from which the kinetic parameters were determined.

Table 7 summarizes the Monod kinetic parameter (μ_m, K_s, Y_g) data for these benzene compounds.

Respirometric Studies with Selected RCRA Phenolic Compounds

The biodegradation of phenol, resorcinol, o-, m- and p-cresols, catechol, 2,4-dimethyl phenol and the reference compound aniline at 100 mg/L concentration levels and exposed to 30 mg/L biomass was followed over a period of 20 days.

All of the phenols were shown to be biodegradable substrates under the conditions of the respirometric testing procedure. The toxicity test control flask respirometric data revealed no inhibitory effects by these compounds at the 100 mg/L levels on the biodegradation of aniline by the sludge biomass.

Table 8 summarizes the bio-kinetic data for the phenols studied, showing the specific growth rate constants as well as the t_0, t_1, and t_2 kinetic parameters. Table 9 provides the Monod kinetic parameter data for these phenolic compounds.

Respirometric Studies with Selected RCRA Phthalate Ester Compounds

Evaluation of the biodegradability and determining of bio-kinetics of degradation of phthalate compounds, dimethyl phthalate, diethyl phthalate, dipropyl phthalate and butyl benzyl phthalate was achieved with use of respirometric oxygen uptake data.

All of the above phthalates were shown to biodegradable under the conditions of the respirometric tests and were shown not to exhibit any inhibitory effects at the 100 mg/L levels on aniline biodegradation by the sludge microbiota.

Tables 10 and 11 summarize respectively the biokinetic (first order) and Monod kinetic parameter data for the selected phthalate esters under study.

Respirometric Studies with Selected RCRA Ketone Compounds

Respirometric oxygen uptake data from the studies with the selected ketone compounds, acetone, 2-butanone, 4-methyl-3-pentanone and a cyclic ketone, isophorone were utilized to determine their biodegradability and biodegradation kinetic parameters.

All the ketones were shown to be biodegradable at 100 mg/L concentration levels in media containing 30 mg/L biomass and did not exhibit any toxicity to aniline biodegradation at these concentrations.

Tables 12 and 13 summarize respectively the first order and Monod kinetic parameter data for these ketones.

CONCLUSIONS

The experimental data of respirometric studies with several classes of organic compounds definitely demonstrate that it is possible to measure the biodegradability (percent biodegradation - as a ratio of BOD to ThOD) and to determine the kinetics of degradation of single organic compounds by using only measurements of oxygen consumption in respirometric batch reactors. The values of the kinetic parameters determined from oxygen consumption data were demonstrated to be similar to those based on the measurements of substrate removal and those made with cell growth data.

The generated data on biodegradation, biodegradation rates and substrate inhibition kinetics through the use of electrolytic respirometry, will enable the classification of biodegradability of toxic priority pollutant and RCRA toxic organic compounds and ultimate projection of the fate of organic compounds of similar molecular structure to those experimentally studied by way of the established predictive treatability models based on structure-activity relationships.

With the electrolytic respirometry approach, data base on the removal of the above compounds by biodegradation fate mechanism can be adequately generated to support the development of predictive models on fate and removal of toxics in industrial and municipal waste treatment systems. A possible relationship between the kinetic parameters and the effect of different factors on these parameters, as determined through electrolytic respirometry and the structural properties of the organic pollutant, can eventually facilitate prediction of the extent and the rate of biodegradation of organic chemicals in the field of wastewater treatment systems from the knowledge of the structural properties of the pollutant organics.

A preliminary predictive biodegradation - structure/activity model based on the group contribution approach was developed from the generated biodegradation kinetic data (first order kinetic parameters) with the use of electrolytic respirometry. It is expected that the model will closely predict the results found experimentally. In this way, the fate of other organic compounds may be anticipated without the time and expense of experimental work.

The electrolytic respirometry biodegradation studies will provide basic pilot scale treatability information and data which will be used to confirm methods to predict treatability and the need for pretreatment of structurally related pollutants (e.g., by structure, anticipated treatability properties, etc.). This study will thus provide a more

extensive list of pollutants than was covered by experimental data, for consideration in guiding the Agency to predict the fate of such compounds without costly experimental testing.

Studies are currently in progress to determine the effect of temperature, and different sources of sludge biomass (domestic and industrial wastewater treatment) on the biodegradation kinetics derived from electrolytic respirometry.

ACKNOWLEDGEMENTS

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TABLE 1

ADVANTAGES OF RESPIROMETRIC METHODS

1. Oxygen uptake can be monitored continuously and constantly and more precisely.

2. Automation provides numerical or binary output data for direct recording or processing (electrolytic).

3. Sample may not require dilution and therefore its oxygen uptake characteristics are measured in a more natural state.

4. Larger sample volumes can be used so that more representative samples are obtained and sampling errors are minimized.

5. The samples are mixed continuously to provide uniform contact of microorganisms, substrate and oxygen.

6. No chemical titrations are required.

7. A continuous record of O_2 uptake is provided with some units having automatic recording devices.

8. Permit treatment plants and in-stream conditions to be simulated more closely than in dilution test.

9. Convenient for measuring the effect of various factors on oxygen uptake, such as dilution, substrate type and concentration, temperature and presence of toxics, effect of ph, nutrient addition, volume and source of sewage seed and seed adaptation.

10. Can be used to determine bacterial growth and substrate removal coefficients.

11. Much lower coefficient of variability can be obtained with respirometry than with the dilution test because of the larger and more representative samples used and the lack of dilution factor.

12. Conditions in nature and in a treatment plant may be simulated more closely in respirometer than in a BOD bottle.

13. Usefull information is often available in very much less than in 5 days.

14. It is possible to stop the test at a recognisable point on the oxygen curve (such as the beginning of the "plateau" which corresponds to the exhaustion of readily oxidized substrate).

15. Recommended for use in: A) routine examination of sewage and trade wastes and in B) control of sewage treatment processes, as well as for C) research studies because of the ease and precision with which variables can be controlled.

TABLE 2 GENERAL CLASSIFICATION OF RESPIROMETERS

Types	Basic Principle of Operation
1. Manometric	Determination of O_2 weight changes in a closed system by measuring or responding to O_2 pressure changes at constant temperature and volume or volume changes at constant pressure.
2. Electrolytic	Same.
3. Dissolved O_2 depletion	Use of dissolved oxygen probe to make direct measurements of depletion of dissolved O_2 from solution.

TABLE 3 TYPES OF RESPIROMETERS BASED ON TECHNIQUES AND APPLICATIONS

I. Respirometers measuring gas exchange

A. Small constant pressure respirometers –
Measurements are made by observing the change in volume of gas phase in contact with the respiring liquid, as gaseous O_2 is absorbed.

B. Small constant volume respirometers –
The changes in pressure due to O_2 uptake is observed. Calibration is necessary.

C. Large respirometers measuring gas exchange –
Recommended especially for studies of treatability.

D. Electrolytic respirometers –
Oxygen pressure is automatically maintained at a constant value by an electrolysis cell.

II. Respirometers in which gas exchange is not measured
Oxygen uptake (BOD) is measured by means of a solid O_2 electrode.

TABLE 4 FACTORS AFFECTING RESPIROMETRIC BOD DETERMINATION

Population dynamics – growth characteristics

Substrate concentration – nutrient deficiency

Rate of oxygen transfer or CO_2 evolution

Type of inoculum
- A. General – Indigenous microbial populations
- B. Addition of specific microbiota to sample
- C. Protozoa

Concentration of inoculum

Dissolved oxygen concentration

Toxicity

Nitrification

Inhibition of protein synthesis

Affect both microoganism growth and substrate utilization rates

Temperature

pH value and buffering capacity

Effect of light

Air bubble effect

Mixing

Turbulence

Nutrient concentration Mineral nutrients

Storage of sample before analysis

Physico – chemical factors

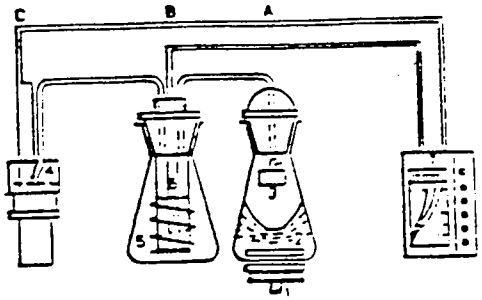
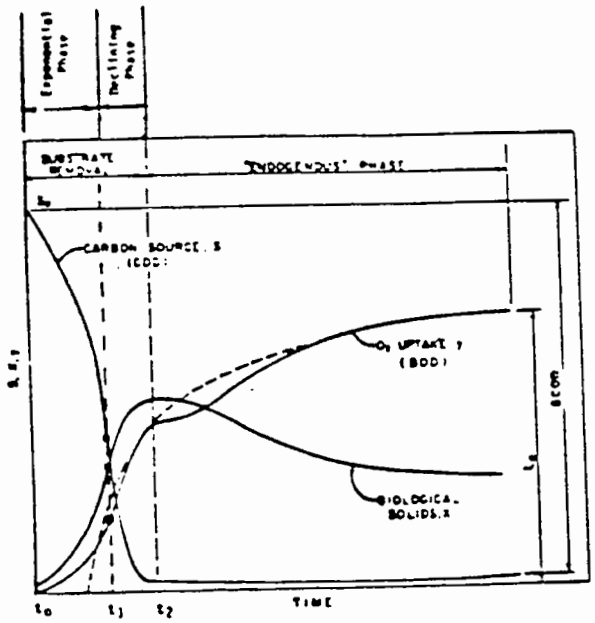


Diagram of a measuring unit
A reaction vessel
B oxygen generator
C pressure indicator

1 magnetic stirrer
2 sample (250 ml)
3 CO_2 absorber
4 pressure indicator
5 electrolyte
6 electrodes
7 recorder

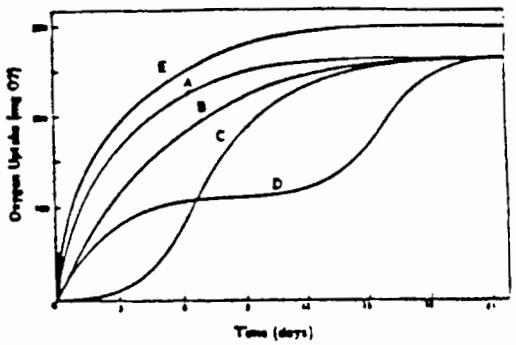
SCHEMATIC DIAGRAM OF A MEASURING UNIT

FIGURE 1



Generalized plot of substrate concentration, biological solids concentration, and oxygen utilization during exertion of biochemical oxygen demand. Circles mark inflection points.

FIGURE 2



A – O_2 uptake curve of a readily oxidizing substrate

B – O_2 uptake curve of a readily oxidizing substrate with lower rate of O_2 uptake than in (A)

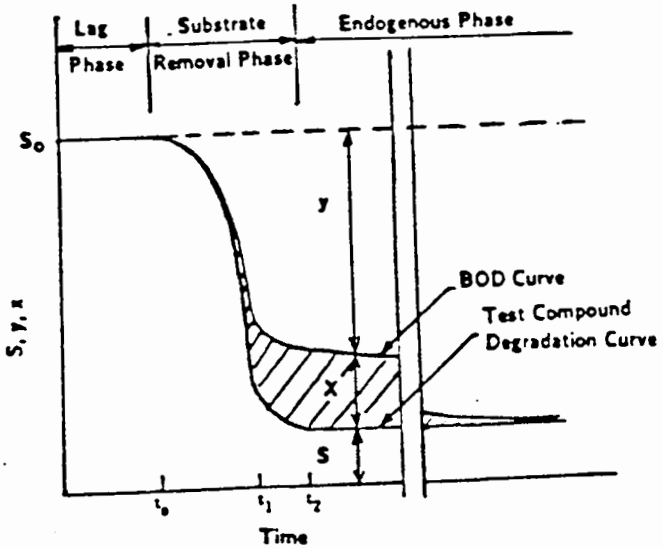
C – O_2 uptake curve with an initial lag period demonstrating oxidation of substrate by microbiota only after undergoing a period of acclimation

D – O_2 uptake curve of a non-homogeneous substrate showing immediate oxidation of part of the substrate followed by acclimation period before oxidation of the remaining part of substrate. Illustration of catabolite repression

E – O_2 uptake curve of biodegradable substrate showing higher rate of O_2 uptake (higher oxidation rate) and an increase of total cumulative O_2 uptake attributed to biocatalytic additives than in (A) and (B); curves for a substrate in presence of only the control seed organisms

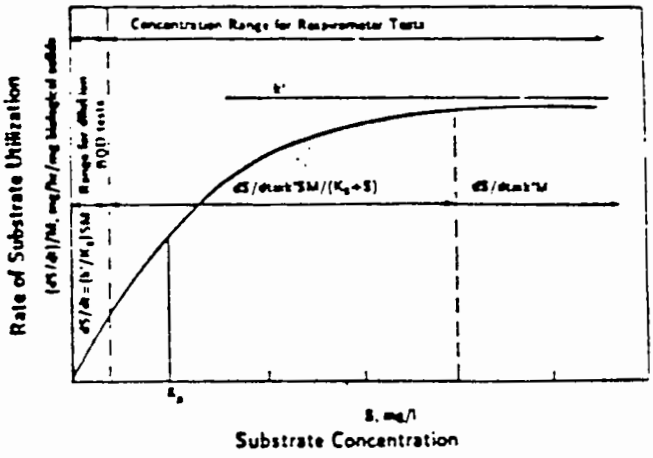
POSSIBLE CURVES OF THE OXYGEN UPTAKE ATTRIBUTED TO THE PROBLEM SUBSTRATE

FIGURE 4



The schematic diagram for the relationship of S, y and x

FIGURE 3



Graphical presentation of the "Monod" substrate utilization equation. The reaction approaches first order at low substrate concentration and a zero-order at high substrate concentrations

FIGURE 5

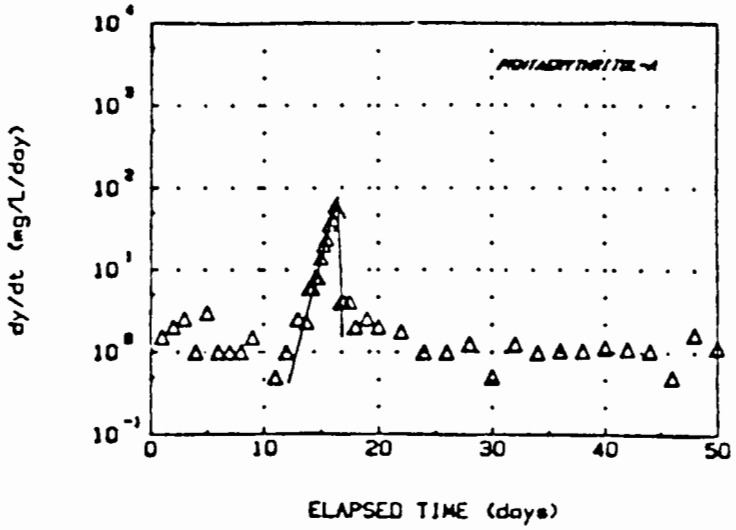


FIGURE 6 Log (dy/dt) vs t Plot
(PENTAERYTHRITOL-A)

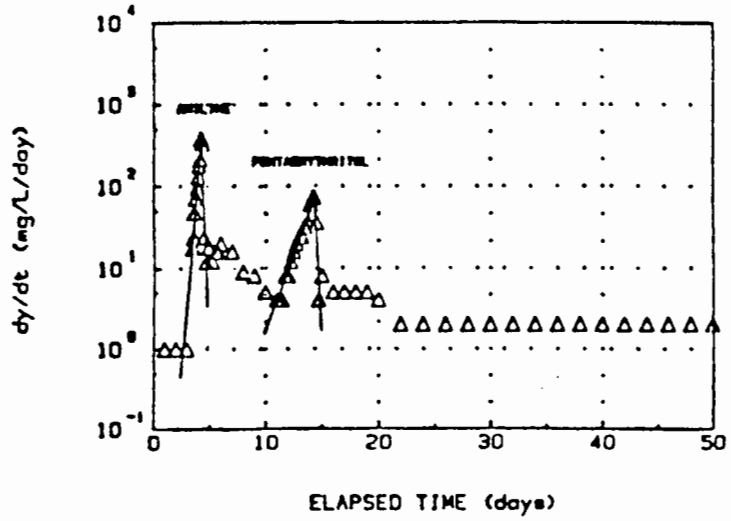


FIGURE 7 Log (dy/dt) vs t Plot
(PENTAERYTHRITOL & ANILINE)

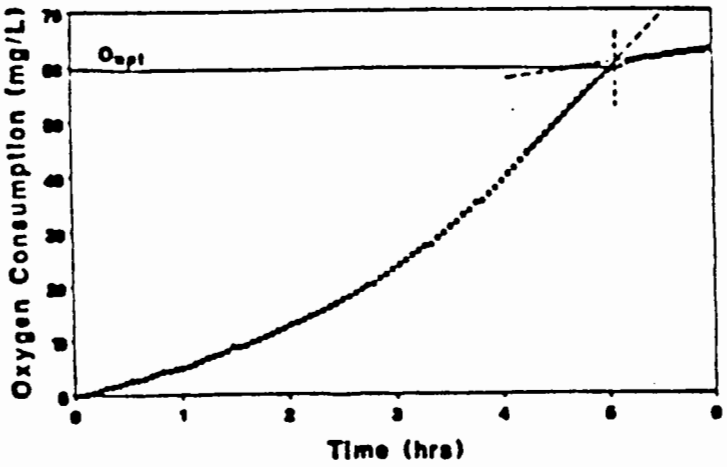


FIGURE 8 DETERMINATION OF THE START OF THE PLATEAU
IN OXYGEN CONSUMPTION FOR USE IN ESTIMATING Y_g

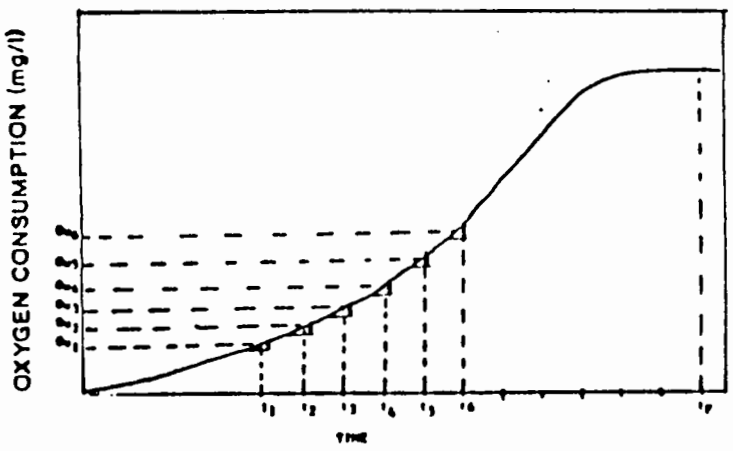


FIGURE 9 PLOT OF OXYGEN UPTAKE RATE (Ou VERSUS TIME)

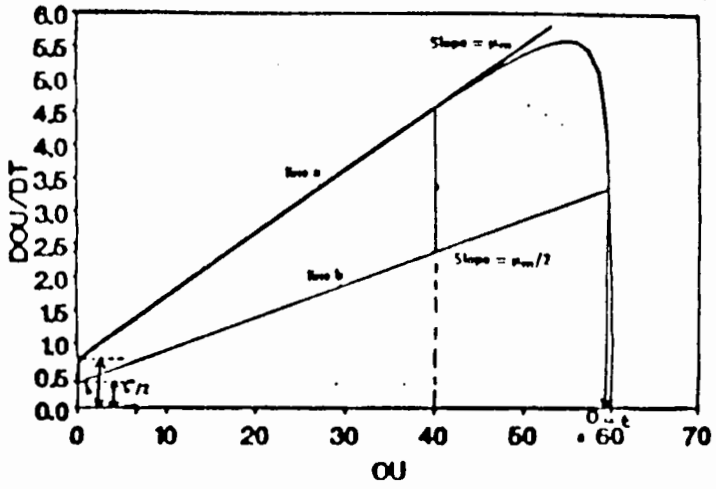


FIGURE 11 Plot showing (ΔOu/Δt) versus Ou_t curve containing a linear section with a slope μ_m to be used in estimating the values of K_s in cases where K_s values are negligible in comparison to S_0.

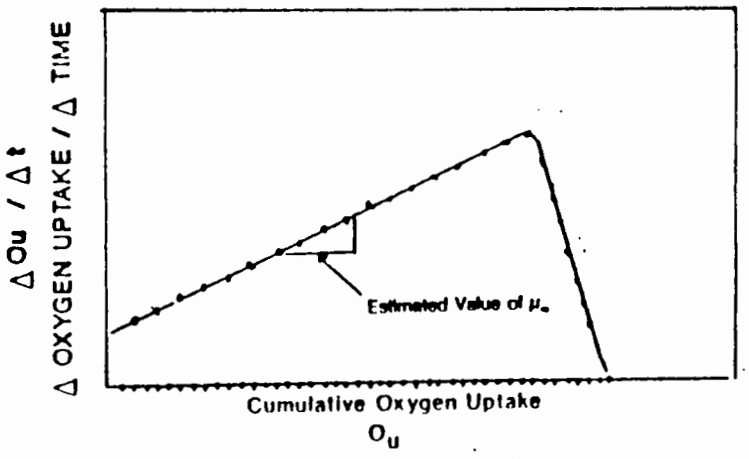


FIGURE 10 PLOT OF ΔOu / Δt VALUES OR SLOPES VERSUS THE CUMULATIVE Ou VALUES

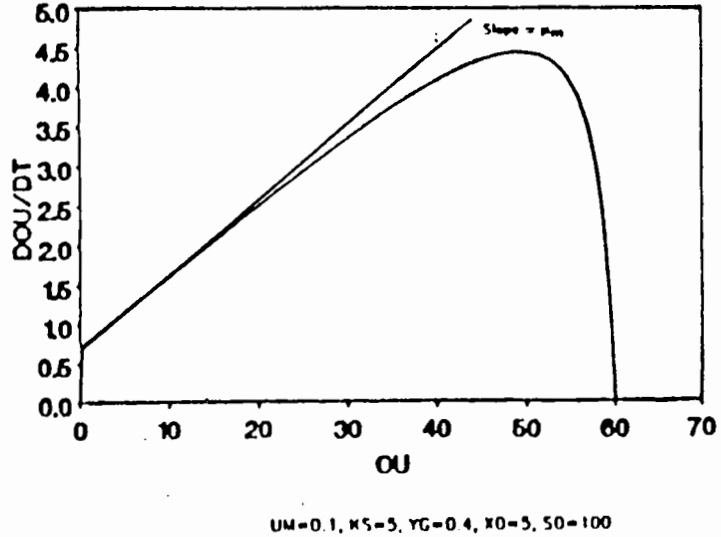


FIGURE 12 Plot of (ΔOu/Δt) versus Ou_t in which the slope deviates from μ_m because K_s values are more significant in comparison to S_0. ($K_s = 5$)

LOGIC FLOW DIAGRAM FOR THE RESPIROMETRIC BIODEGRADATION TESTING PROTOCOL

LEVEL I -- BIODEGRADATION SCREENING METHOD

DETERMINATION OF PRIMARY BIODEGRADATION FROM OXYGEN UPTAKE DATA

DETERMINATION OF ACCLIMATION TIME
ACCLIMATION TIME STUDIES

ACCLIMATION + OR -

(1) DETERMINATION OF ACCLIMATION (LAG) TIME OF MICROBIAL BIOMASS TO TOXIC SUBSTRATE (T_0 VALUES)

(2) DETERMINATION OF THE INITIATION AND TERMINATION TIME VALUES FOR DECLINING GROWTH PHASE OF BOD CURVE (T_1 AND T_2 VALUES)

DETERMINATION OF PERCENT BIODEGRADATION
BIODEGRADABILITY TESTING STUDIES

BIODEGRADATION + OR -

(1) MEASUREMENT OF THE RATIO OF BOD TO THOD IN MG O_2/L OF THE TOXIC SUBSTRATE AS A PERCENT.

(2) MEASUREMENT OF THE PERCENTAGE OF THE RESIDUAL TOXIC SUBSTRATE BY DOC VALUE CHANGES.

(3) MEASUREMENT OF THE PERCENTAGE OF THE RESIDUAL TOXIC SUBSTRATE BY THE SPECIFIC SUBSTRATE ANALYSIS.

DETERMINATION OF MICROBIAL INHIBITION/TOXICITY
SUBSTRATE INHIBITION/TOXICITY STUDIES

INHIBITION + OR -

(1) DETERMINATION OF THE INHIBITORY EFFECTS OF TOXIC SUBSTRATE OR THE TOXICANT BEARING WASTE ON MICROBIAL BIOMASS AS A FUNCTION OF CONCENTRATION.

(2) DETERMINATION OF THE INHIBITORY EFFECTS OF TOXIC SUBSTRATE OR TOXICANT BEARING WASTE ON THE BIODEGRADATIVE ACTIVITY OF BIOMASS ON BIOGENIC SUBSTRATE(S)

(3) DETERMINATION OF SUBSTRATE INHIBITORY LEVELS TO BIOMASS AND TO NORMAL RATE OF BIODEGRADATION

LOGIC FLOW DIAGRAM FOR THE RESPIROMETRIC BIODEGRADATION TESTING PROTOCOL

LEVEL II -- STUDIES OF BIOKINETICS OF BIODEGRADATION BASED ON RESPIROMETRIC OXYGEN UPTAKE DATA

DETERMINATION OF SPECIFIC GROWTH RATES AND THE
INITIAL ESTIMATES OF MONOD KINETICS OF BIODEGRADATION

DETERMINATION OF THE RATES OF EXPONENTIAL AND DECLINING GROWTH PHASE OF BOD CURVE
DETERMINATION OF M AND M' CONSTANTS

APPLICATION OF O_2 UPTAKE DATA AND BOD VALUES TO THE DETERMINATION OF BOD KINETICS. UTILIZAITON OF BIOKINETIC DATA (EXPONENTIAL AND DECLINING GROWTH RATES) FOR BIODEGRADATION EFFICIENCY DETERMINATION.

FIRST ORDER KINETIC RATE CONTSTANTS (SPECIFIC GROWTH RATE PARAMETERS) DETERMINED BY LINEARIZATION OF BOD CURVES THROUGH A RELATIONSHIP OF LOG (DOU/DT) TO T WHICH GIVES STRAIGHT LINES EXPRESSING EXPONENTIAL AND DECLINING PHASES OF BOD CURVES. SLOPES OF THESE LINES REPRESENT M AND M' PARAMETER VALUES RESPECTIVELY.

DETERMINATION OF THE MONOD KINETIC PARAMETERS FROM THE EXPERIMENTAL OXYGEN UPTAKE CURVES
DETERMINATION OF M_H, K_S AND Y PARAMETERS

APPLICATION OF O_2 UPTAKE DATA AND BOD VALUES FOR THE DETERMINATION OF THE INITIAL ESTIMATES OF MONOD KINETIC PARAMETERS DIRECTLY FROM EXPERIMENTAL O_2 CONSUMPTION CURVES WITHOUT THE CONSIDERATION OF INITIAL GROWTH AND GROWTH YIELD ASSUMPTION.

THESE INITIAL KINETIC PARAMETERS ESTIMATE DATA CAN BE SUCCESSFULLY APPLIED FOR BIODEGRADATION EFFICIENCY DETERMINATION IN WASTEWATER TREATMENT SYSTEMS.

SPECIFIC METHODOLOGIES HAVE BEEN DEVELOPED FOR OBTAINING THE INITIAL ESTIMATES OF THE MONOD KINETIC PARAMETERS.

USE OF PRE-ACCCLIMATED BIOMASS OR BIOMASS ACCLIMATED IN RESPIROMETRIC VESSELS.

LOGIC FLOW DIAGRAM FOR THE RESPIROMETRIC BIODEGRADATION TESTING PROTOCOL

LEVEL III -- STUDIES TO QUANTITATE MONOD KINETIC PARAMETERS FROM RESPIROMETRIC OXYGEN UPTAKE DATA

USE OF INITIAL ESTIMATES OF KINETIC PARAMETERS, U_M, K_S AND Y, TO QUANTITATE THESE BY MEANS OF COMPUTER SIMULATION METHODS COUPLED WITH NON-LINEAR CURVE FITTING TECHNIQUES

METHODOLOGY BASED ON THE KINETIC RELATIONSHIP BETWEEN GROWTH RATE, OXYGEN UPTAKE RATE AND TOXIC SUBSTRATE CONCENTRATION IN A MASS BALANCE EQUATION TO DEVELOP INTRINSIC KINETIC PARAMETER DATA FOR PREDICTION OF THE FATE OF THE SUBSTRATE IN FULL SCALE TREATMENT SYSTEMS

METHODOLOGY SUPPORTED BY PROOF-OF-CONCEPT DATA WHICH SHOW AN AGREEMENT BETWEEN THE VALUES OF THE KINETIC PARAMETERS OBTAINED FROM O_2 CONSUMPTION DATA AND THOSE OBTAINED FROM TRADITIONAL MEASUREMENT OF SUBSTRATE REMOVAL (DOC, SCOD, ^{14}C) OR CELL GROWTH.

METHODOLOGY REQUIRES THE USE OF KINETIC EQUATIONS RELATING GROWTH RATE IN PRESENCE OF TOXIC SUBSTRATE, SUBSTRATE UTILIZATION RATE, SOLUBLE PRODUCT FORMATION RATE AND RATE OF OXYGEN UPTAKE IN ORDER TO CALCULATE AND USE THE THEORETICAL O_2 CONSUMPTION DATA (O_2 UPTAKE CURVES) TO QUANTITATE THE BIOKINETIC PARAMETERS.

METHODOLOGY IS BASED ON THE USE OF MEASURED VALUES OF INITIAL GROWTH (X_0) (BIOMASS CONCENTRATION), SUBSTRATE CONCENTRATION (S_{SO}) AND GROWTH YIELD (Y), PRODUCT YIELD (Y_p) (DETERMINED BY FITTING O_2 UPTAKE CURVE AFTER THE PLATEAU (O_2 CONSUMPTION DUE TO ENDOGENOUS METABOLISM AND CELL DECAY).

ONCE X_0, S_{SO}, AND Y_p AND B (CELL DECAY) VALUES ARE KNOWN, THE KINETIC PARAMETERS, U_M, K_S AND Y ARE QUANTITATED BY THE NON-LINEAR CURVE FITTING TECHNIQUES. INITIAL KINETIC PARAMETER ESTIMATES ARE USED IN THE METHOD.

THE NON-LINEAR CURVE FITTING TECHNIQUE (GRID SEARCH METHOD) WAS SHOWN TO BE MOST APPLICABLE, SINCE IT ALLOWS EASY DISCRIMINATION BETWEEN THE CALCULATED AND EXPERIMENTAL O_2 UPTAKE DATA. RSSE ASSOCIATED WITH DIFFERENCES IN CALCULATED AND EXPERIMENTAL O_2 UPTAKE VALUES ARE USED TO OBTAIN NEW ESIMATES OF KINETIC PARAMETERS. THIS PROCEDURE IS REPEATED TILL MINIMUM RSSE IS FORMED.

GRID SEARCH TECHNIQUE - VALUE OF Y IS FIXED. FOR THIS VALUE OF Y, A PAIR OF U_M AND K_S VALUES WHICH GIVE RSSE IS FOUND ON A U_M:K_S PLANE. THE ABOVE PROCEDURE IS REPEATED WITH OTHER VALUES OF Y. VALUES OF U_M, K_S AND Y WHICH GIVE MINIMUM RSSE ASSOCIATED WITH THE DIFFERENCE IN CALCULATED AND EXPERIMENTAL O_2 UPTAKE DATA CONSTITUTE THE BEST VALUES FOR THE KINETIC PARAMETERS.

LEVEL III USES EITHER PRE-ACCLIMATED BIOMASS OR BIOMASS ACCLIMATED IN RESPIROMETRIC VESSELS.

LOGIC FLOW DIAGRAM FOR THE RESPIROMETRIC BIODEGRADATION TESTING PROTOCOL

LEVEL IV -- STUDIES TO DETERMINE THE EXTENT OF BIODEGRADATION

TESTS FOR ASSESSMENT OF ULTIMATE BIODEGRADABILITY DURING RESPIROMETRIC EXPERIMENTS

RADIOISOTOPE RESPIROMETRY
ELECTROLYTIC RESPIROMETRY COUPLED
WITH RADIOISOTOPE TECHNOLOGY

USE OF ^{14}C LABELED TOXIC SUBSTRATES. TRAP $^{14}CO_2$ AND MEASURE ^{14}C INTERMEDIATE METABOLITES DURING INCUBATION. MEASURE RADIOACTIVITY OF THE LIQUID AND GASEOUS PHASES OF THE CONTENTS IN THE RESPIROMETRIC VESSEL SYSTEM BY SCINTILLATION COUNTER TECHNIQUES.

LIQUID AND GASEOUS SAMPLES FOR THE RADIOACTIVITY ANALYSES CAN BE TAKEN INTERMITTENTLY DURING THE RESPIROMETRIC RUN BY MEANS OF SPECIALLY ADAPTED SYRINGES FROM SIDE ARMS OF THE RESPIROMETRIC VESSEL.

RESPIROMETRY COUPLED WITH
SPECIFIC SUBSTRATE ANALYSIS

ANALYSIS OF THE RESIDUAL PARENT TOXIC SUBSTRATE, INTERMEDIATE METABOLITES AND END PRODUCTS OF METABOLISM BY GC, GC/MS AND HPLC TECHNOLOGY.

LIQUID AND GASEOUS SAMPLES OF THE CULTURE SYSTEM CAN BE MANUALLY TAKEN FROM SIDE ARMS OF THE RESPIROMETRIC VESSEL WITH SPECIALLY ADAPTED SYRINGES DURING THE RESPIROMETRIC RUN.

AUTOMATED ANALYSIS OF THE CULTURE SYSTEM CAN BE PERFORMED BY WAY OF SPECIALLY DESIGNED SYSTEM COUPLING THE RESPIROMETRIC VESSELS TO THE ANALYTICAL INSTRUMENTS.

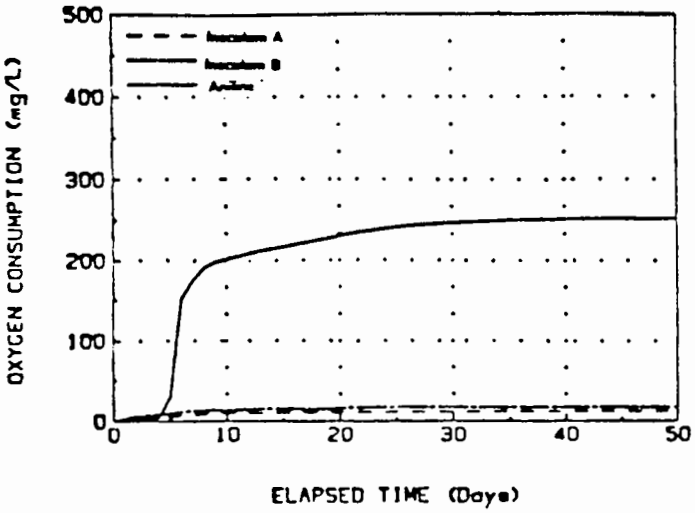


FIGURE 14 OXYGEN UPTAKE DATA ON ANILINE

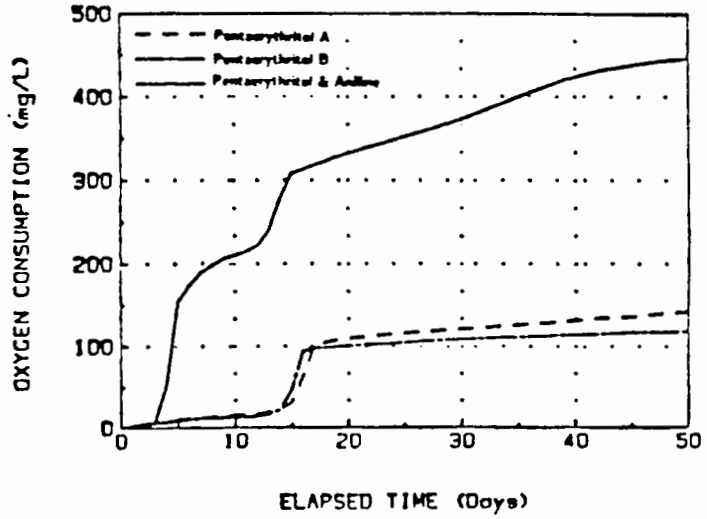


FIGURE 15 BIOLOGICAL OXYGEN UPTAKE CURVE
(Run 1 Sample No. 10-12)

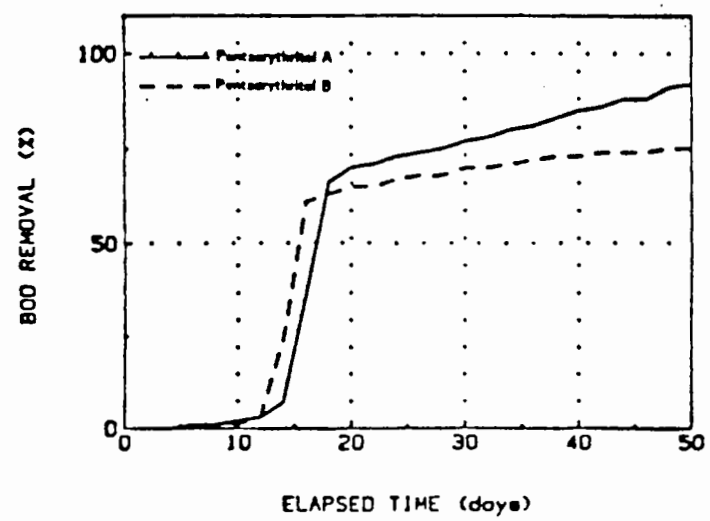


FIGURE 16 BIODEGRATION (% BOD REMOVAL) CURVE
(PENTAERYTHRITOL)

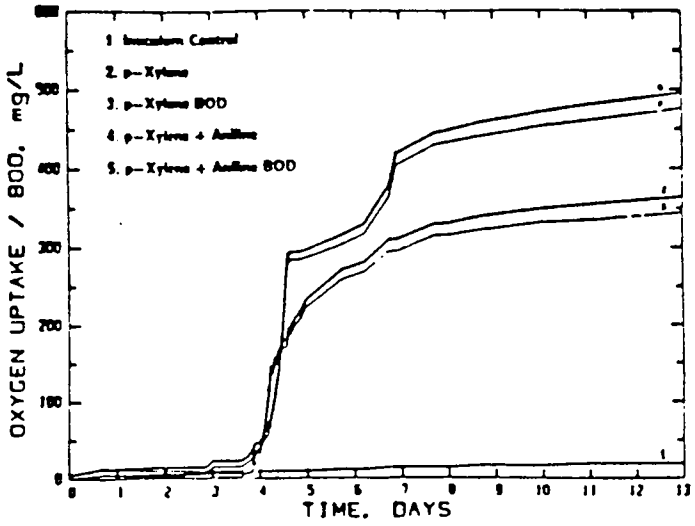


FIGURE 17 BIOCHEMICAL OXYGEN UPTAKE AND BOD DATA FOR P-XYLENE AND P-XYLENE + ANILINE

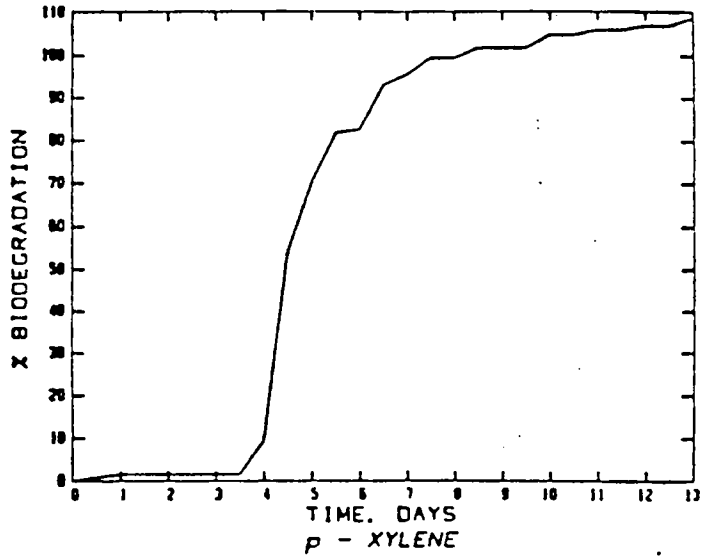


FIGURE 18 BIODEGRADATION DATA FOR P-XYLENE

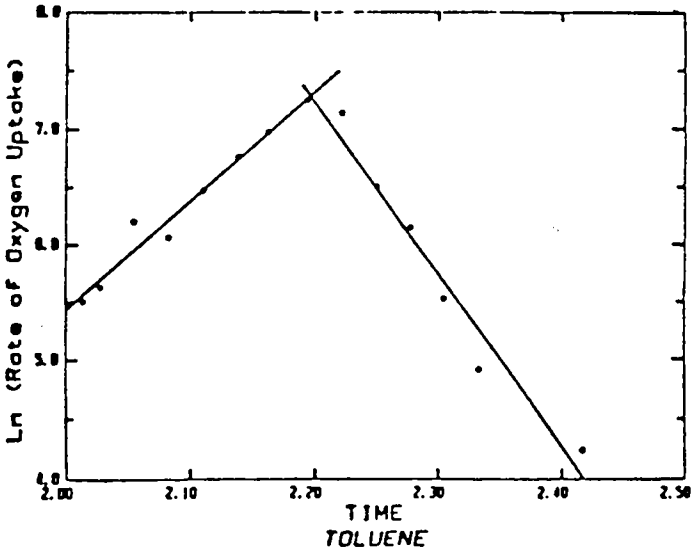


FIGURE 19 PLOT OF LOG ($\Delta Ou/\Delta t$) FOR TOLUENE

TABLE 5. SUMMARY OF RESPIROMETRIC BIODEGRADATION DATA FOR SELECTED BENZENES

PERCENT BIODEGRADATION (BASED ON % BOD REMOVAL)

Time (days)	Aniline	Benzene	Toluene	Ethylbenzene	m-Xylene	p-xylene
0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	2.58	2.11	2.78	2.27	1.35	1.58
2.0	3.43	2.11	8.81	2.27	2.14	1.58
3.0	3.94	4.15	85.11	2.99	69.68	1.58
4.0	14.95	4.97	89.04	3.78	69.68	9.4
5.0	102.0	71.5	94.34	4.29	76.68	70.38
6.0	102.0	74.22	97.92	40.25	82.58	82.68
7.0	102.0	81.85	100.0	73.91	84.67	95.58
8.0	102.0	93.37	101.18	73.78	87.03	99.49
9.0	102.0	93.89	103.48	83.94	87.79	101.76
10.0	102.0	95.45	103.48	88.86	90.12	104.7
11.0	102.0	95.45	103.48	88.86	90.15	105.78
12.0	102.0	96.13	103.48	89.65	90.97	106.7
13.0	102.0	97.46	103.48	91.00	91.80	108.6

TABLE 6. SUMMARY OF BIO-KINETIC DATA FOR SELECTED BENZENE COMPOUNDS

COMPOUNDS	ThOD for 100 mg	t_0 (days)	t_1 (days)	t_2 (days)	μ (day-1)	μ' (day-1)
Aniline						
(Experiment 1)	310	4.00	4.70	4.83	2.78	3.29
(Experiment 2)	310	4.00	4.65	4.79	3.80	8.60
Benzene	308	4.50	4.87	5.00	8.57	25.30
Ethyl benzene	317	4.00	4.21	4.83	8.33	44.80
Toluene	313	2.00	2.20	2.42	8.75	14.93
p-Xylene	317	3.90	4.22	4.83	9.94	4.50
m-Xylene	317	2.00	2.35	2.50	6.60	29.60
tert-Butyl benzene	322	4.40	5.12	5.70	1.21	1.69
sec-Butyl benzene	322	3.50	4.00	5.70	0.78	0.73
Cumene	320	2.40	2.79	3.00	2.31	2.24
Butyl benzene	322	3.30	3.92	4.56	2.42	2.42
1-Phenyl hexane	326	4.00	4.55	5.15	1.85	1.90

μ = specific growth rate constant for exponential growth phase of BOD curve.

μ' = specific growth rate constant for declining growth phase of BOD curve.

TABLE 7. SUMMARY OF MONOD KINETIC PARAMETER DATA FOR SELECTED BENZENE COMPOUNDS

COMPOUNDS	Lag Time (t_0) days	Y_g $\frac{\text{mg biomass}}{\text{mg substrate}}$	μ_m (day-1)	K_s mg/l
Aniline	4.00	0.38	6.15	6.10
Benzene	4.50	0.23	9.30	22.16
Ethyl benzene	4.00	0.23	9.00	11.81
Tolouene	2.00	0.20	9.09	56.74
p-Xylene	3.90	0.26	8.33	67.00
m-Xylene	2.00	0.14	11.50	35.61
tert-Butyl benzene	4.40	0.66	1.80	69.04
sec-Butyl benzene	3.50	0.64	2.39	22.00
Cumene	2.44	0.66	2.44	87.64
Butyl benzene	3.30	0.80	5.17	34.50
1-Phenyl hexane	4.00	0.67	2.15	35.09

μ = maximum specific growth rate.

K_s = half saturation constant; concentration of substrate at $\mu_m/2$.

Y_g = growth yield, mg biomass formed/mg substrate consumed.

TABLE 8. SUMMARY OF BIO-KINETIC DATA FOR SELECTED PHENOLIC COMPOUNDS

COMPOUNDS	ThOD for 100 mg	t_0 (days)	t_1 (days)	t_2 (days)	μ (day-1)	μ' (day-1)
Aniline	310	4.00	4.70	4.83	2.78	3.29
Phenol	238	1.00	1.56	1.67	2.75	8.26
Resorcinol	189	1.50	1.83	2.14	5.42	6.53
p-Cresol	252	1.00	1.34	1.52	4.70	5.78
o-Cresol	252	1.20	1.78	1.90	3.76	3.92
m-Cresol	252	1.44	2.04	2.40	5.17	6.15
Catechol	189	0.85	0.94	1.12	11.80	11.85
2,4-Dimethyl phenol	262	2.00	2.64	2.84	3.21	5.02

μ = specific growth rate constant for exponential growth phase of BOD curve.

μ' = specific growth rate constant for declining growth phase of BOD curve.

TABLE 9. SUMMARY OF MONOD KINETIC PARAMETER DATA FOR SELECTED PHENOLIC COMPOUNDS

COMPOUNDS	Lag Time (t_0) days	Y_g $\frac{\text{mg biomass}}{\text{mg substrate}}$	μ_m (day-1)	K_s mg/l
Aniline	4.00	0.38	6.15	6.10
Phenol	1.00	0.58	9.82	9.43
Resorcinol	1.50	0.48	12.22	6.31
p-Cresol	1.00	0.33	6.11	27.78
o-Cresol	1.20	0.41	4.10	16.41
m-Cresol	1.44	0.46	7.97	17.62
Catechol	0.85	0.49	12.80	43.87
2,4-dimethyl phenol	2.00	0.39	5.62	14.07

μ = maximum specific growth rate.

K_s = half saturation constant; concentration of substrate at $\mu_m/2$.

Y_g = growth yield, mg biomass formed/mg substrate consumed.

TABLE 10. SUMMARY OF BIO-KINETIC DATA FOR SELECTED PHTHALATE ESTER COMPOUNDS

COMPOUNDS	ThOD for 100 mg	t_0 (days)	t_1 (days)	t_2 (days)	μ (day-1)	μ' (day-1)
Aniline	310	4.00	4.70	4.83	2.78	3.29
Dimethyl phthalate	168	3.46	3.98	4.25	2.76	4.71
Diethyl phthalate	195	2.00	2.97	3.30	2.16	2.92
Dipropyl phthalate	211	2.40	2.87	3.40	2.04	2.00
Butyl benzyl phthalate	226	2.00	2.28	2.80	4.12	2.33

μ = specific growth rate constant for exponential growth phase of BOD curve.

μ' = specific growth rate constant for declining growth phase of BOD curve.

TABLE 11. SUMMARY OF MONOD KINETIC PARAMETER DATA FOR SELECTED PHTHALATE ESTER COMPOUNDS

COMPOUNDS	Lag Time (t_0) days	Y_g mg biomass / mg substrate	μ_m (day-1)	K_s mg/l
Aniline	4.00	0.38	6.15	6.10
Dimethyl phthalate	3.46	0.43	7.07	41.68
Diethyl phthalate	2.00	0.46	3.00	11.67
Dipropyl phthalate	2.40	0.48	5.78	15.81
Butyl benzyl phthalate	2.00	0.61	7.80	36.25

μ = maximum specific growth rate.

K_s = half saturation constant; concentration of substrate at $\mu_m/2$.

Y_g = growth yield, mg biomass formed/mg substrate consumed.

TABLE 12. SUMMARY OF BIO-KINETIC DATA FOR SELECTED KETONE COMPOUNDS

COMPOUNDS	ThOD for 100 mg	t_0 (days)	t_1 (days)	t_2 (days)	μ (day-1)	μ' (day-1)
Aniline	310	4.00	4.70	4.83	2.78	3.29
Acetone	221	3.70	3.99	4.18	2.45	3.98
2-Butanone	244	2.00	2.20	2.35	2.41	4.98
4-Methyl-2-pentanone	272	1.85	2.24	2.35	2.31	4.80
Isophorone	278	22.30	23.70	25.40	0.73	0.38

μ = specific growth rate constant for exponential growth phase of BOD curve.

μ' = specific growth rate constant for declining growth phase of BOD curve.

TABLE 13. SUMMARY OF MONOD KINETIC PARAMETER DATA FOR SELECTED KETONE COMPOUNDS

COMPOUNDS	Lag Time (t_0) days	Y_g mg biomass / mg substrate	μ_m (day-1)	K_s mg/l
Aniline	4.00	0.38	6.15	6.10
Acetone	3.70	0.36	4.86	9.76
2-Butanone	2.00	0.39	5.11	10.79
4-Methyl-2-pentanone	1.85	0.45	6.40	24.70
Isophorone	22.30	0.43	1.57	27.42

μ = maximum specific growth rate.

K_s = half saturation constant; concentration of substrate at $\mu_m/2$.

Y_g = growth yield, mg biomass formed/mg substrate consumed.

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16. ABSTRACT

Electrolytic respirometry involving natural sewage, sludge and soil microbiota is becoming prominent in fate studies of priority pollutant and RCRA toxic organics to generate biodegradation/inhibition kinetic data. A developed multi-level protocol is presented for determination of substrate biodegradability and toxicity, microbial acclimation to toxic substrates and first order kinetic parameters of biodegradation and for estimation of Monod kinetic parameters of toxic organic compounds, in order to correlate the extent and rate of biodegradation with a predictive model based on chemical properties and molecular structure of these compounds. Respirometric biodegradation/inhibition and biokinetic data are provided for representative RCRA alkyl, chloro- and nitro-benzenes, phenols, phthalates, ketones, and selected CERCLA leachate toxic organics. Data on the effects of the source of sludge biomass, temperature and concentration of microbial inoculum and toxic substrate on the kinetics of biodegradation are also included.

17. KEY WORDS AND DOCUMENT ANALYSIS

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kinetics	biodegradability, protocol, toxic organic compounds	

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