BIOLOGICAL INHIBITION SCREENING OF INDUSTRIAL WASTE SLUDGES AND SELECTED ORGANICS FOR LAND APPLICATION

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BIOLOGICAL INHIBITION SCREENING OF INDUSTRIAL WASTE SLUDGES AND SELECTED ORGANICS FOR LAND APPLICATION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

C.O.D.	Chemical Oxygen Demand (mg/ℓ)
D.O.	Dissolved Oxygen (mg O_2/ℓ)
D.O. Uptake	Dissolved Oxygen Uptake (mgO_2/gm/hr)
E.P.C.	Estimated Plate Count (microorganisms/mℓ)
S.P.C.	Standard Plate Count (microorganisms/mℓ)
S.R.T.	Sludge Retention Time (days)
V.S.S.	Volatile Suspended Solids (mg/ℓ)

CHAPTER I

INTRODUCTION

General

Many industrial processes produce a waste water stream which is too potent for conventional secondary treatment. Unlike a municipal waste stream, an industrial process contains hydro-carbons which are harder to biodegrade than carbohydrates. As a result, an industrial flow is directed to either biological, physical, and/or chemical pre-treatment systems and the effluent is passed on to a municipal waste water facility. The waste sludges collected from pretreatment schemes are high strength wastes with high levels of COD, BOD, heavy metals, inorganics and organic pollutants. In many cases, these industrial sludges have been classified as hazardous waste material in which case an industry is responsible for this sludge from "cradle to grave." Therefore, the sludge has to be disposed of and/or detoxi-fied in a safe and controlled process. In many areas of this coun-try, there is an abundance of marginal agricultural land which could be used for safe and engineered detoxification of the toxic sub-stances found in industrial sludges. A well engineered land appli-cation system can provide a contained environment for the disposal and degradation of the wastes. Land application is being investi-gated by the Environmental Protection Agency (EPA) as an alterna-tive in the treatment of these strong industrial wastes (1).

Land application has been tried on oily waste products, hazardous material, wood hydrolysis waste waters, and municipal sludges. Each sludge has shown an ability to be degraded by the soil system. The soil system has several advantages in that there is a wide spectrum of bacteria, and the soil provides a large surface area for the biological activity. The soil also tended to localize and degrade pollutants by physical adsorption, chemisorption, biodegradation, and volatilization.

Industrial sludges may contain inhibitory or toxic compounds to the biological processes in the soil microbiota. Many types of organic compounds and metals have been shown to be inhibitory or toxic to microorganisms in an activated sludge system (see Table I and II), but little work had been done on the inhibitory level of contaminnants on the soil microbiota.

The threshold inhibition level was defined as the lowest concentration of compound or waste that caused a reduction in the carbonaceous biological oxidation rate (2). By defining the threshold inhibition level for the soil microorganisms of a particular industrial sludge application site, a maximum loading would be determined. Designing a system with a loading rate below the threshold inhibition level would insure biodegradation of the sludge without inhibiting or having toxic effects on the microbiological population.

Objectives

The main object of this study was to determine and observe the response of soil microbial populations to the presence of industrial sludges. The study was part of a larger study funded by the E.P.A.

TABLE I

THRESHOLD CONCENTRATIONS OF INORGANIC POLLUTANTS THAT ARE INHIBITORY TO BIOLOGICAL TREATMENT PROCESSES

Pollutant	Concentration (mg/ℓ)		
	Activated Sludge	Anaerobic Digestion	Nitrification Processes
Ammonia	480	1500	
Arsenic	0.1	1.6	
Borate (Boron)	0.05-100	2	
Cadmium	10-100	0.02	
Chromium (Hexavalent)	1-10	5-50	0.25
Chromium (Trivalent)	50	50-500	
Copper	1.0	1.0-10	0.005-0.5
Cyanide	0.1-5	4	0.34
Iron	1000	5	
Lead	0.1		0.5
Magnesium		1000	50
Mercury	0.1-5.0	1365	
Nickel	1.0-2.5		0.25
Silver	5		
Sodium		3500	
Sulfate			500
Sulfide		50	
Zinc	0.08-10	5-20	0.08-0.5

Note: Concentrations shown represent influent to the unit processes in dissolved form.

Source: E. L. Stover and Don F. Kincannon, Biological Wastewater Treatment: Process Development and Concept Design. Bioenvironmental and Water Resources Engineering, School of Civil Engineering, Oklahoma State University (1982).

TABLE II.

THRESHOLD CONCENTRATIONS OF ORGANIC POLLUTANTS THAT ARE INHIBITORY TO BIOLOGICAL TREATMENT PROCESSES

Pollutant	Concentration (mg/ℓ)		
	Activated Sludge Processes	Anaerobic Digestion Processes	Nitrification Processes
Alcohols			
Allyl		100	19.5
Crotonyl		500	
Heptyl		500	
Hexyl		1000	
Octyl		200	
Propargyl		500	
Phenols			
Phenol	200		4-10
Creosol			4-16
2,4-Dinitrophenol			150
Chlorinated Hydrocarbons			
Chloroform		10-16	
Carbon Tetrachloride		10-20	
Methylene Chloride		100-500	
1,2-Dichloroethane		1	
Dichlorophen*		1	
Hexachlorocyclohexane		48	
Pentachlorophenol*		0.4	
Tetrachloroethylene		20	
1,1,1-Trichloroethane		1	
Trichloroethylene		20	
Trichlorofluoromethane*		0.7	
Trichlorotrifluoroethane (Freon)		5	
Allyl Chloride			180
Dichlorophen			50
Organic Nitrogen Compounds			
Acrylonitrile		5	
Thiourea		0.075	
Thioacetamid		0.14	
Analine		0.65	
Trinitrotoluene (TNT)	20-25		
EDTA	25	300	
Pyridine		100	

(continued)

TABLE II (continued)

Pollutant	Concentration mg/ℓ) Activated Sludge Processes	Anaerobic Digestion Processes	Nitrification Processes
Surfactants			
Nacconol	200		
Ceepryn	100		
Miscellaneous Organic Compounds			
Benzidine	500	5	
Thiosemicarbazide			0.18
Methyl isothiocyanate			0.8
Allyl isothyiocyanate			1.9
Dithio-oxamide			1.1
Potassium thiocyanate			300
Sodium methyl dithiocarbamate			0.9
Sodium dimethyl dithiocarbamate			13.6
Dimethyl ammonium dimethyl dithiocarbamate			19.3
Sodium cyclopentamethylene dithiocarbamate			23
Piperidinium cyclopenta-methylene dithiocarbamate			57
Methyl thiuronium sulphate			6.5
Benzyl thiuronium chloride			49
Tetramethyl thiuram momosulphide			50
Tetramethyl thiuram disulphide			30
Diallyl ether			100
Dimethylparanitrosoaniline			7.7
Guanidine carbonate			19
Skatole			16.5
Strychnine hydrochloride			175
2 chloro-6 trichloro-methyl-pyridine			100
Ethyl urethane			250
Hydrazine			58
Methylene blue			100
Carbon disulphide			35
Acetone			840
8-hydroxyquinoline			73
Streptomycin			400

TABLE II (continued)

Note: Concentrations shown represent influent to the unit process.

Where indicated with a *, the concentration represents total plant influent.

Source: E. L. Stover and Don F. Kincannon, Biological Wastewater Treatment: Process Development and Concept Design. Bioenvironmental and Water Resources Engineering, School of Civil Engineering, Oklahoma State University (1982).

and conducted at the laboratories of Oklahoma State University entitled "Kinetics of Microbial Degradation of Hazardous Waste by Land Treatment," under the direction of Dr. D. F. Kincannon, Dr. E. L. Stover, and Dr. V. Mast (1). A testing procedure is presented that would determine the threshold inhibition level of industrial sludges and priority pollutants. The procedure involves selection of growth medium, cultivation of soil microorganism, and a testing procedure to define the threshold inhibition point. The test procedure had the advantages of being inexpensive and the evaluation of the inhibition level can be completed in a one-month period as compared to the cost of Gas Chromatography (G.C.) analysis and time period required for actual soil column studies.

Thus, the major objective was to define the threshold inhibition point for industrial waste sludges and selected priority pollutants and directly relate the inhibition level to loading rates of industrial sludges on a land system.

CHAPTER II

LITERATURE REVIEW

Land Application

Land application as a treatment process has been used for many years with the major emphasis on disposal rather than treatment. As early as the 1900's, municipal overland flow systems have been used for treatment of domestic wastes (3). Most of the earlier use of land treatment schemes were designed to treat dewatered sludges and anaerobically digested sludges from municipal waste water treatment (4). Much of the work involved crop production and land reclamation in which loading rates were set to utilize the nitrogen and phosphorus in the wastewater.

The use of land treatment for industrial wastes was a more recent development. There are many wastes amenable to land cultivation. They include food and kindred products, textile finishing, wood preservings, paper and allied products, organic fibers, pharmaceuticals, soap and detergents, organic chemicals, petroleum refining, and leather tanning (5). The suitability of a sludge for land treatment depends on a number of characteristics including, pH, BOD, concentration of chemical elements, soluble salts, and hazardous chemicals, flammability, and volatility (6) (7) (8).

The application of industrial sludges has been studied by many

researchers with the emphasis on oil waste sludges and trace organics. The studies have shown good degradation of the chemical constitutents by biodegradation, sorption, and volatilization. In a study by Bouwer et al. (9), degradation of trace organics occurred and the infiltration rates did not affect the degree of organic removal. Jenkins et al. (10) showed that the removal of toluene and chloroform followed first order kinetics and reported a removal rate of 95.7-100%. They theorized that volatilization was the most likely method of removal. Raymond and Hudson (11) used field plots and applied an oil waste sludge. They found average reductions of 48.5-90% of oil and that all classes of aromatic and paraffinic compounds were degraded. They also showed no movement of the oil in the soil caused by water. Francke and Clark (12) used a vacuum pump oil and reported biological assimilation of the oil by the soil organisms and also showed no movement of oil from water that was passed through the system. These studies and many others not mentioned here have shown that land aplication is a viable alternative in the degradation and/or detoxification of an industrial waste sludge.

Yang and Choa (13) studied the reduction of oil in a soil column and compared the results of sterilized soil versus non-sterilized soil. They reported that the non-sterilized soil had increased reduction rates and concluded that the soil microbiota did have an effect on the degradation of the crude oil, but they pointed out that volatilization is probably a more important mechanism of degradation.

The use of a soil system for industrial waste water sludge was feasible and, if properly engineered, could be a safe method for

disposal and degradation of hazardous compounds.

Though volatilization seemed to be the most important degradation mechanism, the soil microfauna did seem to have a significant role in the degradation process. The biological screening procedure described herein was used to determine the extent of inhibition and/or toxicity of pollutants to the soil organisms.

Inhibition Screening

A variety of abnormalities have occurred in the microorganism from the effects of toxic pollutants. Phenolic derivatives disrupted cell membranes and interfered with oxidase enzymes. Alcohols inhibited respiration and phosphorylation. Large concentrations of hydrogen ions would displace essential ionic species from the sorptive sites on the cell as well as damage important cell structures (14).

There were several publications on the toxic effects of specific compounds. Davis et al. (15) summarized physical and chemical data on benzotriazoles and concluded that health and environmental problems can be caused by benzotriazoles. Caldwell (16) reviewed the response of planktonic microflora from high concentrations of pollutants in the literature since 1970 and discussed the problems comparing the results with the possible effects of natural systems. Little (17) reviewed the current literature of 1977 of bioassay methodology in which the many procedures of conducting toxicity testing were discussed. He noted that there were many problems that occurred in bioassay procedures which included complexity of the testing methods, simulation of the real world environment in bioassay, and the tendency of the natural environment organisms to be exposed

to a wide spectrum of pollutants.

In a study conducted by Huddleston and Meyers (18), it was found that oil waste contained large amounts of heavy metals that could possibly cause inhibition. They found high concentrations of lead, chromium, copper, and zinc, and that the combination of these heavy metals could cause inhibition on the soil microorganisms.

Several workers have used bacterial cultures and studied their response to different environmental stress conditions. Anderson and Domsch (19) studied the response of different additions of glucose and measured the respiration rates. The study showed that a small inoculum of cells can produce a measurable change in respiration rates by glucose. The microbiological inhibition testing procedure used in this study was originally described by Marks (2) to determine the effects of specific compounds or wastewaters on biological treatment processes. The test procedure consisted of monitoring the oxidation rate of a biological seed at various dilutions of wastewater in BOD bottles. The threshold inhibition level was defined and the response of acclimated versus non-acclimated seed material is discussed. Stover (14) (20) (21), and Kincannon et al. (22) have refined the method and have extrapolated the system for use in nitrification studies and land application. Kincannon et al. (22) described the first results of the inhibition screening procedure on industrial sludges. The study explained much of the methodology used in this thesis. The material contained in this thesis was the refinement of the inhibition screening testing for particular organics and industrial waste sludges.

CHAPTER III

MATERIALS AND METHODS

Analytical Methods

Volatile Suspended Solids

The concentration of Volatile Suspended Solids (V.S.S.) was determined by the glass fiber filter technique (glass fiber filters) manufactured by the Whatman Company (2.1 cm diameter, No. 934-AH). The filters were placed in Coors crucibles. The procedure of the test is described in Standard Methods (23).

Chemical Oxygen Demand

Chemical Oxygen Demand (C.O.D.) was determined by the Reactor Digestion Method as described by Hach Company, Loveland, Colorado (24). The test chemically oxidized the samples using sulfuric acid and a silver sulfate catalyst. Potassium dichromate was the oxidizing agent. The samples were heated for two hours at 150°C and then cooled to room temperature. The transmittance was measured on a D/2 Hach Spectrophotometer, and the C.O.D. mg/ℓ was measured by comparison with a standard curve.

Bacteriological Techniques

Sterile Techniques. All glassware and equipment used in contact with bacterial cultures were washed and sterilized for twenty minutes at 15 psi and 121°C in an autoclave oven.

Plate Counts. The enumeration and differentiation of bacteria was accomplished using the spread plate surface counting technique as described in Standard Methods (23).

Difco nutrient agar with 50% Bacto agar was used in all cases. After proper dilutions of samples were prepared, 0.1 and 1.0 mℓ of sample was placed on the agar surface and evenly spread using a turntable and a sterilized glass rod. In all cases, duplicate plates were prepared. These were counted using Quebes Colony Counter, after being incubated at 25°C for 48 hours.

Dissolved Oxygen and Dissolved Oxygen Uptake

Dissolved oxygen and DO uptake were determined using dissolved oxygen electrode model 97-08 by Orion Research, Cambridge, Massachusetts. The measurements were read on a Beckman Expandomatic SS-2. Dissolved oxygen uptake was measured at one minute intervals and the DO depletion was determined per minute.

$$\frac{\text{DO depletion/min} \times 60 \text{ min/hr} \times 1000 \text{ mg/g}}{\text{VSS mg/}\ell} = \frac{O_2 \text{ uptake}}{\text{mg } O_2 \text{ gm/hr}} \quad (3.1)$$

pH

Hydrogen ion concentration was determined by use of an Orion research digital ion-analyzer/501. The meter was standardized before each use.

Dry Weight

Dry weight was determined using Coors crucibles that were cleaned and dried at 600°C for 15 minutes, then the crucibles were cooled in a desicator. One milliliter of sample was placed in the crucible and weighed. The samples were dried at 103°C for two hours and cooled in a desicator. The samples were weighed and the moisture percentage was determined as follows

$$\frac{\text{wet weight - dry weight}}{\text{wet weight}} \; 100 = \% \text{ moisture} \qquad (3.2)$$

Development of Biodegradation

Screening Test

The Biodegradability and Inhibition Screening methodology was developed in a series of steps.

1. Grew soil organisms under favorable environmental conditions which produced an in-situ heterogeneous population for the biodegradability testing.
2. Conducted the biodegradation screening tests.
3. Drew conclusions from testing procedure.
4. If biodegradation did not occur or inhibition was

observed, the test organisms were acclimated or adapted to the industrial waste under investigation.

5. Biodegradation screening tests were conducted with the acclimated seed.
6. Conclusion drawn from the testing procedure.

Figure 1 outlines the procedure and shows the steps that were taken to determine the threshold inhbition level of the industrial sludge.

Cultivation of Organisms

Selection of Medium

The first step of the inhibition procedure was to develop an easily biodegradable feed source that would provide a fast growing, heterogeneous population of soil microorganism. The population had to be large enough to conduct a series of tests and the biological population had to develop quickly so that the test could be conducted in a one-month period. The organisms were cultured from soil used in the EPA Land Application Studies conducted at Oklahoma State University.

The three soils chosen were found at various sites in Payne County, and represented the extremes of the textural classification triangle (Figure 2). The soils studied were Port Series (clay), Mashin Series (silty loam), and Derby Series (sandy loam). Table III describes the soil used in the study. The locations are provided, along with the soil classification, moisture content, and microbial count per gram of soil.

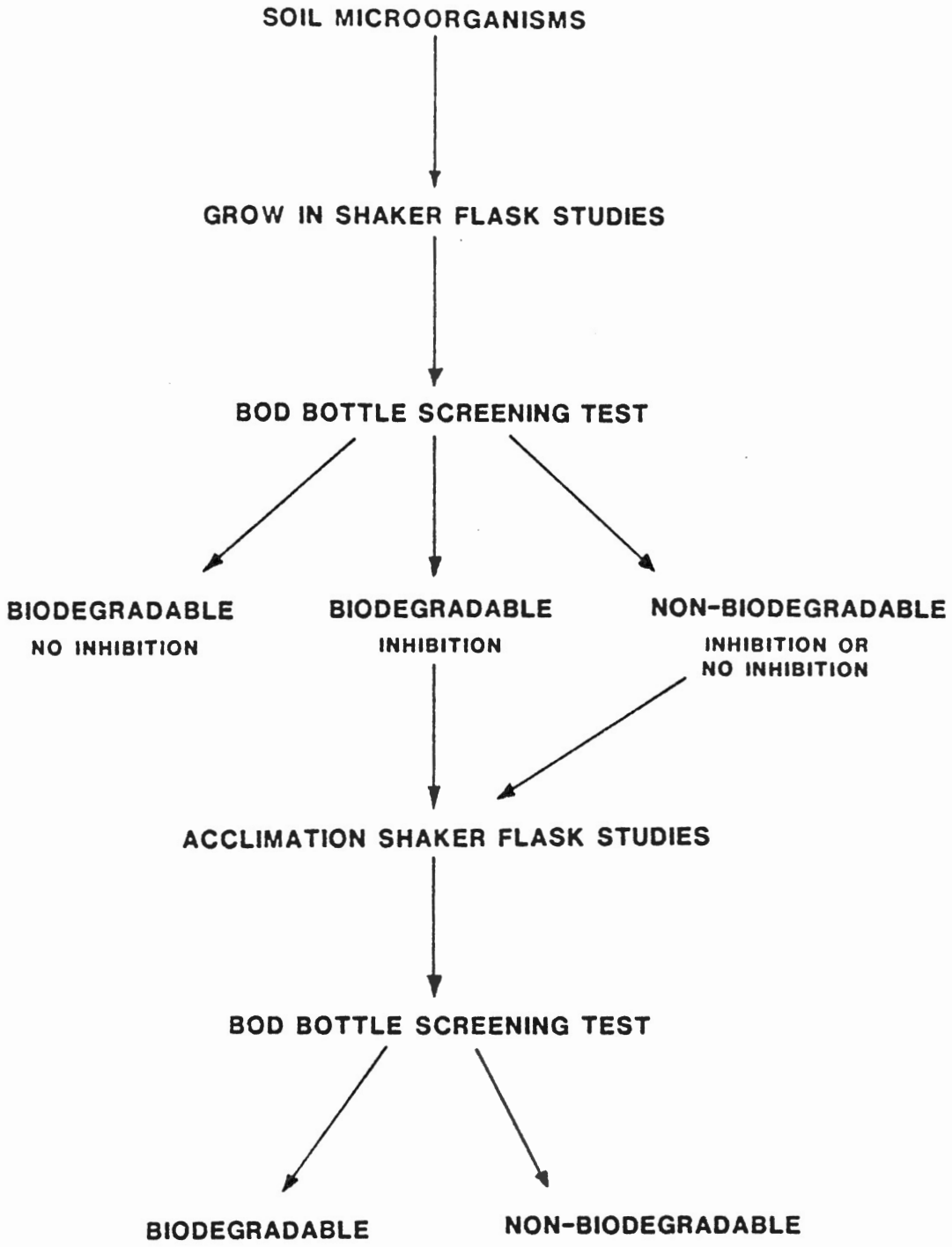
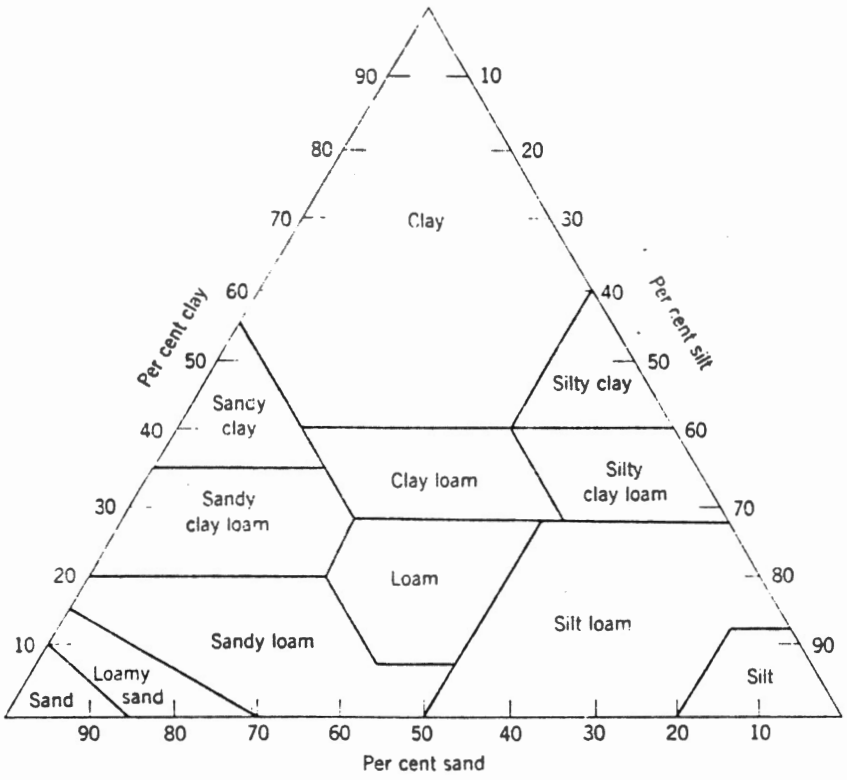


Figure 1. Biodegradability and Inhibition Screening Method



Note: Soils used in the study were
1. Port series
2. Mashin series
3. Derby series

Figure 2. Textural Classification Triangle of the Soils Used in this Study

TABLE III

CHARACTERISTICS OF SOILS

Series	Location	Moisture Content (%)			% Sand	% Silt	% Clay	M pe
		Minimum	Maximum	Average				
Derby	Section 14 Township 17, North	17	8	13	52	40	8	0.
Mashin	Section 10 Township 19, North	26	18	22.5	19	54	27	1.
Port	Section 4 Township 19, North	25	21	23	9	79	15	1.

Four growth media were tested and compared by Standard Plate Counts and Volatile Suspended Solids to determine the rate of growth. Plating and microscopic examinations were used to determine the diversity of the cultured organisms.

The growth media were also judged on the convenience and ability to maintain the purity of the feed medium. The media tested were

1. Nutrient Agar
2. Glucose and Nutrient Salts
3. Soil extract media
4. A synthetic medium designed for aerobic activated sludge lab scale reactors.

The Nutrient Agar (Bacto Nutrient Agar, Difco Laboratories, Detroit, Michigan) was a general purpose medium for the cultivation of microorganism. It contained beef extract, peptone and agar. The nutrient medium was tested because of its ease of preparation and its ability to culture a wide range of microorganisms.

The glucose and selected salts consisted of 80 grams of glucose, 10 grams NH_4Cl, and 2 grams KPO_4 dissolved in one liter of distilled water. This medium was tested because of the availability of the chemicals. The glucose provided the carbon source and added to this were selected essential nutrients. Upon further investigation, the list of essential nutrients was increased until medium D was produced.

The soil extract medium was adapted with refinements for Daniel E. James, Culturing Algae (25), in which the soil was placed in distilled water and was agitated. Then, the mixture was allowed to settle. The supernatant was collected and filtered (0.45 millipore filters) and autoclaved (20 minutes, 18 pso, 250°C). The medium

was tested because of the diversity the medium produced. The soil organism would already be acclimated to the nutrients in the soil system. The soil extract medium would contain natural nutrients of the particular soil system. The medium would hopefully provide a fast growing population that would closely resemble the diversity of the original soil system.

The synthetic medium was developed for biological activated sludge lab scale units. The medium contained a number of carbon sources and salts to provide an easily biodegraded food source. Table IV describes the synthetic medium.

TABLE IV

MICROBIAL GROWTH MEDIA

Constituent	Quantity grams/liter
Glucose	80
Glutamic acid	5
Glycerol	5
Yeast extract	5
Ammonium chloride	10
Manganese sulfate	2
Potassium phosphate	2
Sulfanillic acid	4
Sodium thiosulfate	4

Three stock solutions were prepared. One contained the carbon sources (glucose, glutamic acid, glycerol). Two contained yeast

extract which provided a protein source for the organisms, and three contained the essential nutrients for growth. Equal volumes of the three stock solutions were added to distilled water to give the desired COD of the solution. Approximately 1 mℓ of stock solution in 100 mℓs of distilled water produced a COD of 2000 mg/ℓ. The pH of the feed was adjusted by use of a fifty percent solution of NaOH. The medium was easy to prepare and quality was maintained by refrigeration. The media were judged using the Shaker Flask Cultivation technique.

Shaker Flask Cultivation

To run the series of inhibition screening studies, a large amount of soil organisms were needed. The soil organisms had to be cultured into larger populations because

1. the amount of organisms in a gram of soil was too small to produce the desired D.O. depletion in the screening test.
2. there was no quick and direct method to measure the amount of biomass in the soil.

Therefore, a method had to be developed to produce large populations of cells and to maintain the population for the length of the study. The following procedure is presented.

One gram of soil was placed in 250 mℓ Erlenmeyer flasks, along with 75 mℓs of a 2000 mg/ℓ COD food source. The flask was closed with cotton and placed on a shaker table and ran at low speed (approximately 60 oscillations per minute). After 24 hours, plate counts and Volatile Suspended Solids (VSS) were taken to measure the growth

of the organisms. At 48 hours, 25 mℓs of the culture were transferred to another Erlenmeyer flask which contained 50 mℓs of growth medium (2000 mg/ℓ COD).

Again, plate counts and V.S.S. were conducted 24 hours after the transfers and the culture was again transferred after 48 hours. The time period of transfers of 48 hours was chosen to allow time for plate counts and V.S.S. to be conducted on the off days. Four transfer periods were chosen because this produced adequate growth and removed almost all of the non-biological matter from the cultures. The growth medium was chosen to produce the fastest and largest heterogeneous growth of the soil organisms.

The soil cultures were then transferred to a batch system which contained the growth medium (Figure 3). It was important for the shaker studies to obtain a large population level so that the batch unit could be started. It was also necessary to maintain a desired V.S.S. concentration in the batch reactors.

To produce the desired V.S.S. concentration in the batch reactors, centrifugation was used to initially increase solids concentration and a Sludge Retention Time (S.R.T.) had to be chosen to maintain the desired level.

To initially increase the solids concentration, a large volume of the batch unit was centrifuged at 3000 r.p.m. for 10 minutes. The supernatant was poured off and the concentrated solids were placed back in the reactor. The original level in the batch unit was obtained by adding an appropriate concentration of growth media to give an overall concentration of 2000 mg/ℓ COD within the unit.

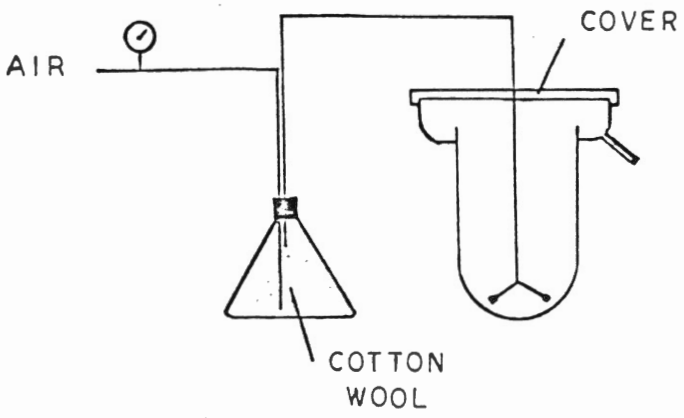


Figure 3. Schematic of the Batch Unit

This procedure only had to be done once or twice to bring the V.S.S. concentration to the desired level. To maintain the desired solids concentration in the reactor, the amount of sludge wastage had to be determined. After some trial and error methods, it was found that running the system on an eight day sludge age maintained the V.S.S. concentration at about 2000 mg/ℓ. The actual procedure was to waste one quarter of the reactor every other day and refill with an appropriate concentration of food source to maintain a 2000 mg/ℓ C.O.D. in the entire reactor. The wasting and feeding every other day allowed tests to be run on the off days and residual C.O.D. concentrations in the batch system after 24 hours would have little effect on the inhibition studies.

The method of preparation of the soil cultures produced a large enough population so that, 1. a series of inhibition screening tests could be run, 2. large populations were maintained over the period of the study and, 3. the microbial population was measured by V.S.S. measurements. The transfer of the shaker flask cultures lasted about eight to ten days, and centrifugation took two to four additional days. Therefore, a large enough population for the screening test was cultivated in a two-week period and could be maintained over the life of the study. The shaker study was also used to evaluate the growth medium that was to be used in the study. The four selected media were used to cultivate the soil organisms. The media was then judged by the growth of organisms measured by V.S.S. concentration and S.P.C. concentrations. The results of the media screening shall be discussed later.

Inhibition Test Procedure

The biodegradability screening procedure of industrial waste sludges for land treatment was a modification of the inhibition bioassay screening procedure employing oxygen uptake as a surrogate parameter of the biodegradation of the industrial sludges, as described by Stover (14) (21). The test used an inoculum of the biological seed that had been cultivated from the particular soil used in the study. The procedure consisted of adding different dilutions of the industrial waste sludge to a series of BOD bottles and inoculating the bottles with a small amount of the cultured microorganisms. A small fraction of glucose was added to the bottles. The glucose was added in such a quantity as to reduce the dissolved oxygen level in the BOD bottle by fifty percent in a three-day test period. All the bottles were filled with standard BOD dilution water as described in Standard Methods (23) and the oxygen depletion was measured with time.

The procedure demanded strict analytical cleaning methods as described in Standard Methods (23). Contamination of BOD bottles by outside sources could interfere with the test. The BOD dilution water was aerated for 45 minutes and then left standing for 30 minutes. The Dissolved Oxygen (DO) was then checked and had to be eight mg O_2/l or above. A 300 mg/ℓ glucose stock solution was prepared with distilled water that was to be added to the test bottles.

As a preliminary step to the screening procedures, a series of BOD bottles were set up with dilution water and six mℓs of the 300 mg/ℓ glucose solution. Various amounts of the cultured microorganisms were added to the test bottles. The V.S.S. of the batch system was

determined from which the inoculum was taken. The bottles were incubated at 20°C for three days and the D.O. depletion was measured. The test determined the necessary seed concentration that would deplete the D.O. in the BOD bottle by fifty percent. The seed inoculum ranged from one to five mls. By conducting V.S.S., measurements on the batch system to be used the day of the test, the amount of seed material could be determined that would give the desired D.O. depletion in the BOD bottle.

Once the appropriate amount of seed material was determined, a series of dilution bottles were set up with increasing concentrations of the waste sludge. Six mls. of 300 mg/ℓ glucose solution was added to each bottle and then filled to just a few mls. short of the capacity of the bottles. Then, the biological seed was added (Table VII). Adding the seed in such a manner prevented any seed material from being exposed to higher concentrations of waste sludge than would be evaluated in each sample bottle. The bottles were then topped off with dilution water and the D.O. level was measured. The initial D.O. levels had to approximate one another so that the individual bottles could be compared. If some of the bottles with higher sludge concentrations did not approach the desired D.O. level, the bottles were aerated by connecting the filled bottles to an empty BOD bottle with a piece of PVC pipe and shaking the two bottles to bring the D.O. level close to the desired level. The bottles were then incubated for three days at 20°C in a dark incubator. At the end of the three-day period, the D.O. was determined. The tests were also run with blanks to test the dilution water and two seed correction bottles to determine the activity of the seed.

TABLE V

PREPARATION OF BOD BOTTLES FOR INHIBITION SCREENING PROCEDURE

Bottle number	Sample Volume (mls)	Dry Weight* (mg)	Biological Inoculum** (mls)	Glucose Solution (mls)	Dilution Water (mls)
1	0.0	--	5	6	289
2	0.01	--	5	6	288.99
3	0.1	--	5	6	288.9
4	1.0	--	5	6	280
5	3.0	--	5	6	286
6	6.0	--	5	6	283
7	10	--	5	6	279
8	30	--	5	6	259
9	60	--	5	6	229
10	100	--	5	6	189
11	200	--	5	6	89
12	250	--	5	6	39

* Dry weight depends on the percentage of water in the waste sludge.

**Biological seed depends on the V.S.S. concentration of the batch unit tested.

Of the three industrial sludges used in this study, two of them showed appreciable amounts of oxygen demand exerted by the waste itself. This could have been caused for a number of reasons.

1. oxidation of sulfur compounds
2. oxidation of nitrogen compounds
3. biological growth in the sludge samples
4. a combination of all three

Therefore, a method had to be developed to deal with this oxygen demand. This posed an interesting problem, for in an enclosed test as in the bottle test, the amount of oxygen was very small and an oxygen demand exerted by the waste itself could easily deplete the D.O. to zero.

The procedure selected was to run duplicate bottles which were not inoculated with seed material. Therefore, the oxygen demand exerted by the waste itself could be subtracted from the D.O. depletion observed in the test bottle with the seed inoculum. At times, the oxygen demand exerted by the waste itself depleted the D.O. of the bottle in the matter of a few hours, in which case the bottles were measured two times daily, and if necessary, re-aerated as previously described. Therefore, an accumulated D.O. value was reported and again subtracted from the inoculated bottles. The difference represents the D.O. used for respiration of the waste material.

Pure Compound Studies

The inhibition screening procedure was also used to study the toxic effects of particular organic compounds. The compounds studied

were toluene, benzene, phenol, and cresol. The screening procedure was set up as previously mentioned, but a strong stock solution of the compound being studied was used as the pollutant. The stock solution was made up with aerated BOD dilution water to save time in re-aerating the samples. The concentrations of the stock solutions were restricted by the solubilities of the organics and were chosen to hopefully cause inhibition at low dilutions and showed no inhibition at high dilutions. At times, this was a trial and error situation until the proper range of the organic concentration was found. The tests were run as previously described.

Application of Screening Procedure

The screening results were graphically represented by plotting dissolved oxygen versus log of the percentage by weight. Figure 4 explains the indications of biodegradability and inhibition of the pollutant in question. Curve A represents the case where no inhibition was observed. Curve B represents the case where the material was biodegradable at lower loadings but becomes inhibitory at a higher loading. In this example, the threshold inhibition level was 1.0 percent by weight. Curve B also shows the effect of chemical oxygen demand. Curve C represents a case where the material was not inhibitory at lower loadings and not biodegradable at these lower loadings. However, as the loading increased, the material became inhibitory, and in this example, the threshold inhibition level was 0.2 percent by weight.

The percent by weight axis was developed by Kincannon et al. (22) to relate the loading of the pollutant with the soil system.

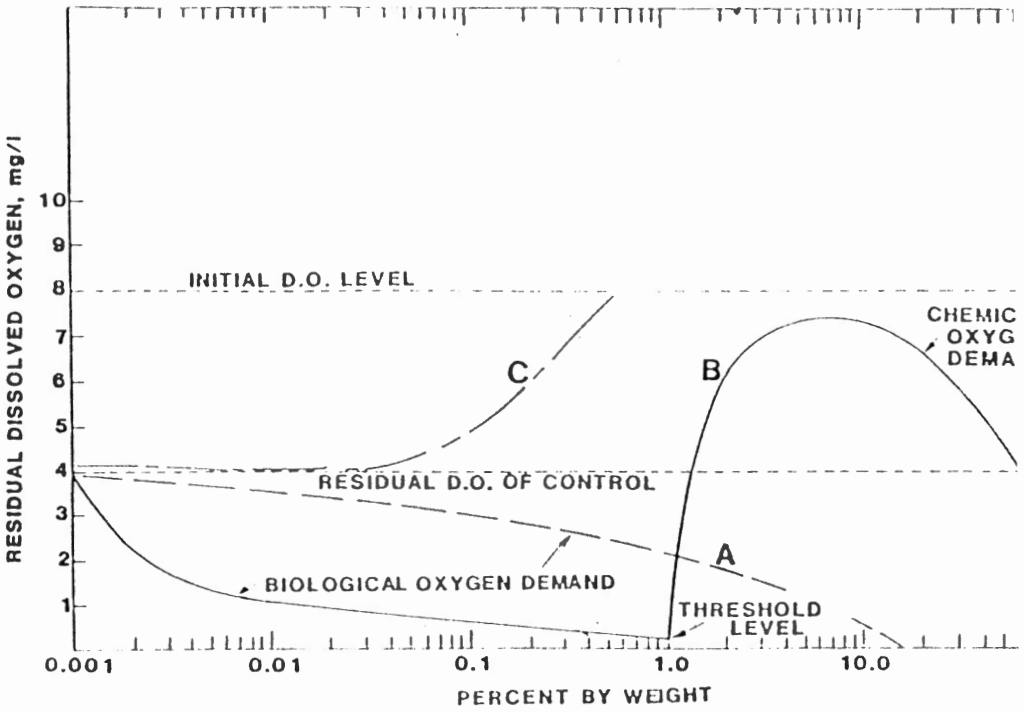


Figure 4. Example of Inhibition Screening Data

The percent by weight calculation was a ratio of the milligrams of pollutant to the milligrams of water in the BOD bottle.

Pure Compound:

$$\text{Percent by Weight} = \frac{\text{Amount of stock solution added (mls)} \times \text{Concentration of stock (mg/ml)}}{300 \text{ gms} \times 1000 \text{ mg/gm}} \; 100 \qquad (3.3)$$

Sludges:

$$\text{Percent by Weight} = \frac{\text{Amount of sludge added (mg)} \times \text{Percentage dry weight \%}}{300 \text{ gms} \times 1000 \text{ mg/gm}} \; 100 \qquad (3.4)$$

The method assumed that 300 mls of dilution water (300 gms by weight) approximates 300 grams of soil. This assumption has not been tested and awaits further study.

Use of the procedure determined

1. if the waste was biodegradable, and if so, up to what concentrations
2. if the waste affected the microorganisms and their ability to degrade an easily biodegradable carbon source (glucose) in the presence of the pollutant.
3. the maximum loading rate of a waste added to a system that would not cause inhibitory or toxic effects.

Acclimation Studies

The test procedure involved an acclimation step to determine if the microorganisms would react differently if grown in the presence of wastes. The cells contained genetic apparatus that determined their ability to cope with a particular environmental change. The crucial steps involved production of enzymes that

1. prepared the compound for entry into the common pathways for the formation of A.T.P. and carbon skeletons for making cellular components
2. prepared the compound for transport through the cell wall and into the cell.

The cell can acclimatize by inducing the needed enzyme or utilizing multispecific enzymes that could act on a variety of similar substrates. There are many organic compounds, when not available as a carbon source, that will require a significant acclimation or adaptive period before biological utilization of the compound can occur. Therefore, a lag period can develop that can affect the screening testing (26).

The acclimation procedure involved cultivating the microorganisms with the presence of the organic pollutants or waste sludges. The non-acclimated inhibition screening results for the particular pollutant are used to determine the rate of gradual increase in the concentration of the pollutant. Figure 4, Curve B, showed the traditional D.O. demand curve with the threshold level occurring at 1.0 percent by weight. Below this loading rate the pollutant would not cause inhibition or toxicity. Therefore, the system was acclimatized below this threshold loading.

The soil organisms were obtained from the batch units set up for the non-acclimated testing. A large inoculum was taken and placed in a Batch reactor and allowed to stabilize on the food sources. After V.S.S. approached 1000 mg/ℓ, the acclimation procedure was started. A concentration of the organic compound or waste sludge was added to the system at every feeding period. Volatile Suspended Solids and Dissolved Oxygen uptake was taken 24 hours after the feeding period to observe the growth of the system and the ability of the organisms to utilize the substrate. The systems were, at first, acclimatized at a concentration of pollutant equal to 10 percent of the threshold inhibition level. The loading would then be increased at each feeding period. When the concentration of pollutant reached 40 percent of the inhibition level, the system was maintained at these conditions until V.S.S. were suitable to run the inhibition test. The inhibition screening test was performed using the method discussed earlier. If the results showed no increase in the threshold inhibition level, the acclimation procedure continued until the concentration of the pollutant reached the threshold point. Then, the test was repeated again. The results of the acclimated versus non-acclimated seed could be compared so that the design engineer would have an idea of how the soil system would act after continuous applications of the waste sludge.

CHAPTER IV

RESULTS

Preparation of Soil Organisms

Selection of Medium

The first objective of the study was to develop a growth medium that would produce a fast growing heterogeneous population of microorganisms. This growth medium was then used to culture soil organisms into large populations. The soil itself could not be used in the inhibition screening for two reasons.

1. The soil contained too few microorganisms for seed source in the BOD bottles.
2. It was difficult and time-consuming to quantify the biomass in one gram of soil by S.P.C.

Therefore, there was an advantage to producing a viable biomass that could be measured using V.S.S. measurements.

The screening of the selected growth media was conducted using the Port Series Soil taken from the flood plain of Stillwater Creek in Payne County, Oklahoma . The soil was classified as a silty loam (Table VI). The soil was used for the media screening studies because it showed the largest microbial count, and the soil characterization (Figure 2) showed it to be located at the extreme of the soil textural triangle (79% silt).

The results of the media screening were shown in Table VI. The V.S.S. concentration in all four selected media were higher at 24 hours than at 72 hours. This probably occurred because of the amount of organic matter in the soil which was added to the shaker flasks. As described earlier, one gram of soil was used in each shaker flask. The organic matter could account for the high V.S.S. values in the first testing period. After transferring the cultures to fesh media, the amount of the extraneous organic matter in V.S.S. measurements became negligible as shown in the decrease in V.S.S. concentrations at 72 hours. From 72 hours onward, the V.S.S. concentrations increased for all four of the media.

Standard Plate Counts were reported in two means. Either as S.P.C. or as E.P.C. (Estimated Plate Counts). The difference was described in Standard Methods (23) and referred to the amount of colonies counted on the plates. If the number of colonies on the plates was in the range of 30-300, the count was classified as a Standard Plate Count (S.P.C.). If the count fell out of this range, it was classified as an Estimated Plate Count (E.P.C.). The classification system described the relative accuracy of the plate counts.

The S.P.C. showed a dramatic increase from 24 hours to 72 hours in all media, unlike the V.S.S. measurements. It appeared that the S.P.C. was not affected by the organic matter of the soil which was to be expected. Also, all of the selected media continued the trend in growth throughout the study.

Though there was no direct correlation between V.S.S. and S.P.C. measurements, general trends regarding growth were shown. Growth of the microorganisms was quick and sizable. Large populations were

TABLE VI

MEDIA SELECTION STUDY WITH THE USE OF SILT (PORT SERIES) SOIL

Growth Medium	Time	Standard Plate Counts (microorganisms/mℓ)	Volatile Suspended Solids (mg/ℓ)
A. Nutrient Agar	24	5.45×10^4 SPC	400
	72	2.38×10^7 SPC	240
	120	6.39×10^8 EPC	640
	168	7.97×10^8 EPC	730
B. Glucose with Selected Salts	24	5.3×10^8 SPC	520
	72	4.7×10^6 SPC	200
	120	6.81×10^7 EPC	350
	168	1.21×10^8 SPC	405
C. Soil Extract Medium	24	6.2×10^4 EPC	450
	72	7.3×10^4 EPC	310
	120	6.51×10^5 SPC	375
	168	2.42×10^6 SPC	390
D. Growth Medium from Lab Studies	24	4.01×10^4 SPC	470
	72	5.96×10^6 SPC	210
	120	7.20×10^8 EPC	680
	168	5.78×10^9 EPC	890

produced with the use of all of the media.

The four media were described previously. They were: A. nutrient agar, B. glucose and selected nutrient salts, C. soil extract medium, and D. synthetic growth medium. The four media were evaluated, and the synthetic growth medium was selected for use in the balance of the study.

Nutrient Agar Medium. The nutrient agar medium produced a rapid growth as shown in the increase in V.S.S. concentrations from 240 mg/ℓ to 730 mg/ℓ, and a four order of magnitude increase in S.P.C. From selective plating and microscopic examination, the medium had a very diverse growth; however, problems arose from the use of the agar. At high C.O.D. concentrations (4000 mg/ℓ and above), the solution became very viscous and was hard to work for use in testing. It appeared to hamper filtering and, therefore, affected suspended solids measurements.

Glucose and Selected Salts Medium. This medium gave a viable growth, but was low when compared to the others. After 168 hours, the medium produced a biomass of 405 mg/ℓ V.S.S. and a count of 1.2×10^8 micro/mℓ S.P.C. as compared to a V.S.S. of 730 mg/ℓ and a S.P.C. of 7.97×10^8 micro/mℓ for medium A (nutrient agar). The glucose medium was easy to prepare and maintain the quality of C.O.D. for use in the shaker and batch systems. These advantages initiated the experimentation of medium D (synthetic growth medium).

The Soil Extract Medium. The soil extract medium promoted growth as was seen from Table II, but the growth was less than the other media

tested. Preparation of the medium was time consuming and was not feasible for the amount of medium that was needed to maintain the cultures. Quality control was difficult, in that it was hard to maintain a constant C.O.D. of the medium after each preparation. The soil extract medium was tested because of the highly diverse growth that it was expected to produce (25). From selected plating and microscopic examination, the soil extract medium appeared to produce a diverse population that resembled the actual soil microbiota.

Synthetic Activated Sludge Medium. The synthetic activated sludge medium was described earlier (Table IV). The medium provided the fastest growth in both S.P.C. and V.S.S. in the testing period. The medium was easy to work with and provided good quality control by refrigeration of the three stock solutions. The medium was chosen for the balance of the study because it met all of the requirements and was an easily biodegradable medium that produced a large biomass in a short period of time.

Preparation of Soil Cultures

After selection of the medium, the soil organisms had to be cultured to produce the desired amount of organisms for the inhibition screening. The results of the shaker studies are shown in Table VII. The V.S.S. concentrations for the three soils decreased at first and then increased to acceptable levels in the ten-day period. This was shown by the increase in the clay soil culture V.S.S. concentration from 540 mg/ℓ after 72 hours to 960 mg/ℓ at the end of the shaker table growth period. S.P.C. concentrations also increased for the

clay soil culture in this period from 6.9×10^5 micro/mℓ to 5.1×10^9 micro/mℓ. At this point the shaker cultures were transferred to batch systems.

TABLE VII

PERFORMANCE OF SOIL CULTURES FROM THE SHAKER FLASK STUDY

Time (hrs)	V.S.S. (mg/ℓ)	S.P.C. (microorganisms/mℓ)	pH
Clay Soil:			
24	2730	3.5×10^4 EPC	7.1
72	540	6.9×10^5 SPC	7.0
120	790	2.4×10^8 EPC	--
168	960	5.1×10^9 EPC	6.9
Silt Soil:			
24	470	4.0×10^4 SPC	6.7
72	210	3.9×10^6 SPC	7.1
120	630	7.2×10^8 EPC	--
168	890	5.8×10^9 SPC	6.8
Sand Soil:			
24	520	7.6×10^4 SPC	6.7
72	305	2.6×10^6 SPC	7.4
120	435	7.5×10^7 EPC	--
168	720	9.1×10^8 SPC	6.9

The batch systems were operated on an eight-day Sludge Retention time (S.R.T.). This relatively slow growth rate would hopefully maintain diversity and allow V.S.S. concentrations to stay within desired limits. The batch systems were monitored for Dissolved Oxygen, pH, V.S.S., and Oxygen Uptake. The results for the clay batch unit were shown in Table VIII. At first the V.S.S. showed no increase, as seen from day eleven to day thirteen of the study. At day fourteen, three-fourths of the reactor volume (1600 mℓs) were centrifuged and the supernatant decanted. The concentrated solids were placed back in the reactor. After two periods of centrifugation, the V.S.S. concentration was over 2000 mg/ℓ.

After day 20 of the study, the system was maintained at the eight day S.R.T. The clay culture V.S.S. concentration varied with time, but the concentration stayed above 1000 mg/ℓ for the balance of the study. The maximum V.S.S. concentration for the clay culture was 1830 mg/ℓ on day 86, and the minimum of 1030 mg/ℓ occurred on the 44th day of the study.

The pH of the unit was monitored periodically during the study. Variations in pH occurred throughout the life of the batch reactor. The trend in pH was a decrease in the first 24 hours after feeding, and then the system would maintain a constant pH for the next 24 hour period. The pH was checked and adjusted when needed. Table IV shows the pH 24 hours after each feeding period.

Dissolved Oxygen (D.O.) was maintained around 7.0 mgO_2/l. The D.O. was checked to insure that the system was receiving enough oxygen from the batch system's air stones. With the D.O. above 7.1 mgO_2/l, the unit was not oxygen limiting.

TABLE VIII

PERFORMANCE OF THE CLAY BATCH UNIT

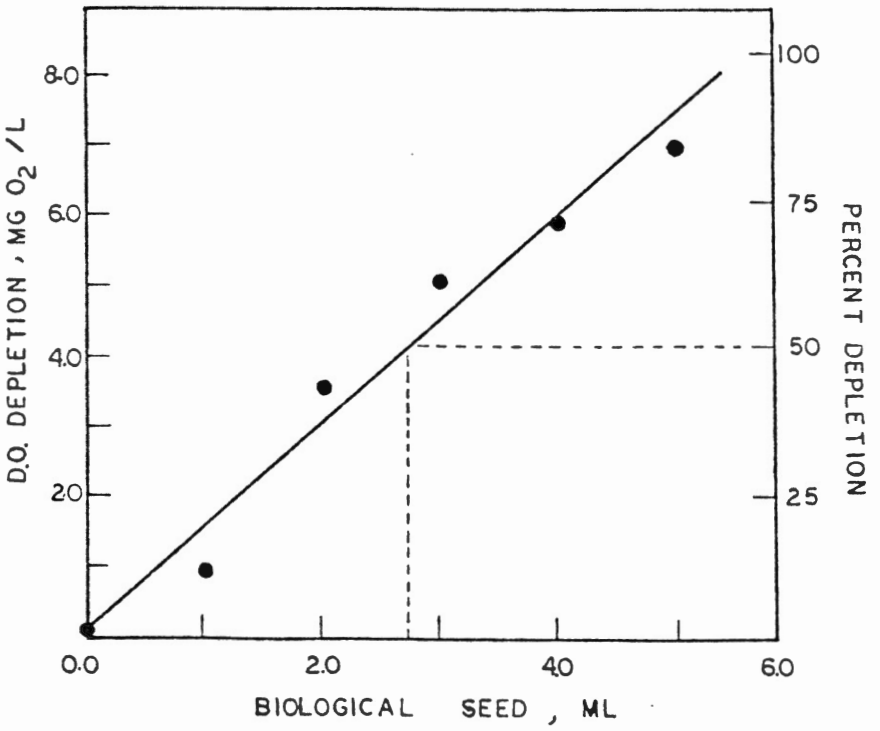
Time (days of study)	V.S.S. (mg/ℓ)	Dissolved Oxygen (mg O_2/ℓ)	D.O. uptake (mgO_2/gm/hr)	pH
transfer to batch system:				
11	560	7.3	--	7.3
12	610	--	--	7.1
13	590	7.0	--	--
centrifugation of system:				
14	1220	--	--	6.4
16	1780	--	--	--
19	2400	7.3	--	--
end centrifugation and run system on eight day S.R.T.:				
20	1650	--	--	7.4
21	1050	--	--	6.9
26	1730	--	--	6.5
37	1200	7.1	10.2	6.6
44	1030	--	--	7.0
51	1130	7.3	21.8	7.1
53	1365	--	--	6.5
55	1465	7.7	7.4	6.3
67	1500	--	--	7.1
79	1700	6.9	11.4	6.3
86	1200	--	--	6.9
87	1830	--	--	7.0
88	1660	8.1	8.2	7.1

Dissolved Oxygen Uptake (D.O. Uptake) was measured to evaluate the ability of the organisms to degrade the synthetic growth medium. The data was collected to compare with acclimation studies discussed later.

Preliminary Study

Figure 5 shows the results of the preliminary study to calculate the proper amount of biological seed that had to be added to the B.O.D. bottle to produce the desired D.O. depletion. The desired depletion was fifty percent of the initial D.O. concentration after three days of incubation. By interpolation of the test result, 2.8 mls. of biological seed material with a V.S.S. concentration of 2300 mg/l produced the desired fifty percent depletion of the initial D.O. concentration. Correlation of this relationship with the amount of biological seed that was reported used in the literature, it was hypothesized that 5 mls of seed was used if the V.S.S. concentration of the batch system was 1000 mg/l (2) (14) (21). The following relationship was developed to approximate the desired seed inoculum for the inhibition testing.

$$\text{Amount of seed inoculum (mls)} = \frac{1000 \text{ mg/l} \times 5 \text{ mls}}{\text{V.S.S. of Batch System (mg/l)}} \qquad (4.1)$$



slope = 1.42 mg O_2/ℓ /mℓ

intercept 0.34 mg O_2/ℓ

95.61 correlation

Figure 5. Preliminary Study to Determime the Amount of Biological Seed to be Used in Screening Testing

Inhibition Screening

Pure Compounds

The microbial populations grown on the synthetic medium were used as the microbial seed for the inhibition screening procedure. The test defines the threshold inhibition point for the pollutant in question. The procedure was first demonstrated on four pure compounds. The chemicals were benzene, toluene, cresol, and phenol. The pure compounds were chosen because they were likely to be in the industrial sludges that were used in this study. One sludge was a wood preserving waste and the two other sludges were generated by the petroleum industry. From the literature, the specific compounds tested were found in the waste from these two industries (27). The results of the inhibition screening for the pure compounds were shown in Figures 6-9.

It was seen in Figure 6 that benzene exhibited a threshold inhibition level of 4.0×10^{-2} percent by weight. This corresponds to a concentration of 400 mg/ℓ. This was illustrated by the sharp rise in residual dissolved oxygen until the initial dissolved oxygen was approached. Below the 4.0×10^{-2} percent by weight (400 mg/ℓ) loading, the benzene was not inhibitory and was biodegraded at various degrees.

At 1.0×10^{-2} percent by weight (30 mg/ℓ), benzene exhibited a small blip which appeared to be a slight reduction in biodegradability. This was illustrated by a gradual rise and fall in the Dissolved Oxygen level. This pattern appeared many times in the study. In addition, the microorganisms from all three soils

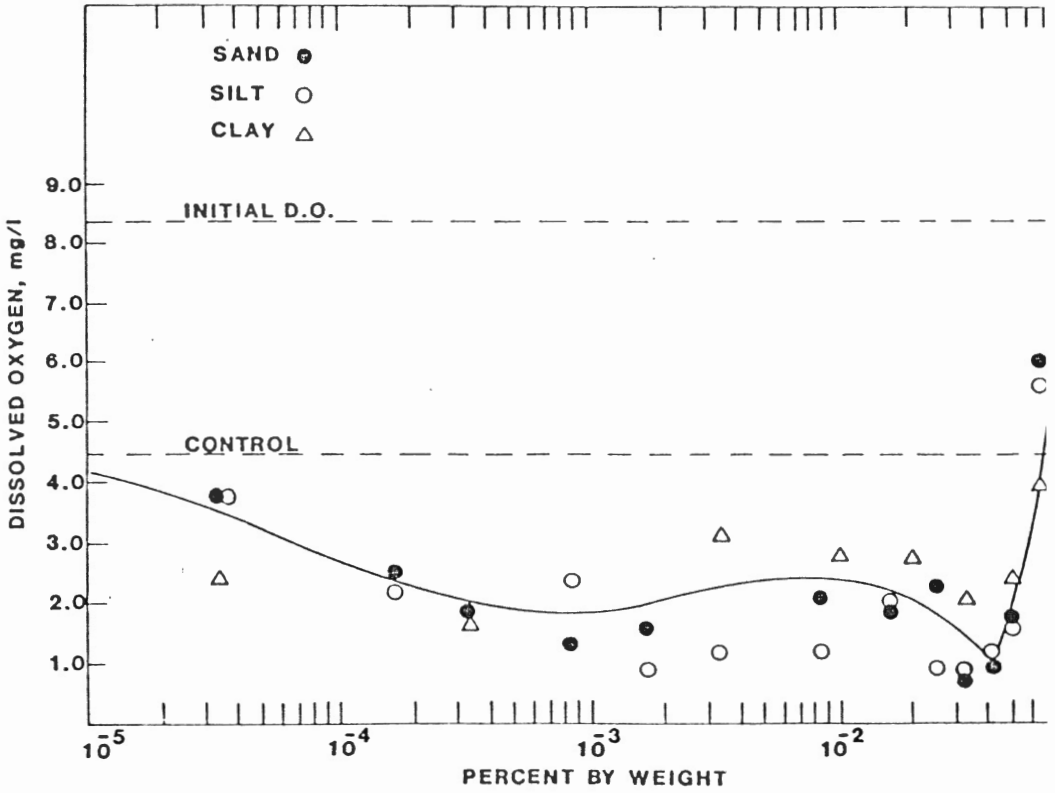


Figure 6. Results of Microbial Inhibition Testing of Benzene

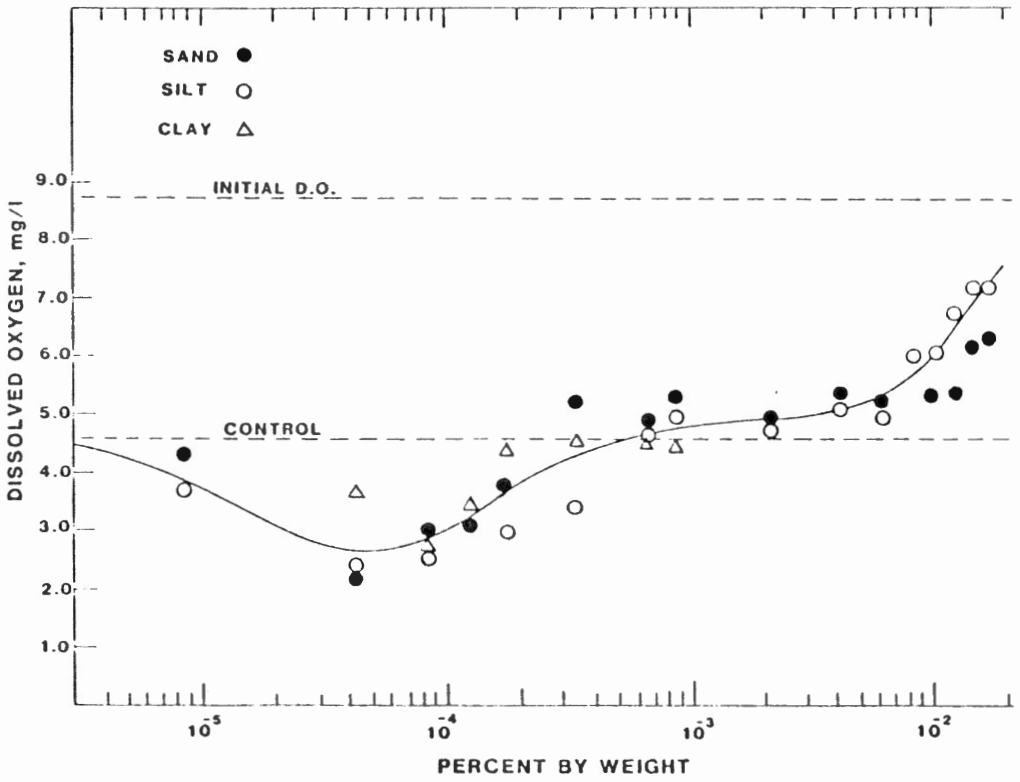


Figure 7. Results of Microbial Inhibition Testing of Toluen

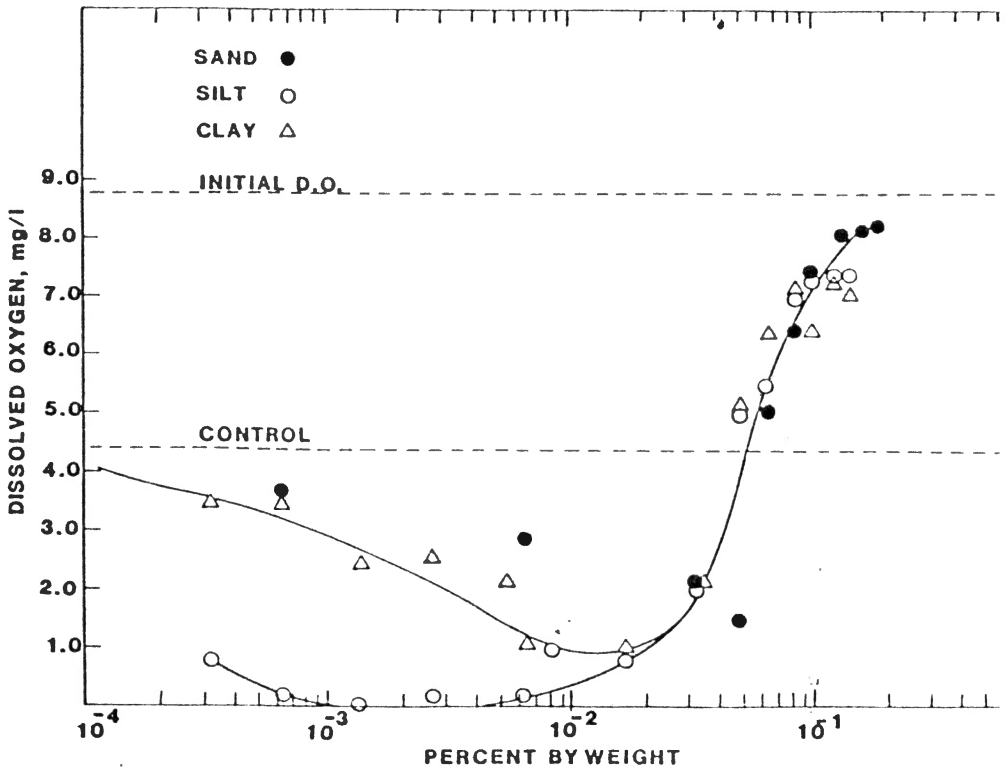


Figure 8. Results of Microbial Inhibition Testing of Cre

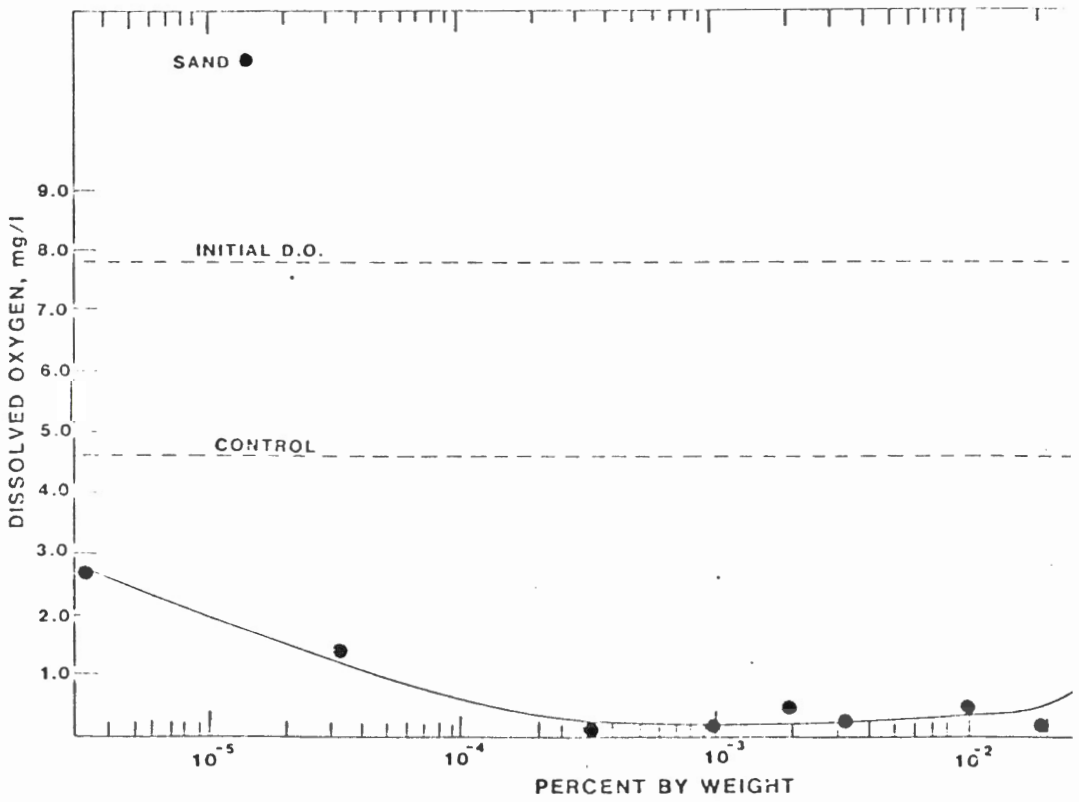


Figure 9. Results of Inhibition Screening of Phenol

reacted in a similar manner.

Figure 7 shows the results of the inhibition screening procedure for toluene. At loading rates lower than 2.0×10^{-4} percent by weight (2.0 mg/ℓ), toluene was biodegraded and was not inhibitory. However, at a loading of 5.0×10^{-4} percent by weight (4.0 mg/ℓ), the toluene was not biodegraded, and the compound did not inhibit the utilization of glucose by the microorganisms. This was seen by the dissolved oxygen leveling out and following the control dissolved oxygen curve. The toluene did not become inhibitory until the loading reached approximately 2×10^{-3} percent by weight (20 mg/ℓ).

The results of the inhibition testing of cresol were shown in Figure 8 and provided some interesting results. The threshold inhibition level for cresol was found to be 2.0×10^{-2} percent by weight (200 mg/ℓ) for all of the soil organisms. At loading rates lower than 7.0×10^{-3} percent by weight (67 mg/ℓ), the silt soil culture biodegraded the cresol at a greater extent than the sand or silt cultures. This was seen in the divergence in the two Dissolved Oxygen curves.

It was seen in Figure 9 that phenol exhibited a threshold inhibition level of about 2.0×10^{-2} percent by weight (200 mg/ℓ). Below 2.0×10^{-2} percent by weight loading, the phenol was not inhibitory and was readily biodegraded. These test results were similar to what other researchers have found with biological growth studies employing phenol (28) (29).

Industrial Sludges

Figures 10, 11, 12, and 13 showed the results of the inhibition screening procedure for the three industrial waste sludges. The three industrial sludges were classified as hazardous wastes by the E.P.A. The sludges included

1. dissolved air flotation (D.A.F.) waste from the petroleum industry
2. a slop oil sludge from clean-up operations of an oil industry
3. a wood preserving industrial waste sludge

Special care was taken in handling the sludges because of the presence of many unknown organics as seen in Gas Chromatography analysis (1).

Figures 10 and 11 showed the results of the microbial inhibition testing of the D.A.F. sludge. It is seen in Figure 10 that, up to a loading of 0.27 percent by weight (27000 mg/ℓ), the D.A.F. oily sludge was not inhibitory and was biodegradable. At low loading rates of 1.0×10^{-2} percent by weight and less, the residual oxygen level was just below the control level. At these loadings (high dilutions) there was possibly too little carbon source in the sludge to cause a noticeable biological oxygen demand. At a loading rate above 3.0×10^{-2} percent by weight, there was enough utilizable carbon to cause biological respiration. Figure 11 was a continuation of the D.A.F. oily sludge testing in which higher loading rates were tested. The threshold inhibition point was defined as 4.0 percent by weight. However, it should be noted that there was only one point to define the inhibition level. Loading rates this high would

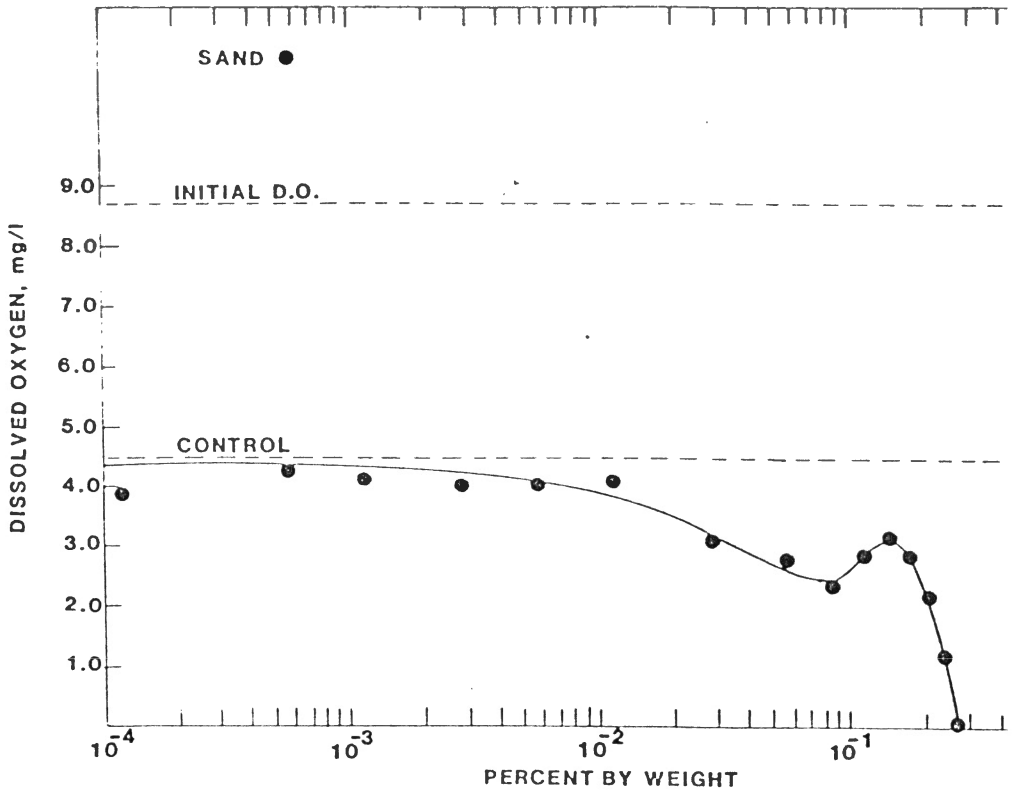


Figure 10. Results of Microbial Inhibition Testing of Oily Sludge

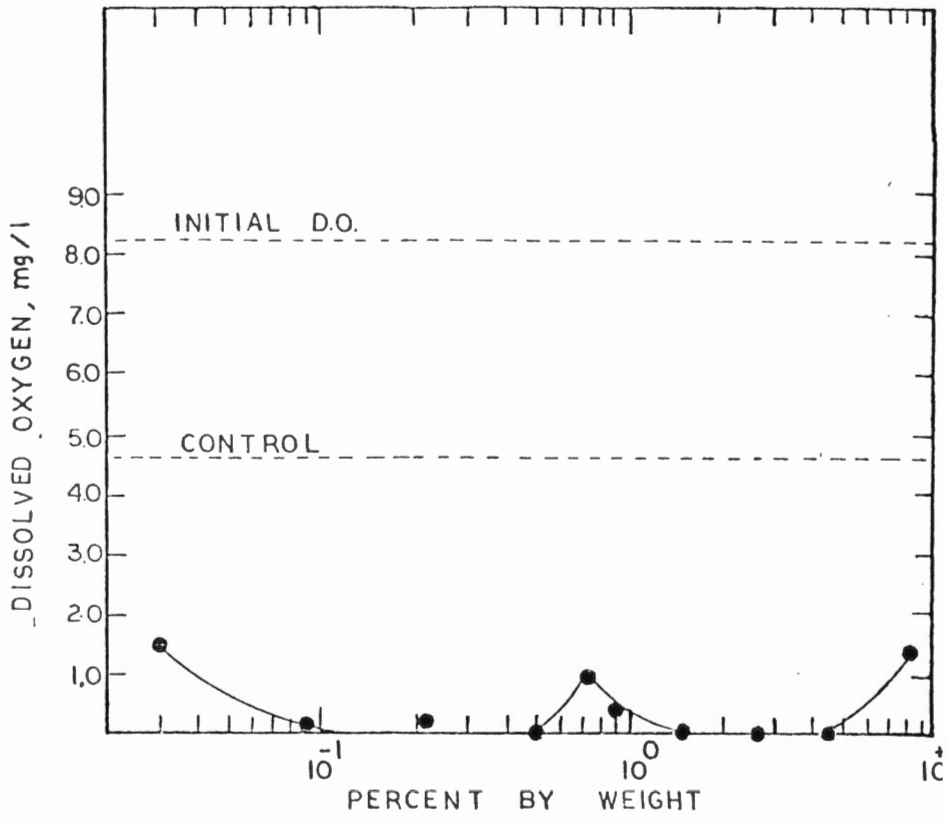


Figure 11. Results of the Continuation of the Microbial Testing of D.A.F. Oily Sludge

not be feasible in an actual land application site because of the hydraulic constraint in application of the sludge caused by excess soil moisture. There was also a small rise and fall in the residual oxygen curve at a loading of 0.7 percent by weight. The threshold level of 4.0 percent by weight may only be a small rise in the residual oxygen curve, in which case higher loadings may not be inhibitory.

The microbial inhibition testing of the wood preserving industrial sludge was shown in Figure 12. The threshold inhibition level occurred at a loading rate of 0.06 percent by weight (6000 mg/ℓ). At lower loadings, the sludge was not inhibitory and was biodegradable. At a high loading rate of 1.0 percent by weight, the industrial sludge caused inhibition or toxicity on the soil microbial culture.

Figure 13 showed the results of the inhibition screening of the slop oil sludge. The test had some interesting results. At loading rates lower than 5×10^{-3} percent by weight, the residual oxygen level approximated the control Dissolved Oxygen level. Then, between the loadings of 0.01 to 0.06 percent by weight, the industrial sludge was extremely biodegradable as shown by the zero residual oxygen level. The threshold inhibition level of the slop oil sludge was 0.06 percent by weight. At a loading of 0.1 percent by weight, the sludge was not biodegradable, and it did not inhibit the microorganisms ability to oxidize the glucose as seen by the Dissolved Oxygen level approaching the control.

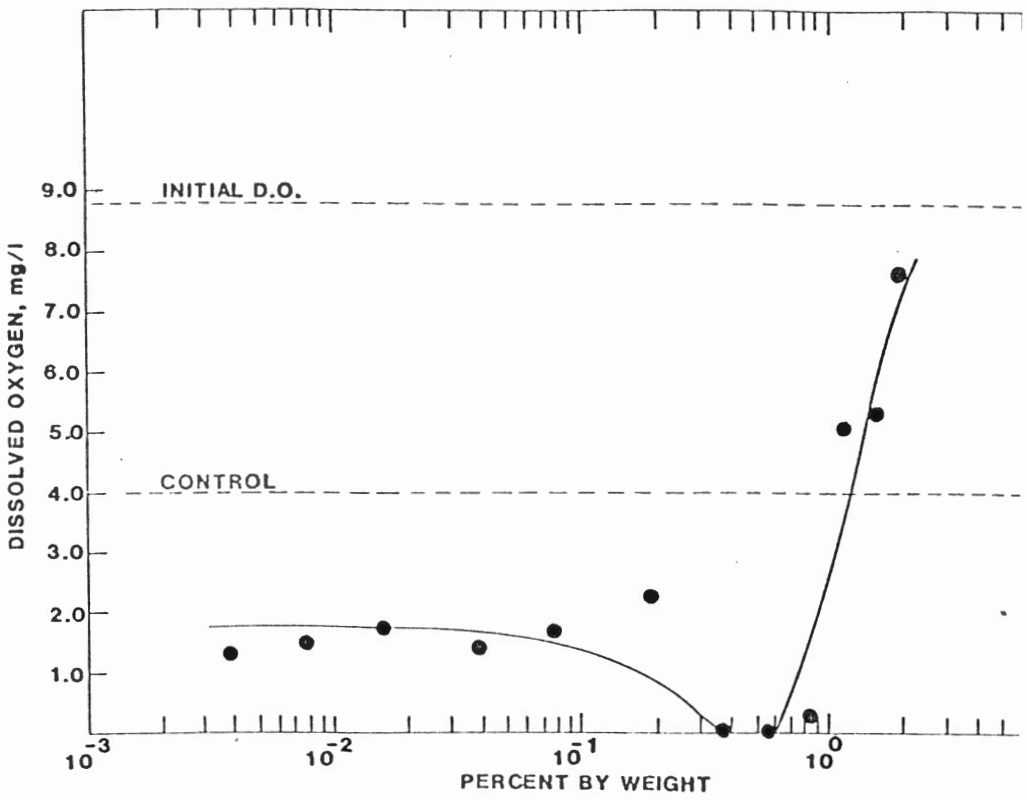


Figure 12. Results of Microbial Inhibition Testing of Wc
Preserving Sludge

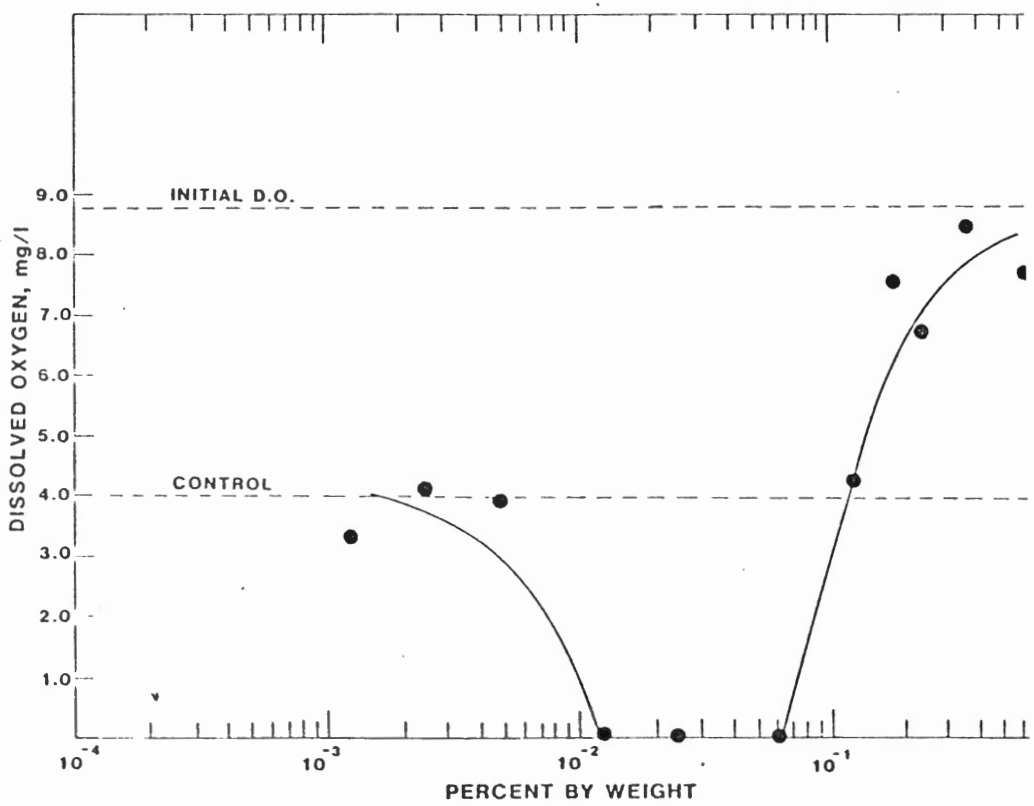


Figure 13. Results of Microbial Inhibition Testing of Slo
Oil Sludge

Acclimation Studies

The acclimation studies were the final step in the inhibition procedure. A batch system was prepared as described earlier and allowed to acclimate to the pollutant. The batch unit was seeded by a combined inoculum from the cultures previously used in the inhibition study. Table IX shows the schedule for the acclimation of cresol and phenol. The concentration of the organic was that found in the batch unit after considering the dilution of the unit. The actual procedure was to make a high strength stock solution and add a small amount of the stock to the feed. The percentage of the inhibition level concentration of the pollutant was determined. The percentage was increased during the feeding periods. Similar schedules were prepared for each acclimation study.

The systems were monitored for V.S.S. concentrations, dissolved oxygen, D. O. uptake, and pH. Table X showed the performance of the acclimated batch cultures. The dissolved oxygen concentration and pH were checked to maintain good growing conditions in the systems. The pH fluctuations were similar to those observed during operation of the unacclimated batch systems. The pH was adjusted when needed. Dissolved oxygen ranged from 6.5 to 8.0 mg O_2/l. The industrial sludge systems seemed to have lower dissolved oxygen readings than the pure compound batch cultures.

The V.S.S. concentration and D.O. uptake were monitored to determine the growth of the systems. The V.S.S. concentrations tended to be constant for all but the phenol and toluene system in which case the units started with low V.S.S. concentrations and gradually

TABLE IX

ACCLIMATION SCHEDULE FOR CRESOL AND PHENOL

days into study	Cresol		Pheno
	concentration (mg/ℓ)	% of inhibition level	concentration (mg/ℓ)
1	40	10	30
3	40	10	30
5	60	15	50
7	80	20	75
11	150	40	100

2	1530	7.4	5.9	6.6
4	1600	--	--	7.1
6	1720	7.1	10.1	6.9
10	1520	7.5	11.7	6.5
Cresol:				
2	1820	7.4	6.1	7.2
6	1740	--	--	7.3
10	1490	7.5	7.2	7.0
14	960	8.1	17.4	7.3
Phenol:				
2	330	--	--	7.0
4	780	7.9	10.0	7.3
6	1020	--	--	6.9
8	1590	--	--	7.2
10	1700	7.2	10.9	6.9
Toluene:				
2	350	--	--	7.2
4	795	7.8	9.8	7.4
6	1290	--	--	6.9
8	1490	7.10	11.3	7.2
12	1520	--	--	7.0
Wood Preserving:				
2	1960	6.5	4.2	7.1
4	1650	--	--	7.1
8	1530	6.7	10.2	7.2
12	1940	4.4	16.6	6.9
Slop Oil:				
2	1500	6.4	4.9	6.8
4	1320	--	--	6.9
8	1520	6.5	10.1	7.0
12	1700	7.1	9.8	6.7
14	1530	--	--	7.1

increased with time. The phenol and toluene systems were the first units to be acclimatized and very small amounts of seed material were used to start the systems. Afterwards, the systems were started with larger amounts of biological seed. This was shown in the increased initial V.S.S. concentrations in the other batch units. The cresol batch system was the only culture to have a decreasing trend in V.S.S. concentration.

The dissolved oxygen uptake data from the unacclimated and acclimated studies was very speculative. The D.O. uptake values were taken 24 hours after the batch systems were fed. This made the measurements dependent on the residual C.O.D. in the batch unit after a 24-hour period. Therefore, it was important to measure D.O. uptake as close to 24 hours after feeding as possible. The initial C.O.D. concentration in the system just after feeding was also important. The initial concentration in the systems of 2000 mg/ℓ C.O.D. was maintained throughout the study. Because of these variables the D.O. values were misleading at times.

D. O. uptake was used as an indicator of the acclimation of the organisms to the various pollutants. The D.O. value represented the endogenous uptake value because it was measured 24 hours after feeding the systems. Taking the D.O. uptake measurement a few minutes after feeding would have been a better indicator of the carbon source utilization by the microorganism. Any future studies which use this parameter should consider the timing of the D.O. uptake measurement.

Table VIII showed the unacclimated D.O. uptake values and Table X showed the acclimated uptake values for the various pollutants.

The acclimated cultures generally had low uptake values after the initial set up of the system, but the uptake increased after four to six days of acclimation. After this time, the D.O. uptake values were in the range of the non-acclimated systems.

Figure 14-17 showed the results of the inhibition testing of the pure compounds using acclimated microorganisms. The seed for each compound was acclimated at concentrations below the threshold inhibition level. The acclimation occurred in a three-week period or until V.S.S. concentrations were obtained above 1000 mg/ℓ.

Figure 14 showed the acclimated inhibition testing for benzene. The threshold inhibition level was found to be 4.0×10^{-2} percent by weight (400 mg/ℓ). The level was the same as that found in the unacclimated study shown in Figure 6. The benzene was biodegraded at various levels below the threshold level with a slight increase in degradation as compared to the unacclimated study. Acclimation of the microorganisms to benzene did not appear to affect the toxicity and/or inhibition level of the compound. This was similar to the results found in the literature by Stover et al. (30).

Figure 15 showed the results of acclimated microbial inhibition testing of cresol. The threshold inhibition level was found to be 2.0×10^{-2} percent by weight (200 mg/ℓ) which was similar to the unacclimated study shown in Figure 8. The acclimation of the organisms to cresol did not affect the inhibition level. This again was similar to the literature values (30). In Figure 8, the unacclimated study showed increased degrees of biodegradability for the silt culture as compared to sand and clay cultures. The acclimated study was similar to the silt culture unacclimated screening test. Therefore,

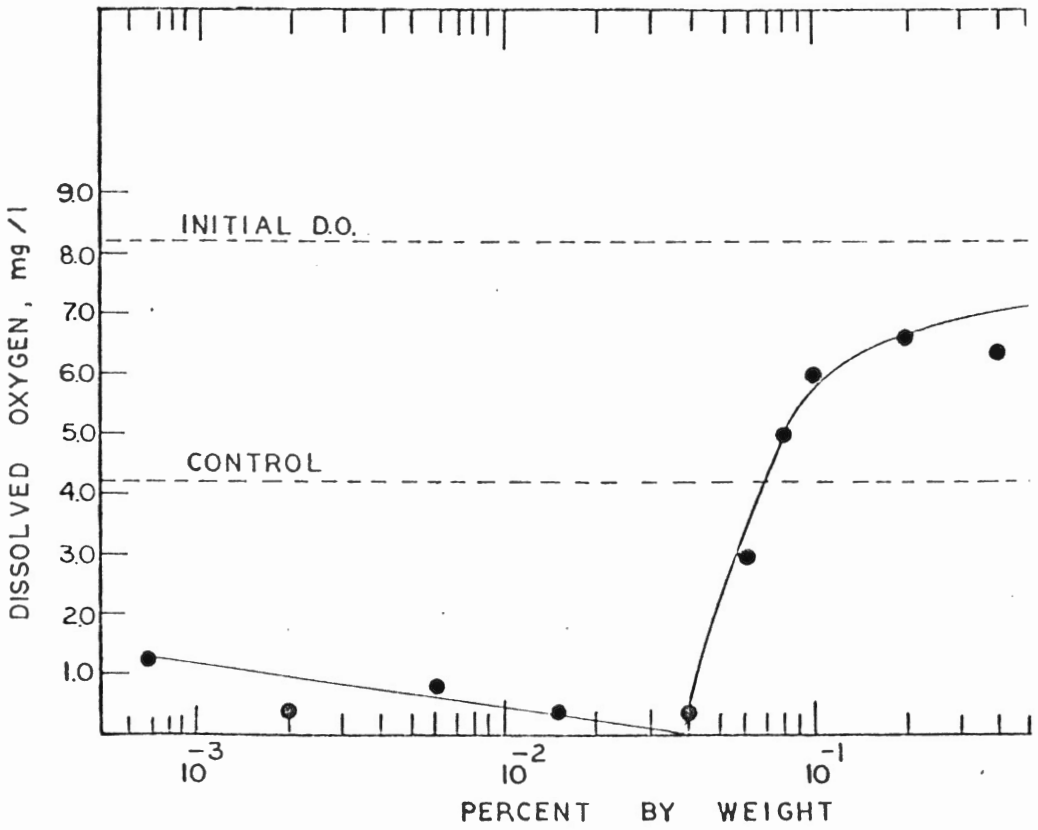


Figure 14. Results of Acclimated Microbial Inhibition Testing of Benzene

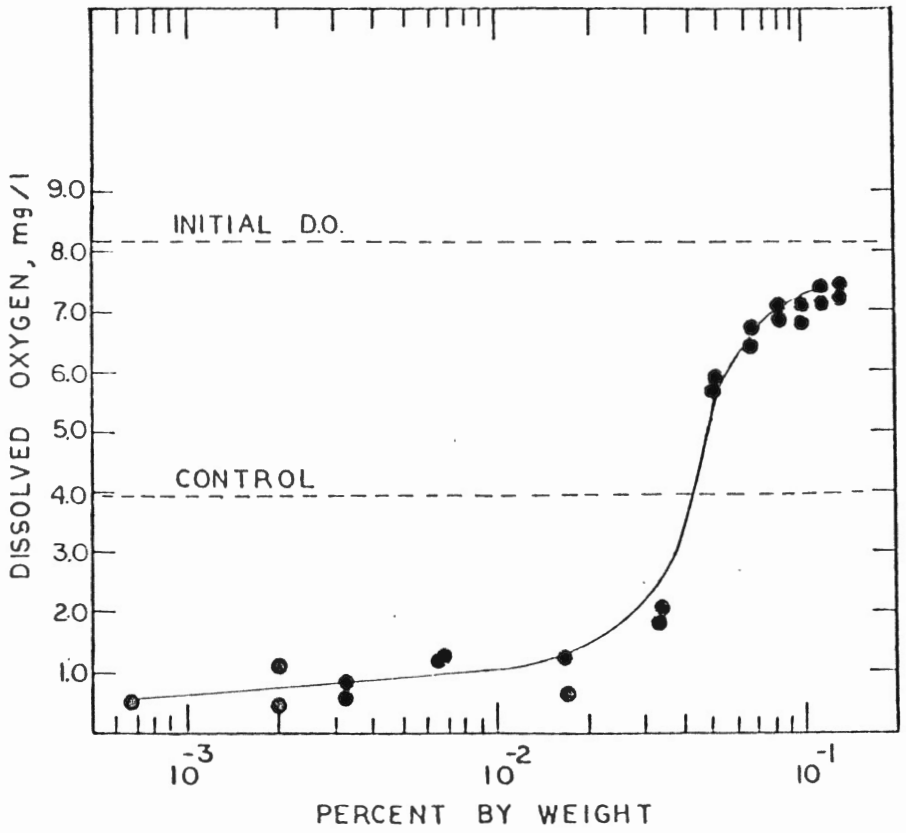


Figure 15. Results of Acclimated Microbial Inhibition Testing of Cresol

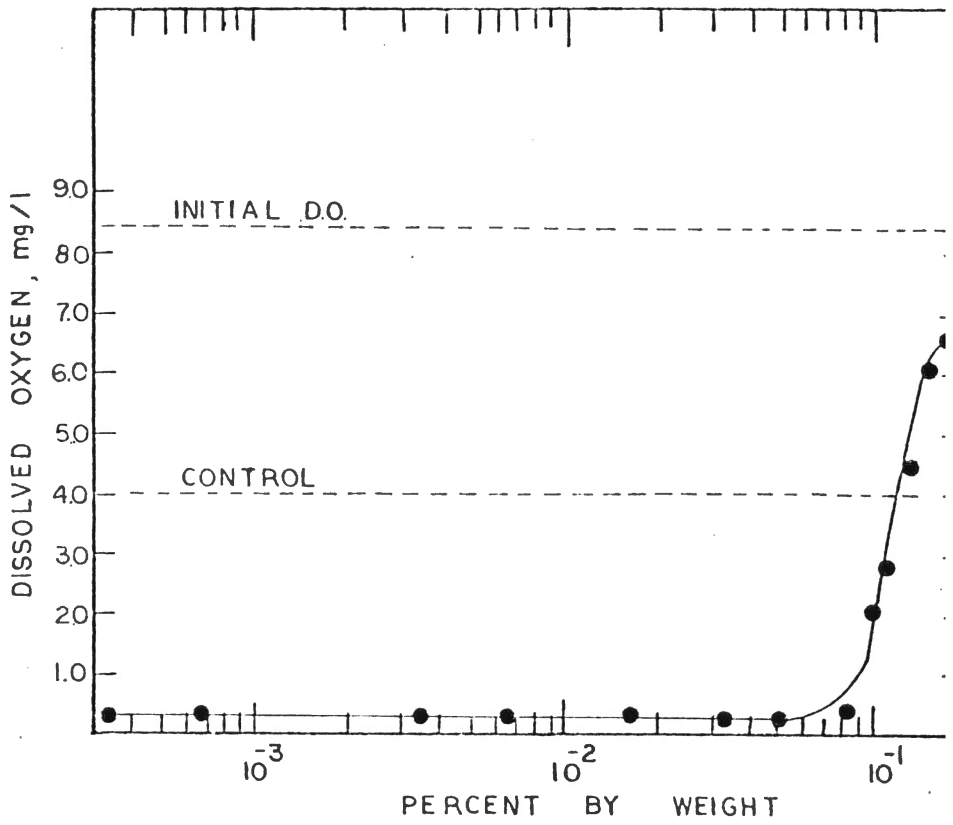


Figure 16. Results of Acclimated Microbial Inhibiti Testing of Phenol

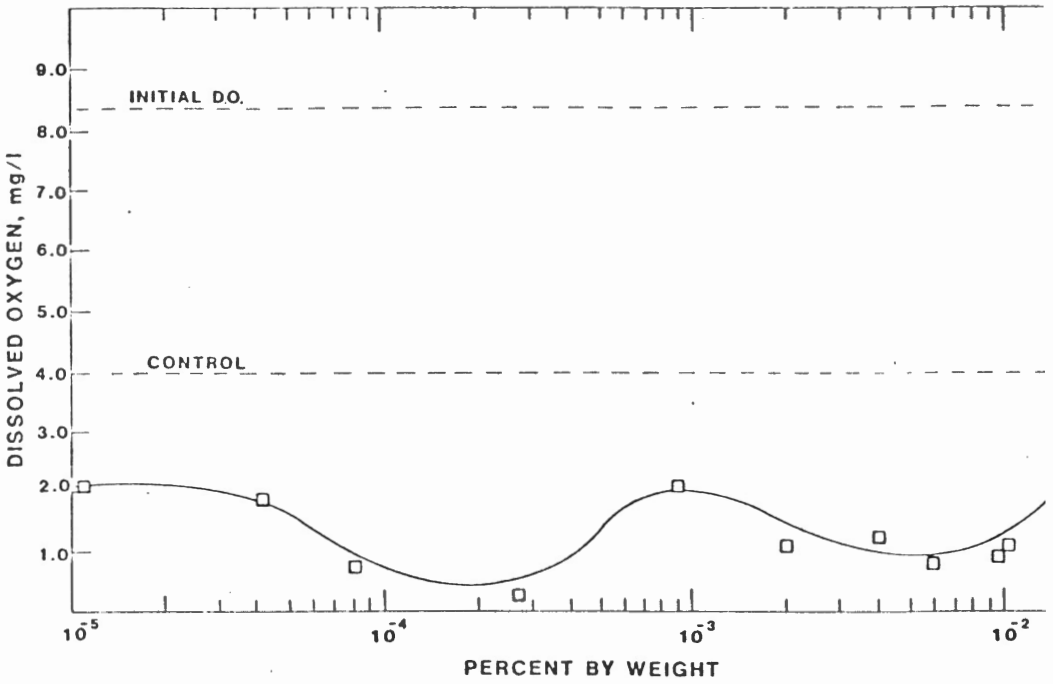


Figure 17. Results of Acclimated Microbial Inhibition Testi
Toluene

acclimation may tend to increase the biodegradability of clay and sandy soils.

The results of the microbial inhibition screening of phenol using acclimated biological seed is shown in Figure 16. The threshold inhibition level was 6.5×10^{-2} percent by weight (600 mg/ℓ). The inhibition level is higher than that found for the unacclimated screening study shown in Figure 9. The threshold inhibition level increased from 2.0×10^{-2} percent by weight (200 mg/ℓ) to 6.5×10^{-2} percent by weight (600 mg/ℓ). This was a significant increase in biodegradability of phenol. In the unacclimated study, a loading of 6.5×10^{-2} percent by weight corresponded to the control dissolved oxygen level, in which case the phenol was not biodegraded but the compound did not inhibit the utilization of glucose. In the acclimated study, the 6.5×10^{-2} percent by weight loading was the threshold inhibition level and, therefore, the phenol was biodegraded. Upon acclimation, the phenol was biodegraded at higher loadings.

Figure 17 showed the screening test for toluene using acclimated seed inoculum. The inhibition testing provided some interesting results. The acclimated threshold level was found to be 1.0×10^{-2} (100 mg/ℓ). At this loading in the unacclimated study (Figure 7), the toluene was not biodegraded and was inhibiting the microorganisms utilization of glucose. At lower loading of toluene, between 5.0×10^{-4} to 2.0×10^{-3} percent by weight (5.0 to 20 mg/ℓ), the unacclimated seed utilized the glucose but could not biodegrade the toluene. In the acclimated study, the toluene was biodegraded at various levels in the mentioned range of loading rates. Therefore, acclimation of

the cultures with toluene increased the organisms' ability to degrade the compound.

Figures 18 and 19 showed the acclimated inhibition screening for two of the industrial sludges used in the study. The D.A.F. oily sludge was not tested in the acclimation study for three reasons.

1. The sludge was very biodegradable as was seen in Figures 10 and 11.
2. At the threshold inhbition point of 4.0 percent by weight, the D.A.F. oily sludge could not be added to a soil system because of hydraulic constraints caused by excessive moisture content.
3. At the high loading, the sludge caused damage to the membrane of the Orion oxygen electrode.

Figure 18 showed the inhibition screening results for the slop oil sludge using the acclimated biological seed. The threshold inhibition level increased from 0.06 percent by weight in the nonacclimated test. This was not a large increase in the threshold level and, as was seen in Figure 13, this could be caused by the lack of data points in the region of the inhibition level of the unacclimated study. It appeared that acclimation did not greatly increase the biodegradation of the slop oil sludge.

The results of the acclimated microbial screening for the wood preserving industrial sludge were shown in Figure 19. The threshold inhibition level had a noticeable increase from the unacclimated study shown in Figure 12. The threshold level increased from 0.06 percent by weight in the unacclimated study to 1.0 percent by weight in the acclimated test. The acclimated study also showed increased

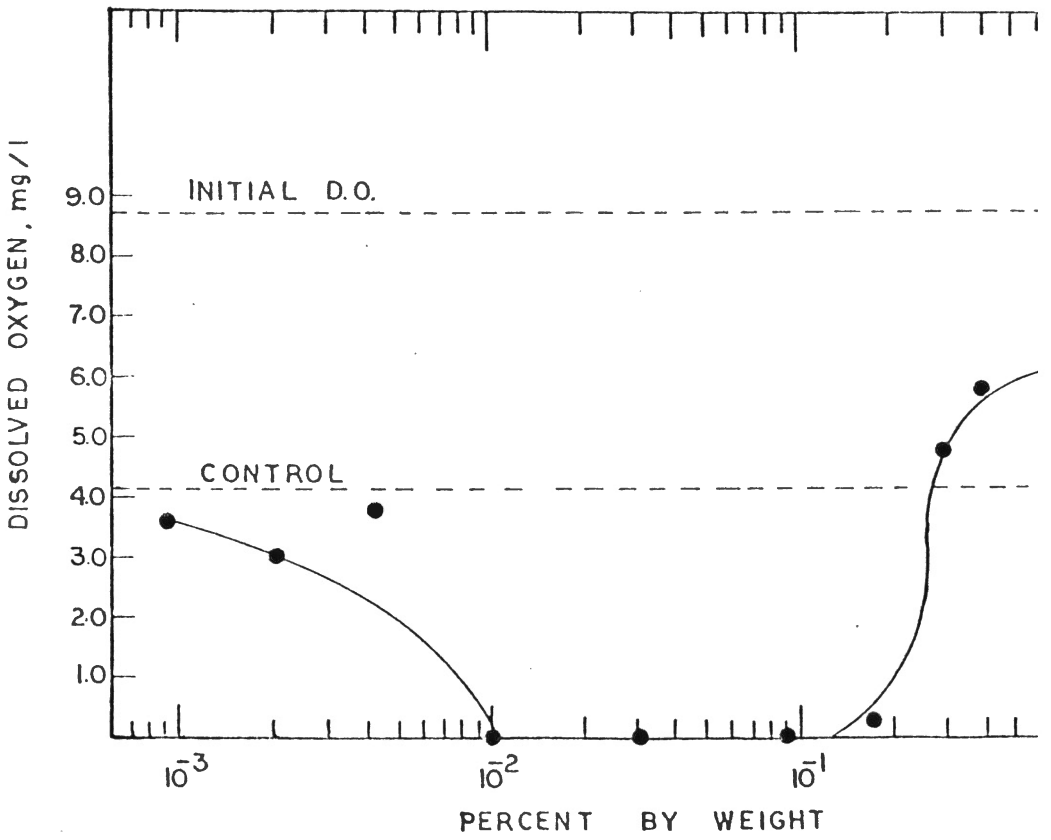


Figure 18. Results of Acclimated Microbial Inhibition Testing of Slop Oil Sludge

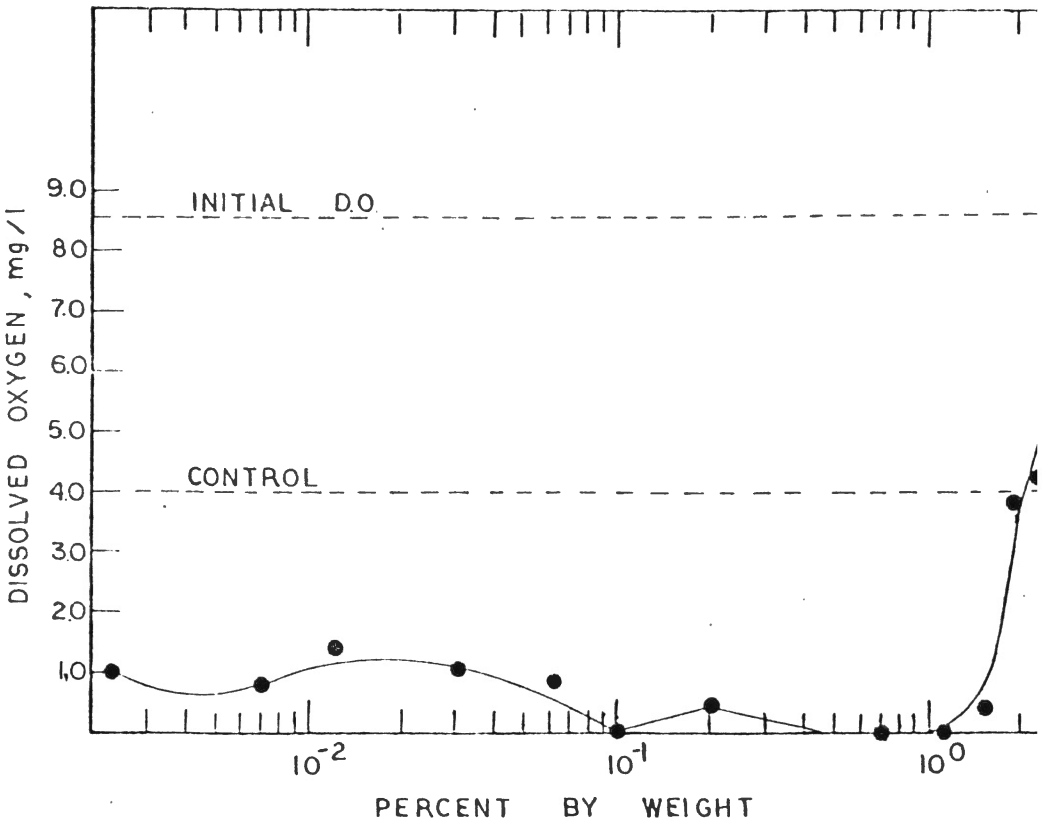


Figure 19. Results of Acclimated Microbial Inhibition Te
of Wood Preserving Sludge

biodegradability at lower loading rates than the unacclimated test. This was seen in slightly lower residual oxygen levels in the acclimated study. This increased degradation could be caused by experimental variations of the two screening runs. Acclimation seemed to increase the biodegradation of the wood preserving waste sludge.

CHAPTER V

DISCUSSION

Application of the inhibition screening procedure in defining the threshold inhibition level of a particular sludge or specific compound can aid in the determination of the maximum allowable loading rate on a particular soil. In designing a land application system for industrial wastes, it is important to determine the toxicity and/or inhibitory level which would prevent biodegradation of the industrial sludge. A properly engineered and maintained land treatment facility with loading rates below the threshold level can provide safe disposal and detoxification of an industrial waste sludge.

It is therefore advantageous to have a reliable test to determine the threshold inhibition level of an industrial sludge on the particular soil system. The inhibition screening procedure described by Marks (2), Stover (14, 21), and Kincannon et al. (22) has the advantages of being simple, quick, and inexpensive. The procedure is a modification of the B.O.D. analysis which is familiar to environmental engineers and technicians. The threshold inhibition level can be defined within a one-month time period. The screening procedure is very easy and simple to conduct compared to column studies, or expensive gas chromatography/mass spec, or atomic adsorption screening studies. The reliability of the screening procedure is assessed by the result of the microbial inhibition testing of phenol. The

threshold inhibition level of 2.0×10^{-2} percent by weight (200 mg/ℓ) compares well with results of growth studies conducted by Kincannon et al. (28) and Rozich et al. (29).

In many of the screening tests there is an intriguing rise and fall in the residual oxygen level at a loading below the threshold inhibition level. The blips occur in the pure compound testing of Benzene (Figure 6), the continued microbial testing of D.A.F. oily sludge (Figure 11), and in two of the acclimation studies, toluene (Figure 17) and Wood Preserving (Figure 19). Two methods have been hypothesized to account for the small rise and fall in the residual oxygen level.

During the carbonaceous oxidation of the pollutant, production of carbon intermediates could inhibit enzyme production and/or function by competitive inhibition. The smaller molecular weight intermediates could out-compete the original substrate for the binding sites on important enzymes. The rate of degradation would be decreased until the original substrate concentration is increased to a level where it can out-compete the intermediates. This agrees with Gaudy (26) who states that competitive inhibition is concentration dependent. In the case of the benzene and the acclimated toluene testing, the intermediate production would be the only compounds in the BOD bottle that could cause the inhibition.

The industrial sludges contain a large array of possible substrates for the microorganisms. Assuming that some of the constituents of the industrial sludge are more biodegradable than others, the blips in the data can be explained by diauxic or diphasic growth as

explained by Gaudy (26). In this case the easily assimilated substrate would be oxidized first, thus lowering the residual oxygen level. Once this initial substrate is removed, the organisms would have to switch to another substrate. This change-over could be associated with a lag period as the microorganisms are adjusting to the next substrate. As a result, the rate of carbonaceous oxidation would decrease until the lag period is over.

Competitive inhibition and diauxic growth are only two possible explanations for the rise and fall in the residual oxidation level. There are no trends in the position of the blips with regards to the threshold level loading and the blips do not appear to be compound specific. Further research in this phenomena could be beneficial in predicting the biodegradation of a particular pollutant.

In the procedure, soil microorganisms are cultured from a soil to a liquid system and large populations are produced to run the screening tests. By using the synthetic growth medium, a heterogeneous population which resembles the microbial soil system is produced. This has the advantages of the cultivation of a large and continually growing pool of microorganisms in which proper growth conditions can be artificially maintained. The system is easy to maintain and acclimation of the culture is a simple procedure.

Using this method, acclimation or adaptive response capabilities for a particular soil system to specific compounds or sludges can be assessed. Predicting the potential for acclimation of the soil microbiota to a pollutant can aid the engineer in the determination of loading rates. If a soil system has the ability to acclimatize to a particular industrial sludge or specific organics, the loading rate

for the land application system can be increased from the initial loading values. The reliability of the acclimation screening procedure can be assessed by looking at the results for benzene and cresol. Acclimating the microorganisms to the specific organics did not increase the threshold inhibition level. This compares well with results obtained by Stover et al. (30) in which acclimation of bacteria to cresol and benzene did not increase observed B.O.D. values.

It is recognized that this is an engineering procedure to assess toxicity and/or inhibition of a particular industrial sludge or specific organic. This is a surrogate parameter that predicts the response of the soil microorganisms to a particular pollutant. As discussed by Caldwell (16) and Little (17), caution must be used when simulating the real world environment. The utilization of a percent by weight inhibition level may not be the same as in a soil system. Future study to determine this relationship would be beneficial in the application of B.O.D. bottle testing to land treatment systems.

CHAPTER VI

CONCLUSIONS

Based upon the results of this study, the following general conclusions were drawn.

The threshold inhibition levels were defined as the lowest concentrations of compound or waste that cause a reduction in the carbonaceous biological oxidation rate. All of the organic compounds and industrial sludges used in this study were found to be inhibitory and/or toxic to the cultured soil microorganisms when loading rates above the threshold inhibition level were applied.

The threshold inhibition level for the industrial sludges and the selected organics were found to be specific to the pollutants.

Land application loading rates for the two oily industrial sludges at the threshold inhibition level were feasible due to hydraulic restraints caused by the excessive moisture content of the sludges.

Acclimation of the soil cultures to the pure compounds increased the biodegradation only in selected cases. The use of acclimation to increase biodegradation was found to be compound specific. Acclimation of the cultures to the industrial sludges increased the biodegradation in the two sludges tested.

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