USE OF BOD TEST TO MEASURE ORGANIC REMOVAL IN LAND TREATMENT SYSTEMS

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CHAPTER I

INTRODUCTION

Present-day man enjoys many comforts and conveniences unknown a century ago. Time and progress have brought with them new challenges. Man has become increasingly aware, especially in the past two decades, of potential environmental hazards and health risks from undue exposure to certain chemicals.

Much recent information suggests that cancer may be largely of environmental origin. Animal data which indicate that some chemicals may cause birth defects are readily available.

In the past, environmental pollution with chemicals was a direct result of progress in the sense that a large and ever growing list of new industrial chemicals is produced each year and with it the possibility of new chemical wastes and by products. Chemicals once thought to be relatively innocuous are now considered as potentially hazardous and toxic and as a possible cause for cancer, birth defects, and other problems.

The methods that were adopted to control the risk from disposal of hazardous wastes like sanitary landfill and incineration were proved to be expensive and also had some pollution problems associated with them.

Land treatment is a less expensive alternative method for ultimate disposal of many industrial wastes. In this process the soil is used to hold the ionic chemicals while microbes degrade the compounds.

Scope and Objectives

The microorganisms in soil seeded with base mix, produced organic material that showed up as total organic carbon (TOC) and chemical oxygen demand (COD) and remained fairly constant throughout the period.

The primary objective of this research was to explain the biodegradability of Dichlorophenol, Dinitrophenol and nitrobenzene in soil systems by the biochemical oxygen demand test.

CHAPTER II

LITERATURE REVIEW

Before the land application of a waste it becomes essential to determine to what extent the soil may be loaded with the hazardous waste without inhibiting the microbial activity of the soil. Land treatment of hazardous waste is designed to utilize the diverse microbial population of the soil to the extent that waste degradation is reduced to below acceptable levels.

An important part of soil-organic chemical interaction involves the rate and mechanism of decomposition of the organic chemical. Decomposition of the organic chemical added to the soil may occur by chemical reactions with soil constituents as a result of biochemical interactions with soil microorganisms, or as a result of photochemical degradation on exposed soil surfaces. Whether chemical, photochemical or biochemical processes are most important in decomposition of a specific organic chemical is probably most dependent upon the chemical characteristics of the organic compound in question, but may also be dependent upon specific soil characteristics as well as the location of the chemical within the soil profile. Very careful investigations are needed to accurately determine whether degradation is actually biological or chemical. However, the interaction of system components is best determined by practical investigations using a set of tests and pilot studies applied on a case by case basis.

Soil factors known to affect the organic chemical degradation include temperature, aeration, microbial population, pH, organic matter, clay, cation exchange capacity and moisture. Soil physical and chemical processes and microbial populations may be similarly affected by these factors. In a environment such as soil, the nature, number, rate and complexity of chemical interactions can be expected to increase or decrease under many of the same conditions causing increased or decreased microbial activity. In 1957 Call (1) stated that "increasing moisture increased Tetrahydro-3, 5-dimethyl-2H-1, 3,5-thiadiazine-2-thione decomposition, and Turner, Corden and Young (2) in 1962 stated that "sodium methyldithiocarbamate decomposition increases only with decreased moisture," and also that "the rate of decomposition of chemicals increases with increased temperature, which was supported by Munnecke and Martin (3) in 1964.

According to Goring (4), Hill et al., (5) Aldrich (6), Burschel and Freed (7), and Edwards (8) the microbial decomposition and activity are generally enhanced by increasing temperature and moisture content. Goring (4) in 1967 also stated the "organic matter content is generally considered a good indicator of microbiological activity and decomposition also seems to be increased with increased organic matter. Soil pH is also known to affect the microbial populations." In 1963 Ashley and Leigh (9) stated that "the decomposition of methyldithiocarbamate is accelerated by metallic cations such as Cu and Fe."

Other explanations about the chemical reactions most likely to occur according to Goring (4); Burchfield (10); Turner and Corden (11); Ashley and Leigh (9); Munnecke and Ferguson (12); Munnecke (13); Turner, Corden and Young (2); Gray (14); Castro and Belser (15); and Torgeson, Yoder and Johnson (16) are decomposition in water or hydrolysis and nucleophillic

substitution by active groups of organic matter. The role of free radicals (hi-energy hydroxyl group) in decomposition of organic chemicals in soil also has been examined recently, though not sufficient data is available to draw any conclusions.

Some other type of chemical reaction known to occur in soil are the non-biological degradation due to the enzymatic action. Soil organic matter includes a wide variety of enzymes not necessarily associated with the living. According to Skujins (17) these may arise from both living and dead. Microorganisms, root exudates, and soil animals and these are stabilized to a remarkable degree by adsorption to buffered particulate matter. Thus the enzymes may survive longer than they normally would in solution and may even become resistant to heat and pH, which would otherwise rapidly deactivate them.

The chemical degradative reactions in soil with the microbial activity has been described by Engst and Kujawa (18) in 1967, primarily by two mechanisms. The use of oxygen being one mechanism. For example, the pretreatment of biorefractory chemical waste water by oxidation. With hydrogen peroxide or sodium hypochloride was found to significantly increase the biodegradability of the waste. Water is another available reagent, where the hydration and hydrolysis can be catalyzed effectively by metallic ions or by even slightly elevated pH kinetics of microbial degradation.

Microbial degradation accounts for much less of organic chemicals from soil. The fate of the organic chemical (i.e., whether or not a chemical is adsorbed, absorbed, activated, deactivated, persistent, short lived, mobile, stationary or eventually a residue problem) may depend upon its transformation by soil microorganisms. The kinetics of decomposition typical for enrichment cultures were established by Audus (19) in

1960 with (2,4-dichlorophenoryl) acetic acid, where the results encouraged the isolation of the soil microorganisms specifically responsible for decomposition of a particular organic chemical. While many microorganisms capable of degrading specific organic chemical have been isolated and characterized, some chemicals failed to support microbial growth or enrichment during the degradation process. Some other chemicals appeared persistent to microbial degradation. Hence, the concept of molecular recalcitrance, molecular fallibility and cometabolism were established by Alexander (20), Alexander and Lustingman (21).

According to Audus (19) first order kinetics apply where the concentration of the chemical being degraded is low relative to the biological activity of soil, which was supported by Hill et al. (5); Burschell and Freed (7); Schuldt, Burchfield and Bluestone (22); Hamaker (23); Sheets (24); Sheets and Harris (25). Michaelis-Menten kinetics seem to apply when the chemical concentration is high, and where the rate of decomposition is independent of concentration. In the dissipation of a chemical from soil, although the loss generally assumes the shape of first order kinetics reaction curve, several mechanisms may be acting on a soil residue at any given time, like volatilization, photodecomposition, mechanical removal by water or wind erosion, leaching, cultivation, adsorption microbial metabolism.

In the dissipation of certain biodegradable chemicals from soil, a lag phase may occur after the initial application in which relatively little chemical is lost. The lag phase is followed by a period of rapid disappearance, as a result of microbial metabolism. For example, in the biodegradation of 2,2-dichloropropionic acid, a lag phase was seen initially followed by rapid loss of chemical by Kaufman (26) in 1964. This type of degradation, however has been observed under isolated cultures with

relatively high concentration of the compound, where the influence of other factors may not be limiting, which certainly doesn't mean that biodegradation is not a significant factor in the dissipation of most of the organic chemicals in the soil, but it is to indicate that the other processes such as volatilization, adsorption, leaching, etc., may limit the availability of the chemical to biodegradation.

Readily biodegradable chemicals are generally degraded more rapidly and without initial lag phase in subsequent application to the soil. This phenomenon has been observed with numerous organic chemicals by Audus (19), Kaufman (26) and Kaufman and Kearney (27).

Hurle and Rodemacher (28) in 1970 compared the dissipation of 4,6-dinitro-o-cresol and (2-4,dichlorophenoryl) acetic acid in soil treated for the first time and pretreated soils from field plots. An interesting factor they noted was that, the pretreatment had no effect on the rate of 2,4-dinitro-o-cresol dissipation from soil, but (2,4-dichlorophenory) acetic acid dissipation was more rapid in the previously treated soil than in the soil treated for the first time.

The actual significance of this phenomenon under field conditions appeared to be dependent upon several factors like

1. rate and frequency of application
2. the time elapsed between applications
3. the cropping systems
4. the survival of an enriched population
5. the complexity of the metabolic reaction involved
6. the physical-chemical behavior of the chemical in soil, and
7. possible interactions of one chemical with another

It has been observed that dissipation of subsequent chemical application occurs more rapidly in soils initially treated at higher applica-

tion rates; which agrees with the fact that microbial populations tend to respond both quantitatively and qualitatively in accordance with the supply of readily available substrate.

The rate and frequency of application and the time elapsed between application could be expected to influence the survival of enriched microbial populations. This was observed by Kaufman (26, p. 24) in 1980 and he stated that an enriched population can survive under cropped field conditions for a period of two or more years.

The survival of an enriched population is affected by several factors. For example, in the case of soil borne plants pathogens, an alternate host, crop debris, or resistant structure are a few factors which enable such organisms to survive from one growing season to the next. Though not much data is available about the survival mechanism of chemically enriched populations, in the absence of such structural features, the chemically enriched populations is assumed to survive either on the trace amounts of the originally enriching substrate, alternate substrates which enable the population to retain its number and/or enzyme potential; though this would be abnormal under natural conditions. Under conditions where the chemicals is adsorbed, the survival of the enriched populations takes place on the slowly desorbed materials. All the chemicals for which enriched population have survived under field conditions are subjected to very limited adsorption. Thus it is reasonable to expect the complete dissipation of the substrate within a relatively short period of time.

The chemical and physical behavior of a chemical in soil may also preclude its biodegradation and/or the development of an enriched population. Structural characteristics may cause a chemical to be recalcitrant to biodegradation. It has been found that there are few organic chemicals

which are not biodegradable at least to some extent. Adsorption to soil constituents is probably the most significant factor limiting the biodegradation of some chemicals in soil. The availability to the microbial population of a substrate in sufficient quantities at a consistent frequency could be a determining factor in the development of an enriched population. For example, 4,6-dinitro-o-cresol is readily degraded by isolated soil microorganisms. In degradation in soil occurs at a slow rate only and wasn't affected by previous treatment. It was stated by Harris and Warren (29) in 1964 (p. 43) that "the biodegradation of 4,6,dinotro-o-cresol in soil is a function of soil pH." Adsorption of this chemical in soil is pH dependent with greatest adsorption (99%) occurring on illite and montmorillonite clays at pH 4.6, where as no adsorption occurs at a pH of 7.3. The availability of 4,5-dinotro-o-cresol to biodegradability may thus be limited in acidic soils, but unlimited in alkaline soils.

The kinetics involved in the development of soil microbial populations capable of degrading organic chemicals, though not fully understood, but the available information indicates that it basically follows the sigmoid growth patterns of isolated microbial cultures initially exposed to fresh, suitable substrates under favorable environmental conditions. An initial lag phase occurs when microbes are initially exposed to fresh medium during which there is little or no growth of the microbes and no degradation takes place.

Following the lag phase is the logarithimic phase, where the microbes proliferate at a maximum rate increasing by geometric progression during which the most rapid utilization o- the substrate occurs. Then the

microbial population enters the stationary phase, where there isn't any growth of the microbes. At this stage if the microbial population is resupplied with additional fresh substrate it will continue to proliferate with either a shorter lag phase or no lag phase at all. In absence of additional substrate the population will enter a death or decline phase, the rate depending upon the species.

Chemical Structure - Biodegradability

Ideally chemicals considered for land treatment should be non toxic, remain stationary at the site of application and degrade rapidly once they are applied. Selection of such chemicals necessitates an understanding of each chemical. Chemical-physical characteristics and the structure activity relationships underlying toxicity, mobility and degradability. Kaufman and Plimmer (30) in 1972 compared the structural features necessary for toxicity to target organisms with those permitting degradation in the environment for several large classes of pesticides. The studies showed that differences existed among the various chemical classes. In some chemical classes those structural features contributing to toxicity were coincident with those necessary for degradability whereas in other chemical classes they were diametrical.

Degradation of Combination Chemicals

Combinations of wastes in soil may result from either the successive application of individual wastes with concomitant cumulation of their residues, or the intentional application of combined wastes. Problems involving the degradation, persistence or toxicity of organic chemical may arise when several wastes or their residues are present in the soil.

Colby et al. (31) in 1967 observed interactions in terms of plant response for some mixtures of organic chemical while most of the interactions resulted in increased phyiotoxicity, a few involved in reduced toxicity.

Interactions leading to decreased persistence may result from chemical reactions or enhanced biodegradation of the combined waste. In 1966 Miller and Lukens (32, p. 36) stated that "the deactivation of sodium methyl dithio carbonate by certain halogenated hydrocarbons is an example of this type of chemical reaction." Sodium methyl dithiocarbamate is degraded when combined with 1,3-dichloropropene, 1,3-di-bromo-3-chloropropene, ethylene bromide or related polyhalogenated alkenes. Another practical application of this phenomenon was the incorporation of small amounts of [(4-chloro-o-roly)oxy] acetic acid into (2,4,5-trichlorophenory) acetic acid solutions facilitated a more rapid degradation of the highly persistent (2,4,5-torchlorophenoxyl) acetic acid.

Foster (33) in 1962 explained this type of reaction as the co-oxidation whereby microorganisms may metabolize a compound without being able to utilize the energy derived from the chemical reaction to sustain growth.

Kaufman (26), Kaufman and Sheets (34), Kaufman and Miller (35) observed the increased persistence of one or more chemicals applied in combination. All of the interactions presently known involve the parent organic molecule (i.e., the interaction of one organic molecule with another). The diversity and multiplicity of organic chemical residues and their degradation produces increases the probability that similar interactions may also occur at various other stages of microbial degradation.

Increased persistence of chemicals may result from several types of interactions.

1. Chemical and physical interaction of the organic molecules occurring in combination may preclude their normal degradation in soil and thus increase their persistence in soil.

2. The biocidal properties of the chemicals to soil microorganisms may preclude their biodegradation.

3. Direct inhibition of the adaptive enzymes of effective soil microorganisms.

4. Inhibition of the proliferation process of effective microorganisms.

An apparent increased chemical persistence may also be explained by reactions of organisms sensitive to the chemical combinations. The sensitivity of organisms to a given chemical may be greater in the presence of a second chemical. Thus, the organism may be affected for a longer period of time due to the enhanced activity of lower chemical concentrations during the time when the soil chemical concentration is actually decreasing.

The aforementioned review of the biodegradability of individual organic chemicals and the combined organic chemicals seems mainly to depend on the chemical structure, some being rapidly degraded and others recalcitrant to degradation. However, to explain the biodegradability of certain organic chemicals graphically, to witness the rate of degradability, to know if any of the selected compounds had any kind of inhibition effects and to gain some knowledge specifically on how long does it take for an organic chemical to totally biodegrade. This research proposal was warranted.

CHAPTER III

MATERIALS AND METHODS

General Experimental Plan

The primary thrust of the project was to use biochemical oxygen demand test as a surrogate parameter to evaluate the biodegradability of specific organic compounds by using biological soil reactors.

Soils were placed in glass biological soil reactors and the wastes were applied to the top and worked into the top 20 centimeters. The wastes selected for the study was synthetic. The biological soil reactors were 3.5 inches in diameter and 7" long. Provisions were made so that the soils could be monitored at various depths.

The soil material utilized for the purpose was port type passing through vs. No. 40 sieve. EPA base mix (37) with concentration of about 250 mg/ℓ was added to the soil as a seed, mainly to activate the microorganisms in the soil. The stock solution of the base mix was prepared in a 2 litre bottle consisting of the following:

Ethylene glycol - 226 ml/2ℓ

Ethylene alcohol - 226 ml/2ℓ

Glucose - 226 gms/2ℓ

Glutomic acid - 226 ml/2ℓ

Acetic acid - 226 ml/2ℓ

Phenol - 45.2 gms/2ℓ

Phosp-oric acid - 31.5 ml/2

Salts:

$CaCl_2$ - 8 mg/ℓ

$MnSO_4$ - 8 mg/ℓ

$FeCl_3 \cdot 6H_2O$ - 0.49 m/ℓ

$MgSO_4 \cdot 7H_2O$ - 80 gm/ℓ

Five ml. of the carbon source and 3 ml. of salt stock solution were used to prepare one litre of the base mix. This had a concentration of about 250 mg/ℓ.

The ratio of soil to base mix was generally maintained at 5:1 ratio, i.e., for about 1000 gms of soil approximately about 200 ml. of the base mix was added so that the final product turned to be a smooth, moderately solid paste and had a moisture content of about 10-20%.

Application of the Waste: The three organic chemicals chosen for the study were:

1. 2,4-Dichlorophenol
2. 2,4-Dinitrophenol
3. Nitrobenzene

Waste (Organic Chemicals)	Soil	Loading
2,4-Dichlorophenol	Port	1000 µg/gm of soil
2,4-Dinitrophenol	Port	1000 µg/gm of soil
Nitrobenzene	Port	1000 µg/gm of soil
Combined Soil Systems		
2,4-Dichlorophenol + 2,4-Dinitrophenol + Nitrobenzene	Port	1000 µg of each comp/gm of soil
2,4-Dichlorophenol + 2,4-Dinitrophenol + Nitrobenzene	Port	500 µg of each comp/gm of soil

The study was mainly divided into two parts. In the first part of the study the three organic chemicals were applied individually along with the base mix the loading rate being 1000 µg/gm of soil and the biochemical oxygen demand was monitored up to 15 days and in the later part of the study, the three organic chemicals were combined together and loaded along with the base mix at a rate of 1000 µg of each comp/gm of soil and 500 µg of each comp/gm of soil, the biochemical oxygen demand being monitored up to 34 days.

Setting of the Columns

The soils seeded with the base mix and loaded with the hazardous waste were set up in glass columns so as to avoid any kind of interference with the analysis. Cheese cloth was used at the bottom of each column to hold the soil in the column.

A triplicate analysis was suggested for each waste, hence a total of nine columns were set for the three compounds the loading rate being 1000 µg/gm of soil.

Another column was set, which contained only the soil and the seed (EPA base mix).

In the later part of the study, where the three compounds were combined six columns were set in a similar fashion, at two different loading rates of 1000 µg/gms and 500 µg/gms, the analysis still being in triplicates.

The columns were mixed thoroughly daily for uniform aeration and a moisture content of 10-20% was maintained in all the columns, by adding some water.

Experimental Procedures

In the beginning of the analysis samples were drawn from the column at time zero, three, six, nine and fifteen days. Three different dilutions of 0.1 gm, 0.3 gm and 0.6 gm of soil were taken from each column. The electronic balance Mettler AE 160 was used to weigh the soils.

BOD Test

At the start of each analysis, the BOD bottles were cleaned with cleaning acid, washed thoroughly with tap water and rinsed with distilled water. The cleaning acid was prepared in accordance with the standard methods (38).

The soils weighed were put in the BOD bottles.

Dilution water was prepared in a 20-litre aspirator bottle. Distilled water was used as dilution water, and the reagents used to make the BOD dilution water were in accordance with the standard methods (38). Compressed air was applied to the dilution water to aerate it, for about an hour, so that the dilution water had a DO of about 7.5 - 8.5 mg/ℓ.

In the whole analysis no additional seed of any kind was used.

The dilution water was transferred into all the BOD bottles and the initial DO was read with the help of a oxygen electrode. (Oxygen-Electrode; Orion Research, Model 97-08-00).

The bottles were sealed air tight and incubated at ambient temperature (20 ± 1C) DO determinations were performed every day for five days.

In the last part of the research with combined wastes, dilution of 0.1 gm, 0.2 gm, 0.4 gm of the soil were used and the analysis was extended

to 34 days, the samples being taken at an interval of 3 days for the first 15 days and for the rest of the analysis samples were collected after a period of 7 days.

CHAPTER IV

RESULTS

These experiments were designed to evaluate the BOD test as a surrogate parameter for measuring the organic contaminates remaining in a land treatment system.

The entire research was divided into two parts: in the first part of the research four different soil systems were considered. The first soil system had 2,4 Dinitrophenol and the base mix, the second system had 2,4 Dichlorophenol and base mix, the third system had nitrobenzene and the base mix and the fourth system had base mix alone. The amount of the organic contaminates present in each soil system was 1000 µg/gm of soil. In the second part of the research the three organic compounds were combined and loaded at two different loading rates of 1000 µg/GM of each compound per gram of soil and 500 µG of each compound per gram of soil. The results presented are the oxygen utilization (in other words biochemical oxygen demand) expressed in MG/GM of dry soil.

Figures 1-4 illustrate oxygen utilization in MG/GM of soil for the soil systems loaded with base mix and the base mix plus 2,4 Dinitrophenol, 2,4 Dichlorophenol and Nitrobenzene. The samples were taken on the day of loading. No lag period of oxygen consumption occurred with any of these systems for the concentrations considered. During first two days the oxygen consumption or BOD exertion increased significantly, especially for lower dilutions, after which the consumption slowed down. These curves pretty much follow the first order decreasing rate of kinetics like any

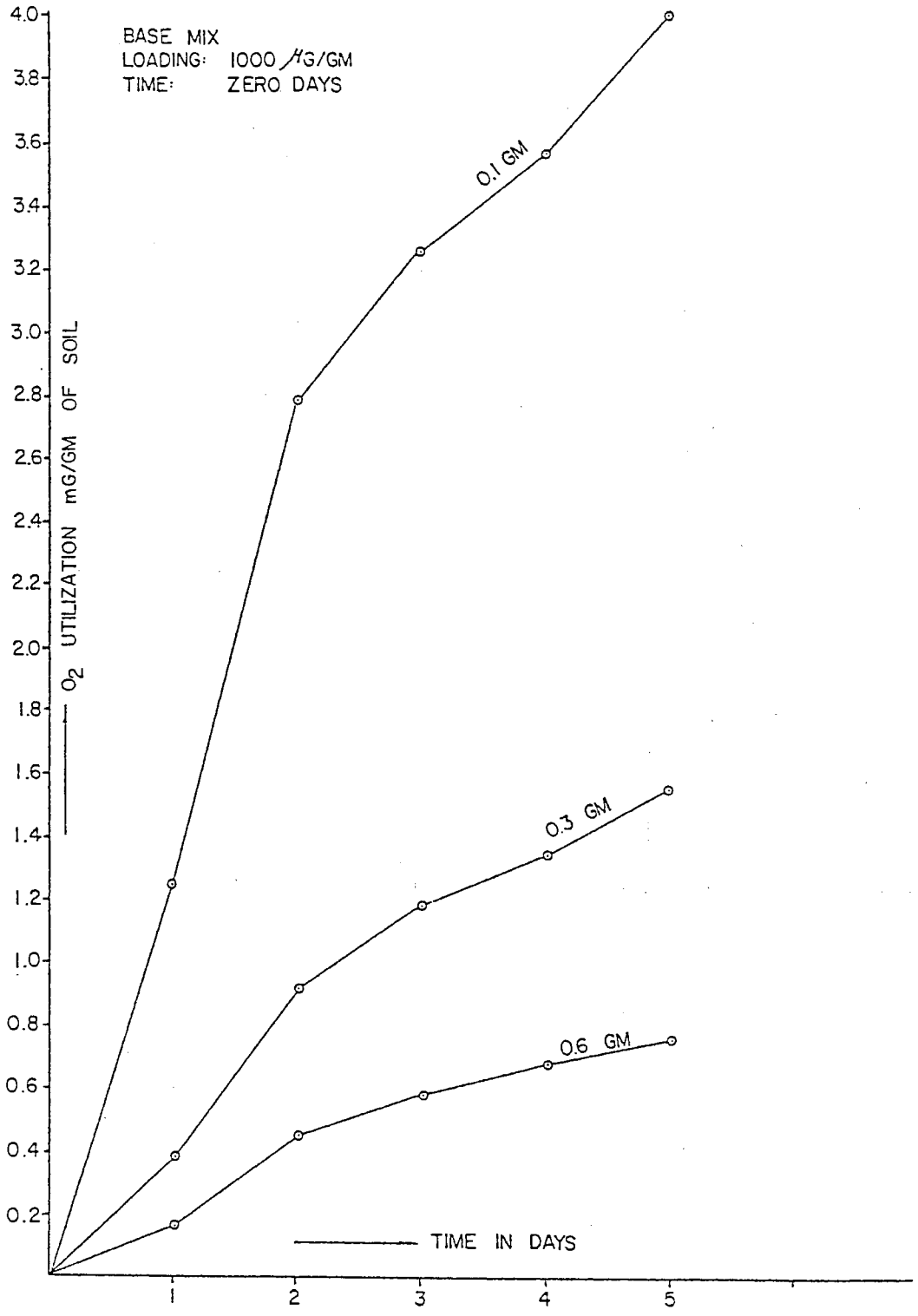

Figure 1. Oxygen Utilization Vs. Time Curves for the Base Mix at Time Zero

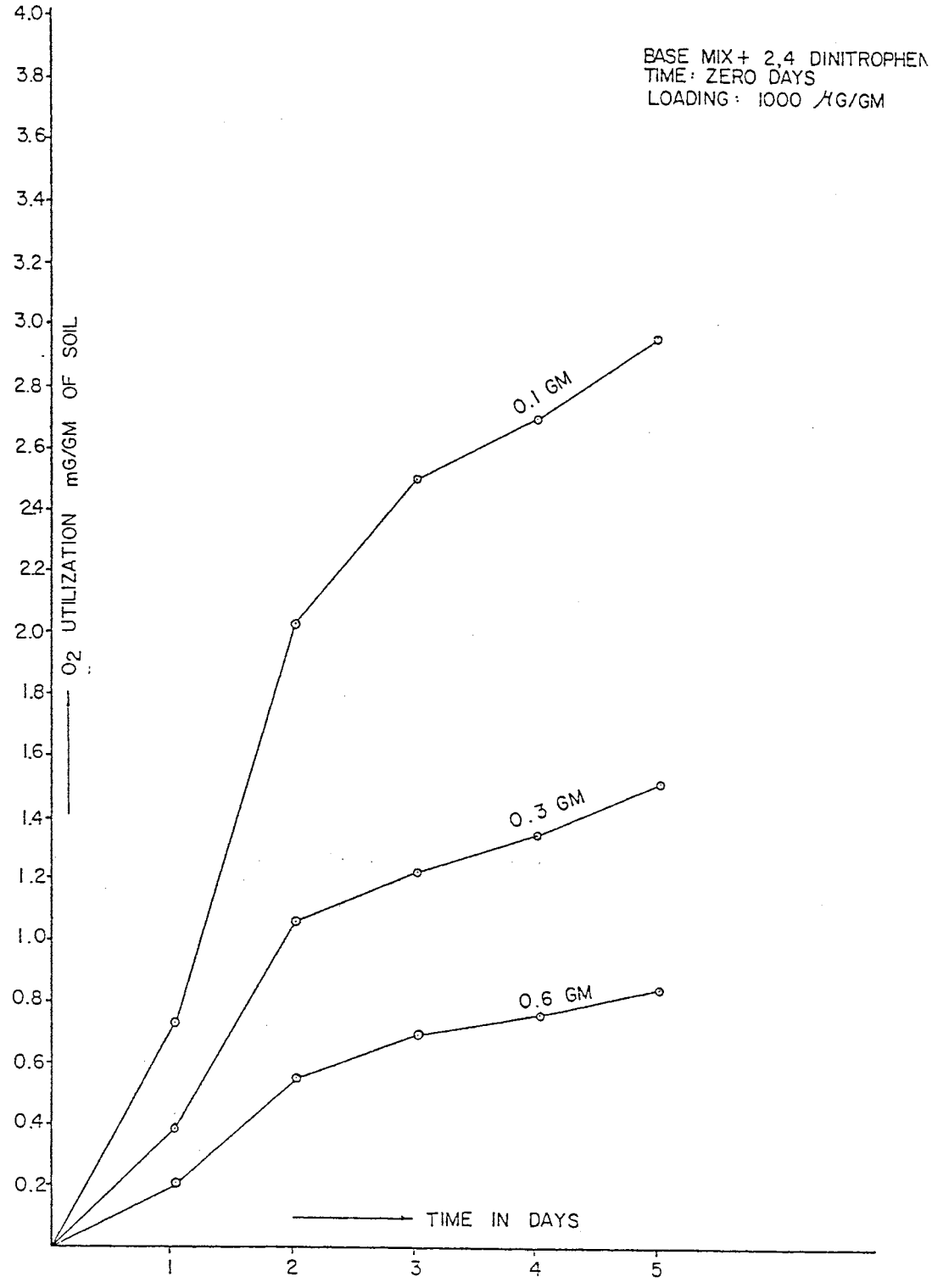

Figure 2. Oxygen Utilization Vs. Time Curves for Base Mix Plus 2,4 Dinitrophenol at Time Zero

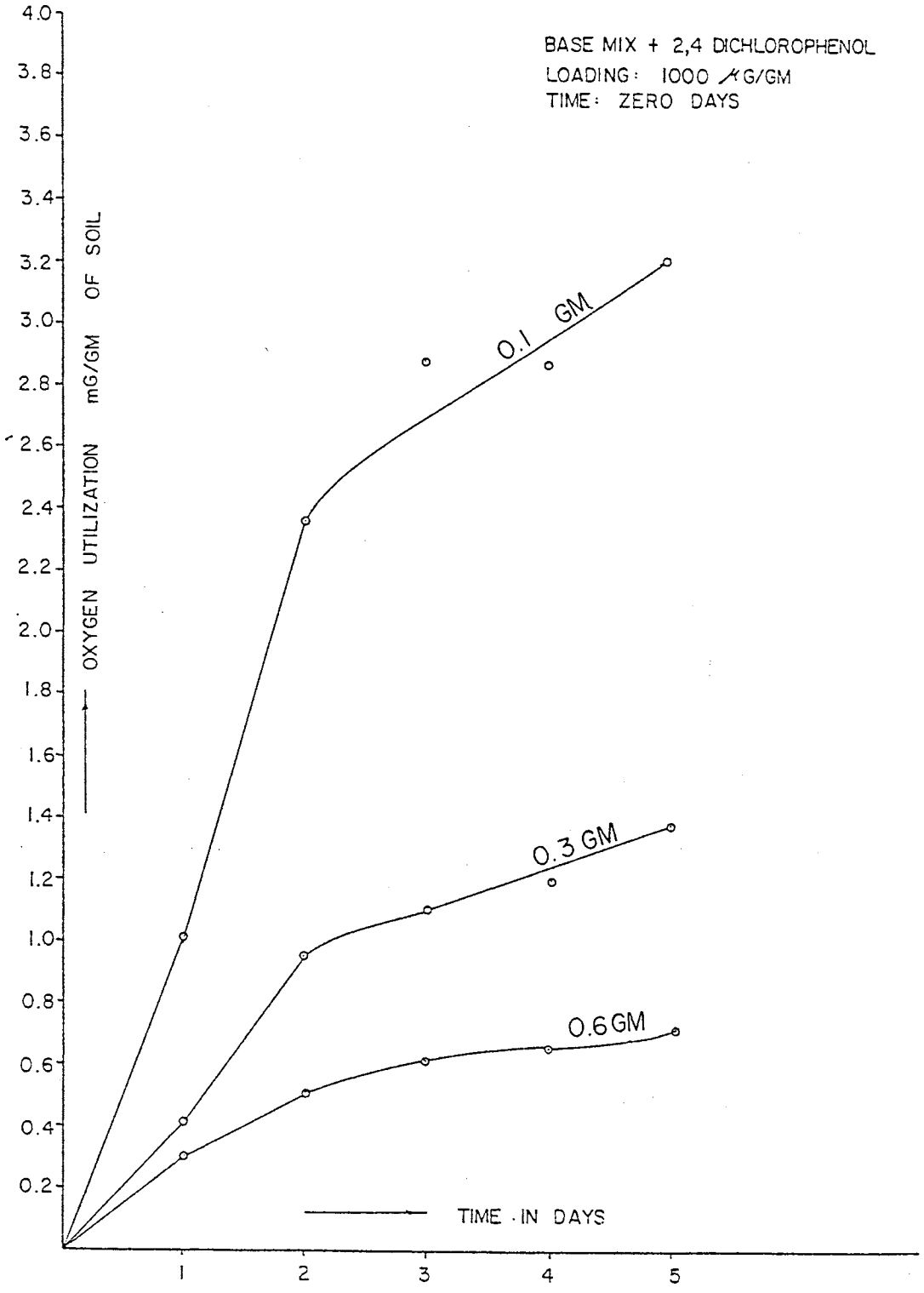

Figure 3. Oxygen Utilization Vs. Time Curves for Base Mix Plus 2,4 Dichlorophenol at Time Zero

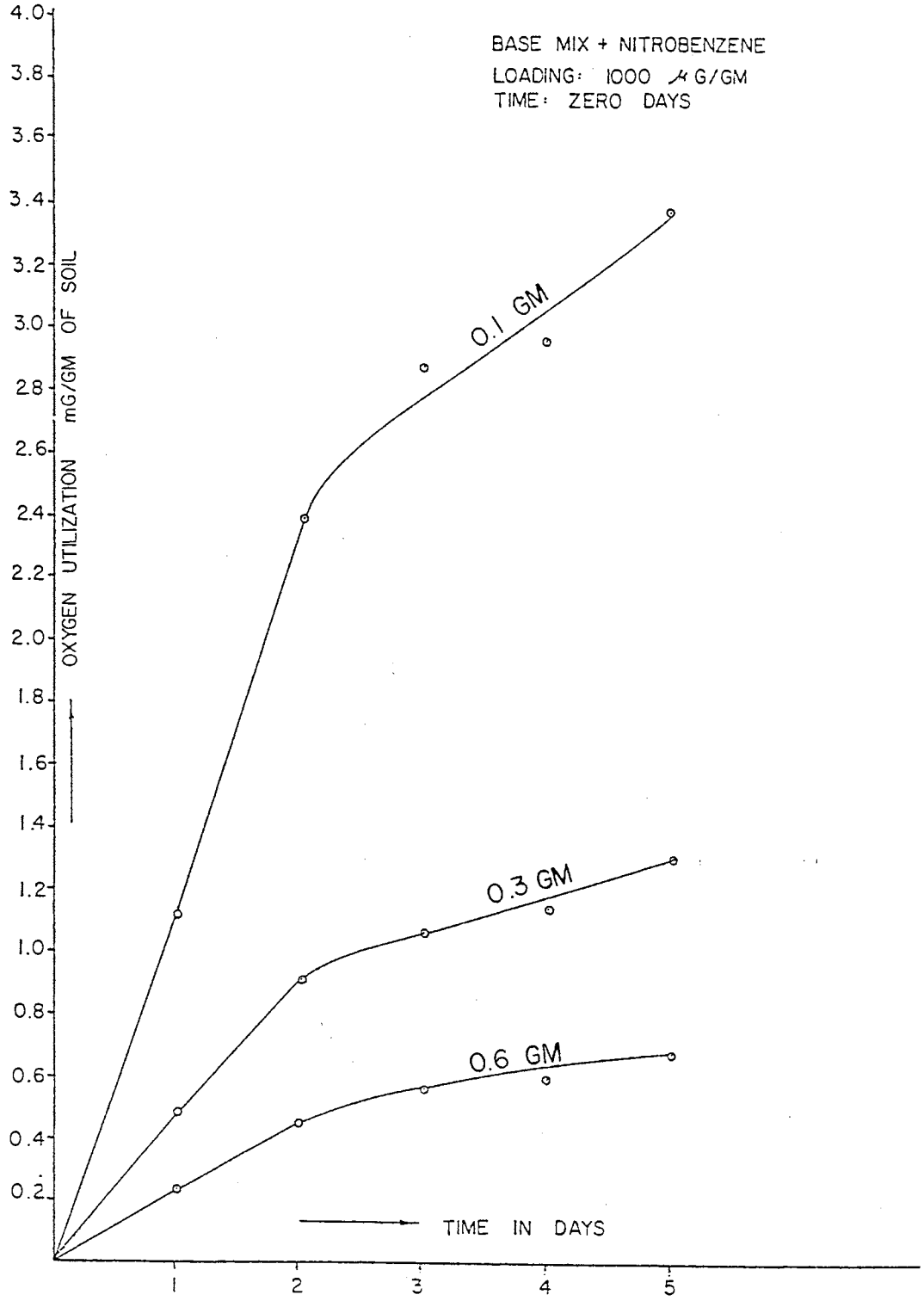

Figure 4. Oxygen Utilization Vs. Time Curves for Base Mix Plus Nitrobenzene at Time Zero

other BOD curve. From the figures it can also be noticed that the lower dilutions or the lower concentrations of the sample had higher BOD exertion. Also the base mix system had higher BOD exertion as compared to the other systems. The results of this analysis are tabulated in Table I.

The oxygen utilized by the soil systems three days from the time of application of the waste is listed in Table II. Again no lag in oxygen consumption was observed. The consumption rate increased significantly for the first two days and then slowed down. The BOD was comparatively less than the previous analysis, but the lower concentrations of the sample still had higher BOD exertions, rather than equal exertion.

The analysis was repeated after six days from the initial application of the waste. In this case the oxygen consumption was increasing for the first two days. There was not any noticeable change in oxygen consumption during the third day. The consumption slightly increased during the fourth day and remained pretty much the same for the fifth day. The BOD of the soil systems continued to decrease with the BOD still being low for higher concentrations of the soil sample. The results of this analysis are tabulated in Table III.

After a period of nine days the analysis showed a lag in oxygen consumption for the first day for the systems containing the three organic compounds. The base mix system showed no sign of oxygen consumption during the first day. The consumption started to increase from the second day, and the three organic compound systems showed an increase in amount of oxygen used, as compared to the six day period analysis, while the base mix system continued to decrease. The results of this analysis are tabulated in Table IV.

TABLE I

SUMMARY OF THE OXYGEN UTILIZATION DATA OF THE FOUR INDIVIDUAL SOIL SYSTEMS FOR THE TIME ZERO DAYS

Name of the Compound	Actual Wt. of Sample	Moisture Content of Sample	Dry Wt. of the Sample (Gms)	Oxy Dep. $\frac{Mg}{Lit} \times \frac{300\ ml}{Dry\ Wt.\ of\ Soil} \times \frac{Lit}{1000\ ml}$ Oxygen Depletion: Mg/Gm				
				1st Day	2nd Day	3rd Day	4th Day	5th Day
2,4 Dinitrophenol	0.1790	15.79%	0.1507	0.7166	2.09	2.488	2.687	2.946
2,4 Dinitrophenol	0.3487	15.79%	0.2936	0.378	1.052	1.205	1.338	1.49
2,4 Dinitrophenol	0.6540	15.79%	0.5507	0.1961	0.544	0.686	0.751	0.833
2,4 Dichlorophenol	0.1218	13.26%	0.1056	1.079	2.357	2.869	2.869	3.21
2,4 Dichlorophenol	0.3135	13.26%	0.2719	0.419	0.948	1.103	1.191	1.379
2,4 Dichlorophenol	0.6172	13.26%	0.5353	0.299	0.509	0.616	0.65	0.717
Nitrobenzene	0.1255	14.28%	0.1075	1.116	2.372	2.874	2.958	3.376
Nitrobenzene	0.3473	14.28%	0.2977	0.483	0.906	1.068	1.138	1.31
Nitrobenzene	0.6585	14.28%	0.5644	0.233	0.457	0.563	0.59	0.68
Base Mix	0.1105	14.00%	0.0972	1.234	2.777	3.24	3.549	4.166
Base Mix	0.32	14.00%	0.2816	0.372	0.905	1.171	1.33	1.544
Base Mix	0.6532	14.00%	0.5748	0.156	0.443	0.574	0.678	0.756

TABLE II

SUMMARY OF THE OXYGEN UTILIZATION DATA OF THE FOUR INDIVIDUAL SOIL SYSTEMS FOR THE TIME THREE DAYS

Name of the Compound	Actual Wt. of Sample	Moisture Content of Sample	Dry Wt. of the Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy Dep. $\frac{Mg}{Lit} \times \frac{300\ ml}{Dry\ Wt.\ of\ Soil} \times \frac{Lit}{1000\ ml}$				
				1st Day	2nd Day	3rd Day	4th Day	5th Day
2,4 Dinitrophenol	0.1097	13.05%	0.0953	0.629	1.416	1.762	1.825	2.203
2,4 Dinitrophenol	0.3273	13.05%	0.2845	0.189	0.421	0.632	0.643	0.79
2,4 Dinitrophenol	0.6810	13.05%	0.5921	0.11	0.253	0.329	0.334	0.461
2,4 Dichlorophenol	0.1082	11.53%	0.0957	0.564	1.253	1.598	1.598	2.068
2,4 Dichlorophenol	0.3019	11.53%	0.2670	0.168	0.483	0.651	0.674	0.786
2,4 Dichlorophenol	0.6018	11.53%	0.5324	0.10	0.231	0.298	0.309	0.422
Nitrobenzene	0.1206	12.12%	0.1059	0.509	1.161	1.586	1.586	2.124
Nitrobenzene	0.3378	12.12%	0.2968	0.181	0.404	0.566	0.566	0.788
Nitrobenzene	0.6157	12.12%	0.5410	0.105	0.221	0.304	0.304	0.432
Base Mix	0.1173	9.4%	0.1062	0.706	1.412	1.836	1.977	2.118
Base Mix	0.3877	9.4%	0.3512	0.128	0.341	0.512	0.597	0.64
Base Mix	0.6053	9.4%	0.5484	0.10	0.223	0.365	0.41	0.464

TABLE III

SUMMARY OF THE OXYGEN UTILIZATION DATA OF THE FOUR INDIVIDUAL SOIL SYSTEMS FOR THE TIME: SIX DAYS

Name of the Compound	Actual Wt. of Sample	Moisture Content of Sample	Dry Wt. of the Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy Dep. $\frac{Mg}{Lit} \times \frac{300\ ml}{Dry\ Wt.\ of\ Soil} \times \frac{Lit}{1000\ ml}$				
				1st Day	2nd Day	3rd Day	4th Day	5th Day
2,4 Dinitrophenol	0.1114	10.71%	0.0994	0.603	1.237	1.237	1.659	1.69
2,4 Dinitrophenol	0.3245	10.71%	0.2867	0.207	0.321	0.341	0.528	0.569
2,4 Dinitrophenol	0.6260	10.71%	0.5589	0.107	0.203	0.203	0.327	0.327
2,4 Dichlorophenol	0.1071	9.49%	0.0969	0.619	0.99	0.99	1.3	1.3
2,4 Dichlorophenol	0.3154	9.49%	0.2854	0.22	0.336	0.336	0.494	0.494
2,4 Dichlorophenol	0.6217	9.49%	0.5627	0.106	0.159	0.159	0.261	0.261
Nitrobenzene	0.1081	12.84%	0.0942	0.764	1.082	1.082	1.401	1.401
Nitrobenzene	0.3082	12.84%	0.2686	0.212	0.335	0.335	0.491	0.491
Nitrobenzene	0.6009	12.84%	0.5237	0.126	0.183	0.183	0.252	0.252
Baxe Mix	0.1081	8.9%	0.0984	0.457	1.067	1.067	1.371	1.371
Base Mix	0.3158	8.9%	0.2876	0.26	0.365	0.417	0.521	0.521
Base Mix	0.6183	8.9%	0.5632	0.5632	0.133	0.186	0.292	0.292

TABLE IV

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE FOUR INDIVIDUAL SOIL SYSTEMS FOR THE TIME: NINE DAYS

Name of the Compound	Actual Wt. of Sample	Moisture Content of Sample	Dry Wt. of the Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy Dep. $\frac{Mg}{Lit} \times \frac{300\ ml}{Dry\ Wt.\ of\ Soil} \times \frac{Lit}{1000\ ml}$				
				1st Day	2nd Day	3rd Day	4th Day	5th Day
2,4 Dinitrophenol	0.1131	12.69%	0.0987	0.091	0.486	1.003	2.37	2.735
2,4 Dinitrophenol	0.3344	12.69%	0.2919	0.03	0.184	0.308	0.801	0.883
2,4 Dinitrophenol	0.6084	12.69%	0.5311	0.02	0.118	0.197	0.480	0.525
2,4 Dichlorophenol	0.1030	11.34%	0.0913	0.08	0.427	0.755	2.135	2.3
2,4 Dichlorophenol	0.3131	11.34%	0.2775	0.04	0.172	0.281	0.789	0.875
2,4 Dichlorophenol	0.6155	11.34%	0.5457	0.02	0.082	0.164	0.401	0.439
Nitrobenzene	0.1095	12.97%	0.0952	0.1	0.315	0.724	2.205	2.3
Nitrobenzene	0.3004	12.97%	0.2614	0.052	0.114	0.241	0.814	0.895
Nitrobenzene	0.6226	12.97%	0.5418	0.03	0.083	0.155	0.448	0.487
Base Mix	0.1138	11.84%	0.1003	0	0.448	0.747	1.196	1.345
Base Mix	0.3058	11.84%	0.2695	0	0.222	0.389	0.445	0.5
Base Mix	0.6118	11.84%	0.5393	0	0.11	0.139	0.222	0.25

During the final period of this analysis (i.e., after fifteen days from the initial application of the waste), the data shows a steady increase in oxygen uptake from the first day for the systems receiving the three organic compounds. Also there was an increase in the amount of oxygen used up by the systems containing 2,4 Dichlorophenol and nitrobenzene as compared to the nine day period analysis. However, the 2,4 Dinitrophenol system showed a decrease in the amount of oxygen used, especially for the lower dilutions of the soil sample. Also the system containing the base mix alone showed no oxygen consumption for the first two days of testing. But the BOD of this base mix system continued to decrease reaching as low as 0.52 Mg/GM of soil. Like all the previous analysis, the BOD exertion was higher for lower concentrations of the sample. The results of this analysis is tabulated in Table V.

The oxygen depletion as measured in MG/LIT was approximately the same for all the concentrations of the soil samples considered, which resulted in higher concentrations of the soil samples having lower BOD exertion (expressed in MG/GM of dry soil). Figures 5, 6, and 7 show the BOD_5 vs time (sampling period) (i.e., rate of biodegradation with time). From the figures it can be seen that the three systems containing 2,4 Dinitrophenol, 2,4 Dichlorophenol and nitrobenzene more or less follow the same rate of biodegration as the base mix system for a time period of six days. After six days the BOD_5 of the base mix system levels off for three days and then starts to decrease again, while the BOD_5 of the three organic compound systems increased after the six day time period. The BOD_5 of the 2,4 Dinitrophenol system did decrease again for the 0.1 GM concentration of the sample, but did not for other sample sizes.

The microorganisms in the three soil systems containing 2,4 Dinitrophenol 2,4 Dichlorophenol and nitrobenzene were not measuring the organic

TABLE V

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE FOUR INDIVIDUAL SOIL SYSTEMS FOR THE TIME: FIFTEEN DAYS

Name of the Compound	Actual Wt. of Sample	Moisture Content of Sample	Dry Wt. of the Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy Dep. $\frac{Mg}{Lit} \times \frac{300\ ml}{Dry\ Wt.\ of\ Soil} \times \frac{Lit}{1000\ ml}$				
				1st Day	2nd Day	3rd Day	4th Day	5th Day
2,4 Dinitrophenol	0.1345	13.27%	0.1166	0.283	0.54	0.926	1.312	2.058
2,4 Dinitrophenol	0.3211	13.27%	0.2784	0.161	0.269	0.463	0.646	0.969
2,4 Dinitrophenol	0.6019	13.27%	0.5220	0.12	0.206	0.293	0.419	0.574
2,4 Dichlorophenol	0.1339	11.32%	0.1187	0.328	0.834	1.415	1.794	2.527
2,4 Dichlorophenol	0.3071	11.32%	0.2723	0.176	0.308	0.506	0.694	1.046
2,4 Dichlorophenol	0.6089	11.32%	0.5399	0.11	0.194	0.25	0.394	0.561
Nitrobenzene	0.1158	14.45%	0.099	0.484	0.787	1.363	1.818	2.606
Nitrobenzene	0.3221	14.45%	0.2755	0.217	0.359	0.555	0.762	1.034
Nitrobenzene	0.6135	14.45%	0.5248	0.11	0.177	0.274	0.371	0.52
Base Mix	0.1275	12.65%	0.1113	0	0	0.269	0.404	0.539
Base Mix	0.3144	12.65%	0.2746	0	0	0.109	0.218	0.382
Base Mix	0.6157	12.65%	0.5378	0	0	0	0.11	0.2

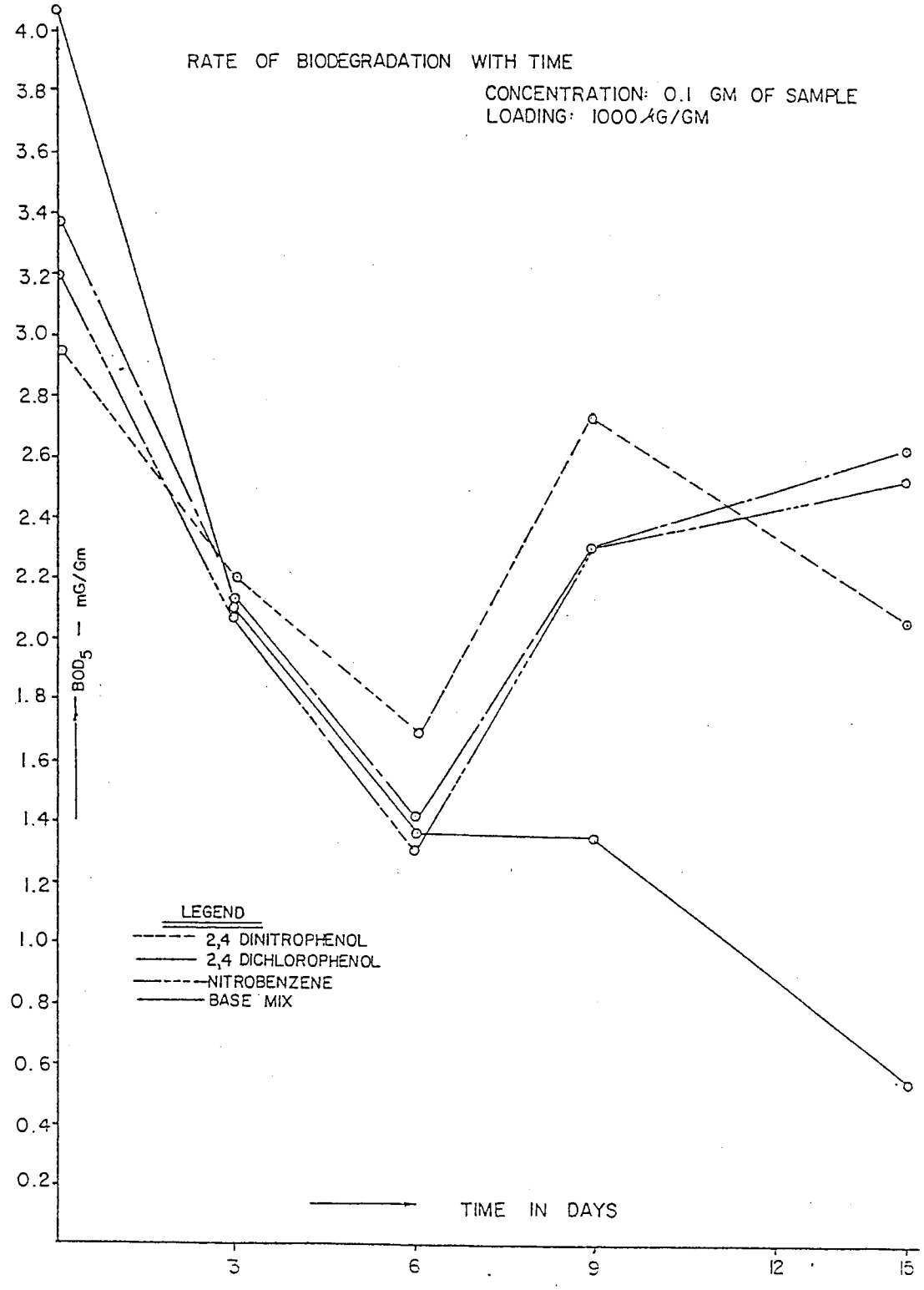

Figure 5. Rate of Biodegradation With Time
0.1 Gm of Sample

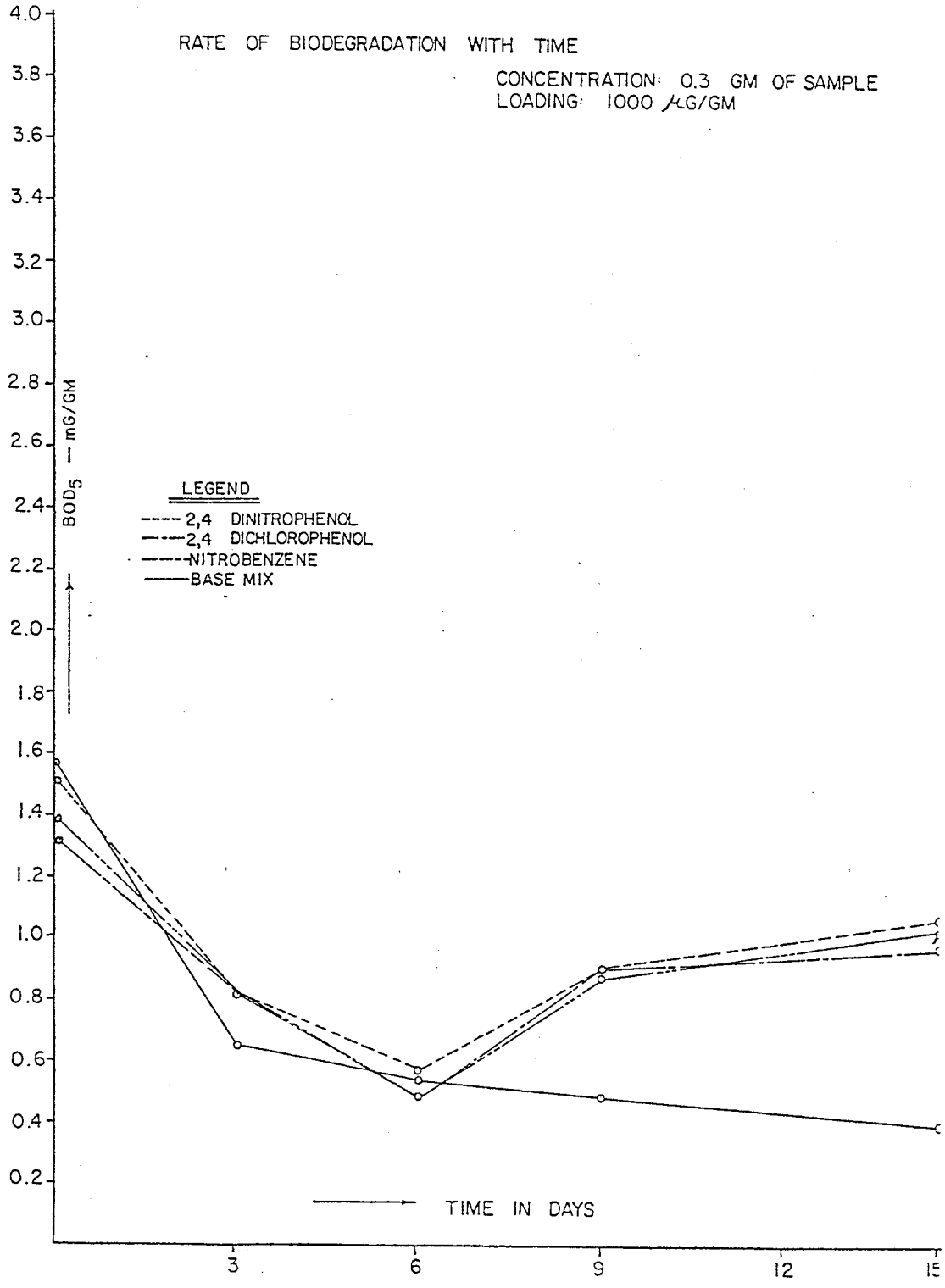

Figure 6. Rate of Biodegradation With Time 0.3 Gm of Sample

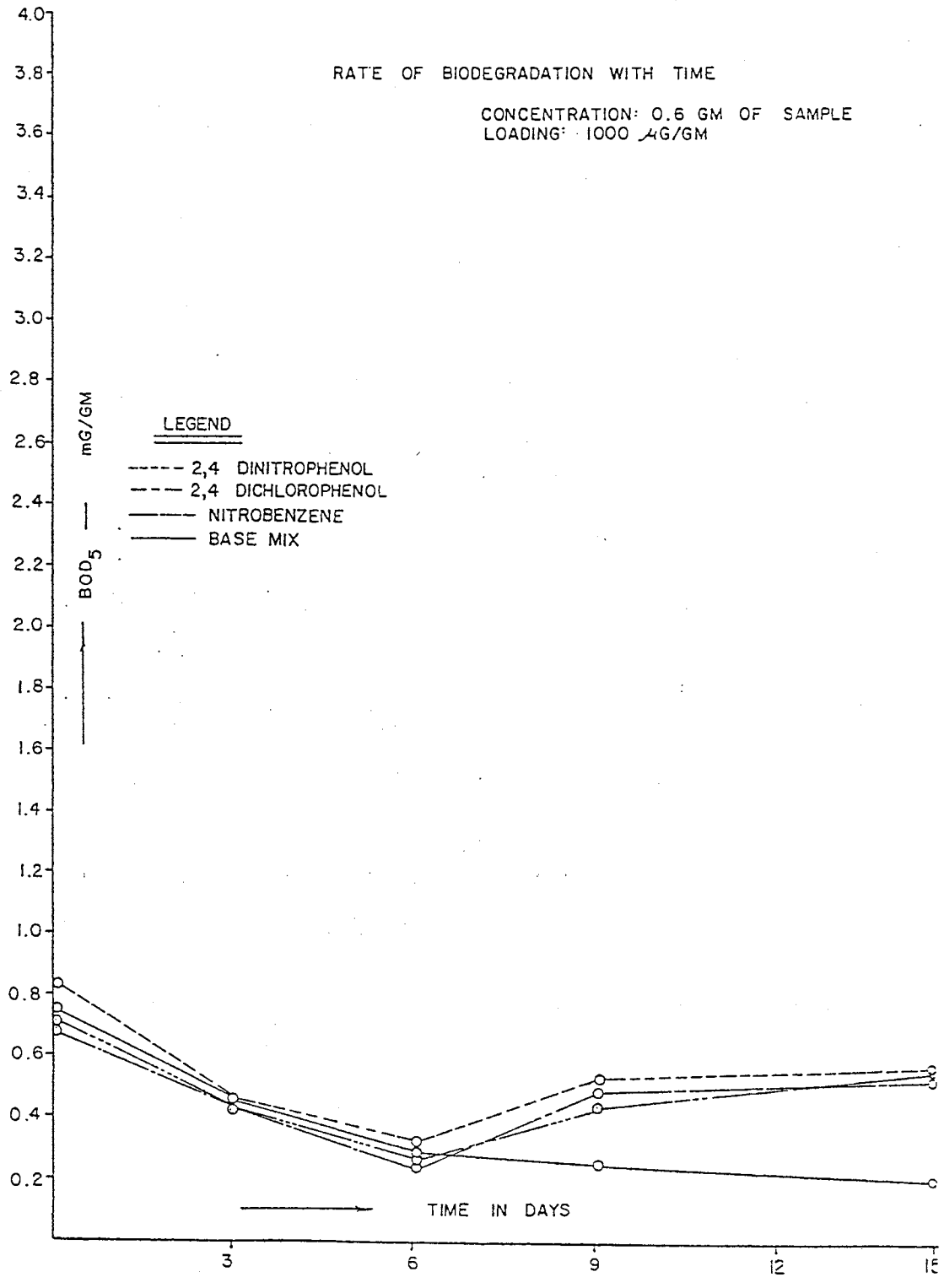

Figure 7. Rate of Biodegradation With Time 0.6 Gm of Sample

compounds for the first six day time period. The oxygen consumption was mainly due to the base mix present in these soil systems. A possible reason these soil systems turned out to have a slightly lower BOD_5 than the base mix system, although the other systems had equal amounts of base mix present in them, is that the organic compounds present in the system along with the base mix, were inhibiting the microorganisms and preventing them from using the base mix. After the six day time period the BOD_5 in the base mix systems levels off for three days, during which the microorganisms started to utilize the organic compounds present in these systems, and this can be seen by a sudden increase in the BOD_5 of the soil systems containing the three organic compounds, For all the dilutions considered. After the nine day period the BOD_5 of the base mix system did decrease again. And so did the BOD_5 of the soil system containing 2,4 Dinitrophenol plus base mix. Rest of the soil systems containing the 2,4 Dichlorophenol and nitrobenzene, including the higher sample sizes of the system containing 2,4 Dinitrophenol showed a slight increase in BOD_5 compared to the nine day period. The data for these studies are tabulated in Table VI.

Combined Reactors

In the second part of the research similar studies were conducted combining the three organic compounds and loading them at two different rates, 1000 µg of each compound per gram of soil and 500 µg of each compound per gram of soil. The dilutions considered for the analysis were 0.1 gm, 0.2 gm and 0.4 gm of soil sample.

At the time of loading (i.e., time zero) the BOD exertion was monitored with time up to twenty days for both the loadings. In all the

TABLE VI

BOD_5 OF THE FOUR INDIVIDUAL SOIL SYSTEMS

Name of the Compound	Dry Wt. of the Sample (Gms)	Biochemical Oxygen Demand - Mg/Gm				
		0 Days	3 Days	6 Days	9 Days	15 Days
Base Mix	0.1	4.166	2.118	1.371	1.345	0.539
	0.3	1.544	0.64	0.521	0.5	0.382
	0.6	0.756	0.464	0.292	0.25	0.2
2,4 Dinitrophenol	0.1	2.946	2.203	1.69	2.735	2.058
	0.3	1.49	0.79	0.569	0.883	0.969
	0.6	0.833	0.461	0.327	0.525	0.574
2,4 Dichlorophenol	0.1	3.21	2.068	1.3	2.3	2.527
	0.3	1.379	0.786	0.494	0.875	1.046
	0.6	0.717	0.422	0.261	0.439	0.561
Nitrobenzene	0.1	3.376	2.124	1.401	2.3	2.606
	0.3	1.31	0.788	0.491	0.895	1.034
	0.6	0.68	0.432	0.252	0.487	0.52

cases the BOD exertion had flattened out by 17 days of testing with no increase in BOD to 20 days.

BOD exertion or oxygen utilization for the combined systems are shown in Figures 8 and 9. It can be seen that a lag period of oxygen consumption occurred for both loadings. This lag period of oxygen consumption occurred for the first two days after which there was a significant increase in the consumption of oxygen during the third day, followed by a very slight increase during the fourth and fifth days. The BOD values determined at the end of five days still turned out to be higher for lower concentrations of the sample. The data seemed to follow a first order decreasing rate of kinetics like any other BOD curve. Also these lag periods could have a minor effect on the five day BOD values. This data is tabulated in Table VII.

Oxygen uptake data for samples taken three days after application of waste are tabulated in Table VIII. From the data it can be seen that for the higher loading i.e., 1000 µg/gm of soil a lag period in the oxygen consumption occurred again for the first two days of testing with a considerable increase in the consumption rate during the third day. The lower loading system (i.e., the system with 500 µg/gm of soil), did not show any significant lag; however, in both loadings a small amount of removal was seen with oxygen uptake still being higher for the lower concentrations of the sample.

The six day sampling period analysis did not show any significant difference from the three day period analysis. The higher loading still showed a lag period during the first two days of testing with a considerable increase in oxygen consumption during the third day. The lower loading column did show a steady uptake, but the five day BOD values were not

TABLE VII

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: ZERO DAYS

Actual Wt. of Sample (Gm)	Moisture Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion Mg/Gm Oxy Dep. Mg/L x 300 ml/Dry Wt. of Soil x Lit/1000 ml.																		
			1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day	8th Day	9th Day	10th Day	11th Day	12th Day	13th Day	14th Day	15th Day	16th Day	17th Day	18th Day	19th Day	20th Day
Loading Rate: 1000 µg/gm																					
0.1035	18.78%	0.0840	0.7	1.071	4.285	4.46	4.82	5.89	6.60	7.67	8.035	9.28	9.28	9.46	9.82	9.82	9.82	10.89	11.07	11.07	11.07	11.07
0.2018	18.78%	0.1639	0.353	0.732	3.11	3.29	3.47	3.84	4.12	4.30	4.48	5.21	5.30	5.39	5.49	5.49	5.49	5.94	6.13	6.13	6.13	6.13
0.4012	18.78%	0.3258	0.12	0.506	2.11	2.2	2.3	2.8	3.08	3.26	3.49	3.82	3.86	3.91	4.05	4.05	4.05	4.18	4.28	4.28	4.28	4.28
Loading Rate: 500 µg/gm																					
0.1190	16.26%	0.0996	0.6	0.753	2.4	2.56	2.71	3.31	4.06	4.36	4.66	5.27	5.27	5.27	5.57	5.57	5.87	6.47	6.92	6.92	6.92	6.92
0.2236	16.26%	0.1872	0.25	0.56	1.6	1.76	1.92	2.08	2.48	2.64	2.72	3.28	3.28	3.44	3.52	3.6	3.84	4.0	4.16	4.16	4.16	4.16
0.4050	16.26%	0.3391	0.11	0.353	1.41	1.45	1.54	1.81	2.079	2.167	2.21	2.47	2.52	2.6	2.69	2.74	2.83	3.0	3.09	3.09	3.09	3.09

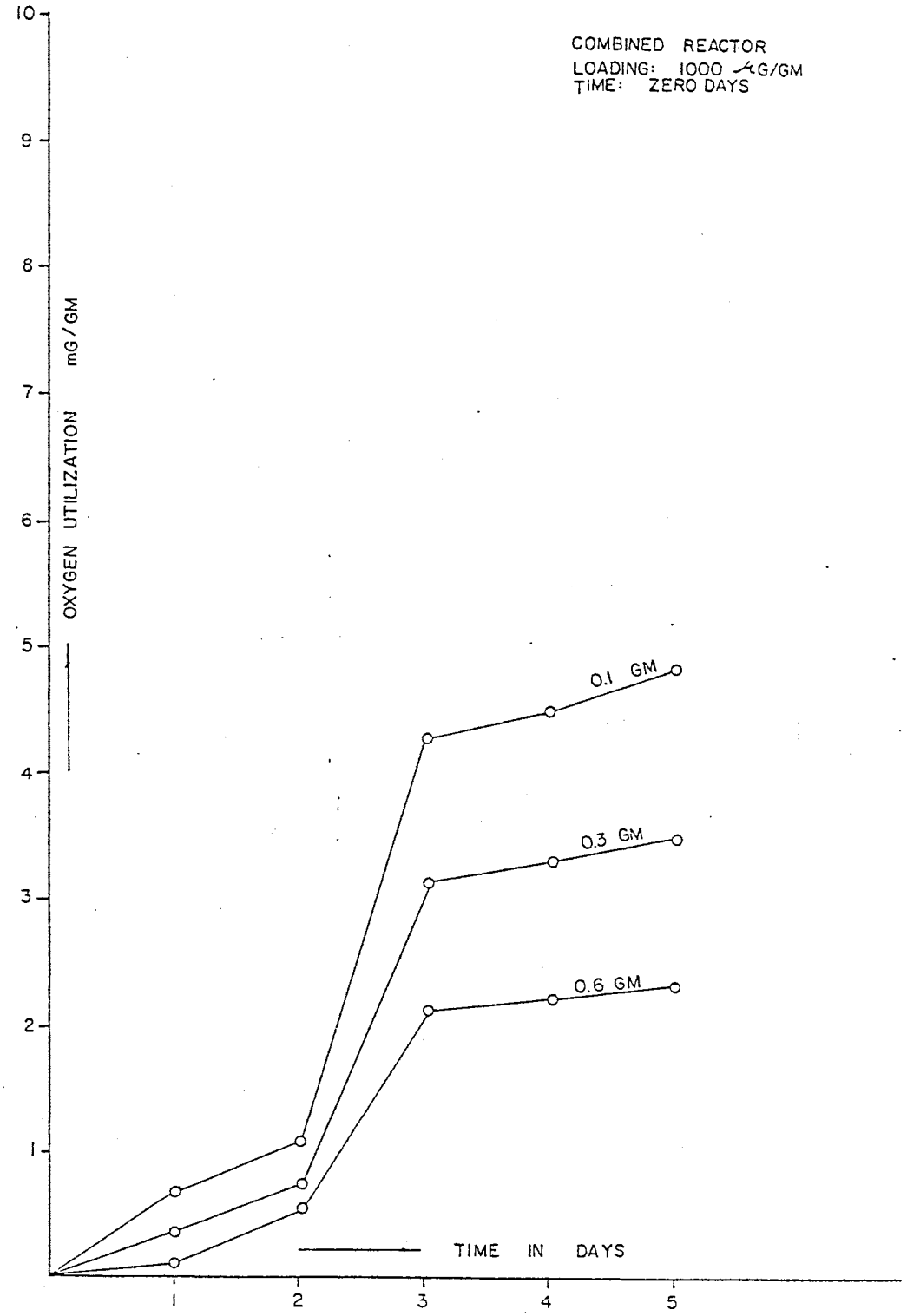

Figure 8. Oxygen Utilization Vs. Time Curves for the Combined Reactors at Time Zero Days for 1000 µg/gm of Loading

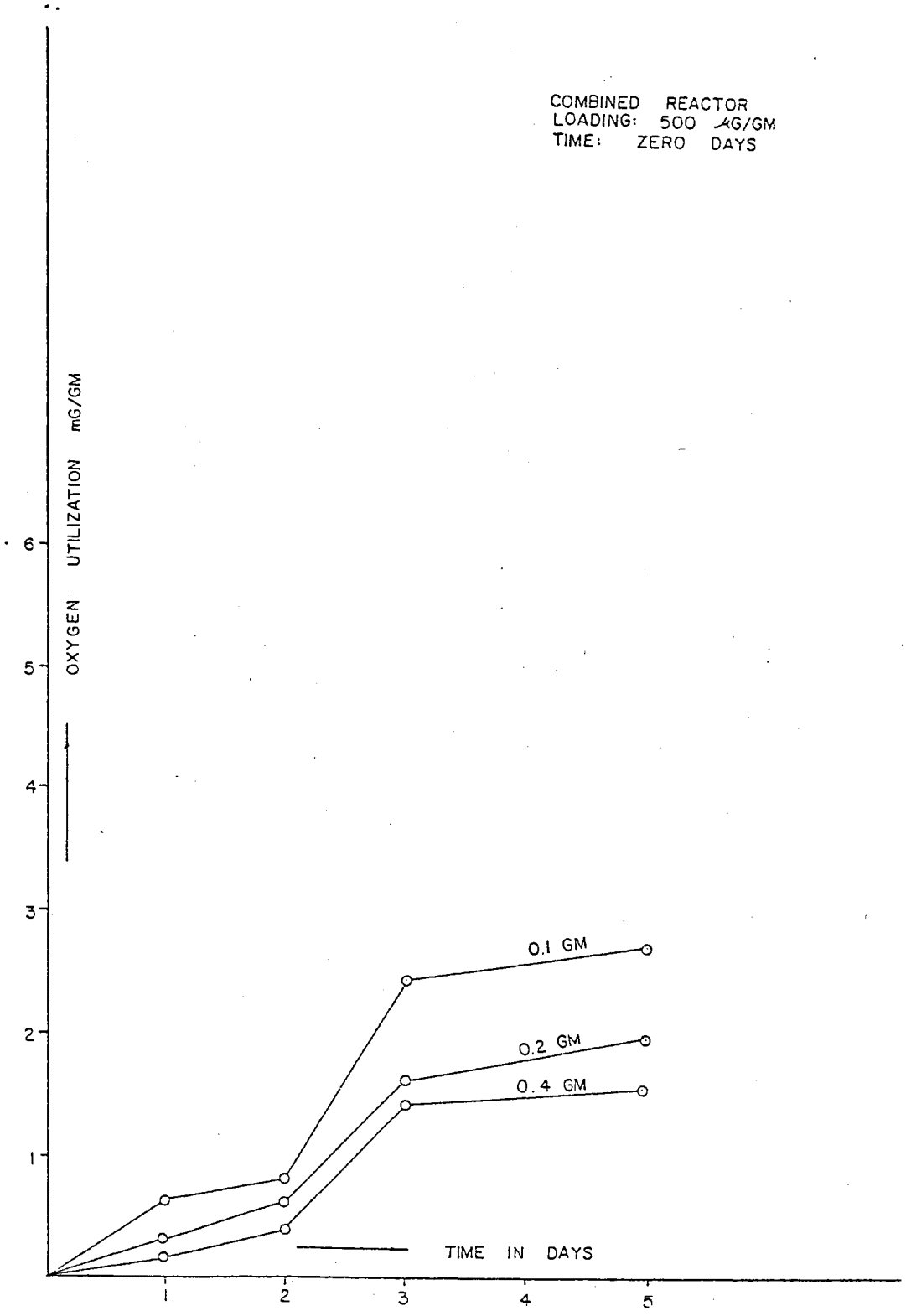

Figure 9. Oxygen Utilization Vs. Time Curves for the Combined Reactors at Time Zero Days for 500 µg/gm of Loading

TABLE VIII

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: THREE DAYS

Actual Wt. of Sample (Gms)	Moisture Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
Loading Rate: 1000 µg/gm							
0.1173	14.29%	0.1005	0.512	0.637	2.537	2.776	3.761
0.2110	14.29%	0.1808	0.213	0.414	1.991	2.157	2.621
0.4112	14.29%	0.3524	0.127	0.263	1.031	1.66	1.95
Loading Rate: 500 µg/gm							
0.1106	12.81%	0.0964	0.495	0.65	1.4	1.587	2.42
0.2100	12.81%	0.1830	0.207	0.480	1.14	1.278	1.72
0.4079	12.81%	0.3556	0.11	0.251	0.97	1.037	1.366

much different from the three day BOD_5 values. Also in both loadings the lower concentration of the sample turned out to have higher BOD_5 values. The results of this analysis are tabulated in Table IX.

The results of the nine day sampling period analysis are tabulated in Table X. An important observation of this analysis was that there wasn't any lag period in oxygen consumption for either loadings. Both loadings showed a steady oxygen consumption. The five day BOD values were slightly higher than the previous analysis, especially for the higher loading. This probably tells us that the initial lag period in oxygen consumption could effect the five day BOD values. Also in both the loadings the lower concentrations of the sample turned out to have higher BOD values.

The analysis was repeated after twelve days from the application of the waste. (The column with higher loading (i.e., 1000 µg/gm) of soil again showed an initial lag period for the first couple of days. The oxygen consumption was pretty steady for the lower loading, and also there was some removal of the organic compounds compared to the previous analysis for this system. The column with higher loading didn't show any significant removal of the compounds. Again in both the loadings, the lower concentration of the sample had higher five day BOD values. The results of this analysis are tabulated in Table XI.

The results of the fifteen day period analysis are listed in Table XII. No significant lag period occurred in either loadings. The five day BOD values were slightly less than the twelve day BOD_5 values. In most cases, the BOD_5 values still being higher for lower concentrations of the sample.

Since no significant removal of the compounds was occurring, the next

TABLE IX

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: SIX DAYS

Actual Wt. of Sample (Gms)	Moisture Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml.}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml.}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
			Loading Rate: 1000 µg/gm				
0.1133	11.99%	0.0997	0.452	0.601	2.557	2.798	3.851
0.2242	11.99%	0.1973	0.191	0.380	1.687	1.839	2.25
0.4033	11.99%	0.3549	0.12	0.278	1.479	1.606	1.952
			Loading Rate: 500 µg/gm				
0.1042	8.71%	0.0951	0.512	0.689	1.419	1.608	2.61
0.2130	8.71%	0.1944	0.20	0.469	1.018	1.126	1.635
0.4055	8.71%	0.3701	0.11	0.229	0.956	1.037	1.305

TABLE X

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME NINE DAYS

Actual Wt. of Sample (Gms)	Moisutre Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml.}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml.}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
			Loading Rate: 1000 µg/Gm				
0.1106	11.29%	0.0981	1.4	1.77	2.782	3.058	4.067
0.2095	11.29%	0.1858	0.60	1.259	1.937	2.115	2.502
0.4016	11.29%	0.3562	0.226	1.187	1.6	1.768	1.962
			Loading Rate: 500 µg/Gm				
0.1026	9.27%	0.0930	1.225	1.225	1.967	2.19	2.677
0.2078	9.27%	0.1885	0.601	0.923	1.432	1.591	1.83
0.4026	9.27%	0.3652	0.328	0.706	0.944	1.026	1.273

TABLE XI

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: TWELVE DAYS

Actual Wt. of Sample (Gms)	Moisture Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml.}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml.}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
			Loading Rate: 1000 µg/Gm				
0.1231	13.8%	0.1061	0.404	0.593	2.262	2.686	3.11
0.2185	13.8%	0.1883	0.286	0.493	1.991	2.150	2.405
0.4045	13.8%	0.3486	0.18	0.473	1.462	1.721	2.237
			Loading Rate: 500 µg/Gm				
0.1132	10.3%	0.1015	0.891	1.273	1.773	2.068	2.305
0.2038	10.3%	0.1828	0.4	0.59	1.28	1.312	1.394
0.4013	10.3%	0.3599	0.191	0.5	0.816	1.083	1.33

TABLE XII

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: FIFTEEN DAYS

Actual Wt. of Sample (Gms)	Moisture Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml.}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml.}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
			Loading Rate: 1000 µg/Gm				
0.1021	13.5%	0.0883	0.428	0.713	1.732	2.48	3.567
0.2055	13.5%	0.1775	0.242	0.523	1.064	1.605	2.129
0.4057	13.5%	0.3509	0.18	0.478	0.991	1.367	1.92
			Loading Rate: 500 µg/Gm				
0.1079	9.9%	0.0972	0.4	0.864	0.956	1.419	2.098
0.2098	9.9%	0.1890	0.3	0.571	0.682	1.072	1.428
0.4016	9.9%	0.3618	0.14	0.464	0.547	0.936	1.243

analysis was done after a period of seven days from the last analysis. The results of this analysis show that no lag period of oxygen consumption occurred for either loading. A decrease in the amount of oxygen uptake was seen in all cases, signifying the removal of the organic compounds. BOD_5 values were still higher for lower concentrations of the sample. The data of this analysis are listed in Table XIII.

A similar analysis after twenty-seven days from the initial application of the waste was conducted. During this analysis no sign of oxygen consumption was seen during the first day of testing for both the loadings. The consumption was pretty steady from the second day, slowing down after the fourth day. There was a decrease in the oxygen uptake at the end of five days as compared to the twenty-one day period analysis. There has certainly been some removal of the organic compounds, but as usual the lower concentrations of the sample still had higher BOD_5 values. The results of this analysis is listed in Table XIV.

A final analysis after thirty-four days from the application of the waste was performed and the results are tabulated in Table XV. Like the twenty-seven day period analysis, no sign of oxygen consumption occurred during the first day. The consumption was pretty steady from the second day on. At the end of five days there was a significant decrease in the amount of oxygen consumed or the BOD exerted, as compared to the previous analysis, which definitely signifies a good removal of the organic compounds. Like any other analysis performed this analysis also showed higher BOD values for the lower concentrations of the sample.

The important factor to be noted in the analysis dealing with the combined organic compounds is unlike the first part of the research, the oxygen depletion in mg/lit was higher for higher concentrations of the

TABLE XIII

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: TWENTY-ONE DAYS

Actual Wt. of Sample (Gms)	Moisture Content of (Gms)	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml.}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml.}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
			Loading Rate: 1000 µg/Gm				
0.1020	12.28%	0.0894	0.801	1.0	2.382	2.449	2.519
0.2178	12.28%	0.1910	0.5	0.64	1.41	1.41	1.49
0.4159	12.28%	0.3648	0.2	0.312	0.953	0.953	0.995
			Loading Rate: 500 µg/Gm				
0.1027	10.27%	0.0921	0.4	0.814	1.889	1.954	1.954
0.2054	10.27%	0.1843	0.241	0.341	1.139	1.139	1.188
0.4010	10.27%	0.3598	0.14	0.32	0.875	0.917	1.083

TABLE XIV

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: TWENTY-SEVEN DAYS

Actual Wt. of Sample (Gms)	Moisture Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml.}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml.}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
			Loading Rate: 1000 µg/Gm				
0.1009	13.28%	0.0875	0	0.617	0.96	1.542	1.577
0.2012	13.28%	0.1744	0	0.481	0.688	1.032	1.049
0.4020	13.28%	0.3486	0	0.37	0.481	0.757	0.846
			Loading Rate: 500 µg/Gm				
0.1037	12.98%	0.0902	0	0.698	0.997	1.662	1.696
0.2044	12.98%	0.1778	0	0.472	0.674	0.843	0.928
0.4048	12.98%	0.3522	0	0.3	0.408	0.021	0.809

TABLE XV

SUMMARY OF THE OXYGEN UTILIZATION DATA FOR THE COMBINED SOIL SYSTEMS FOR THE TIME: THIRTY-FOUR DAYS

Actual Wt. of Sample (Gms)	Moisture Content of Sample	Dry Wt. of Sample (Gms)	Oxygen Depletion: Mg/Gm Oxy. Dep. $\frac{Mg}{Lit} \times \frac{300\ ml.}{Dry\ Wt.\ of\ Soil} \times \frac{1\ Lit}{1000\ ml.}$				
			1st Day	2nd Day	3rd Day	4th Day	5th Day
Loading Rate: 1000 µg/Gm							
0.1087	10.57%	0.0972	0	0.413	0.864	0.925	1.018
0.2069	10.57%	0.1850	0	0.34	0.567	0.616	0.648
0.4051	10.57%	0.3622	0	0.2	0.356	0.422	0.596
Loading Rate: 500 µg/Gm							
0.1018	10.54%	0.0910	0	0.4	0.462	0.659	0.857
0.2019	10.54%	0.1806	0	0.202	0.415	0.631	0.664
0.4040	10.54%	0.3614	0	0.14	0.298	0.398	0.54

sample considered, but this depletion was not enough to give the same biochemical oxygen demand as the lower dilutions after calculating the five day BOD values. This again resulted in the lower dilutions of the soil sample having higher BOD values expressed in mg/gm of dry soil.

Figures 10 and 11 show the BOD_5 (in mg/gm of dry soil) versus time (sampling period) in days for both loadings.

Figure 10 (1000 µg/gm of soil loading) shows the three different concentrations of the soil samples considered. From the figure it can be seen that no significant amount of BOD_5 was being removed during the initial sampling periods. After a period of nine days a rapid decrease in BOD_5 occurred. The BOD_5 reached values as low as less than 1 mg/gm of soil at the end of the analysis.

Figure 11 shows the plot of BOD_5 in mg/gm versus time (sampling period). In days for the lower loading (i.e., 500 µg/gm) the three curves shown in the figure represent the three different concentrations considered. It can be seen that there was a slight decrease in the BOD_5 from the beginning of the analysis. The removal rate was slow until a time period of twenty-one days. After the twenty-one day period a rapid decrease in the BOD_5 can be seen. The data for these studies are tabulated in Table XVI.

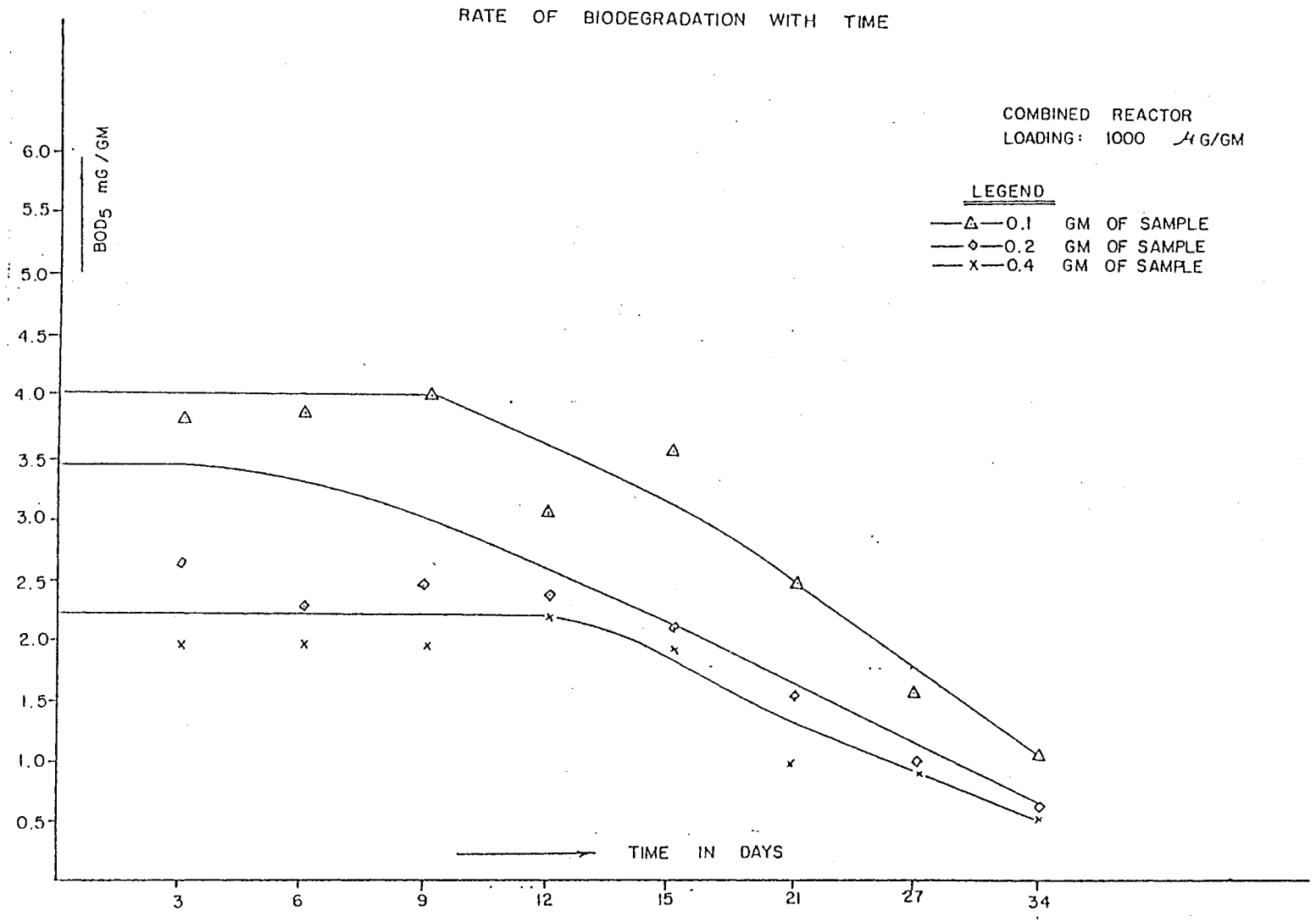

Figure 10. Rate of Biodegradation With Time Combined Reactor Loading 1000 µg/gm

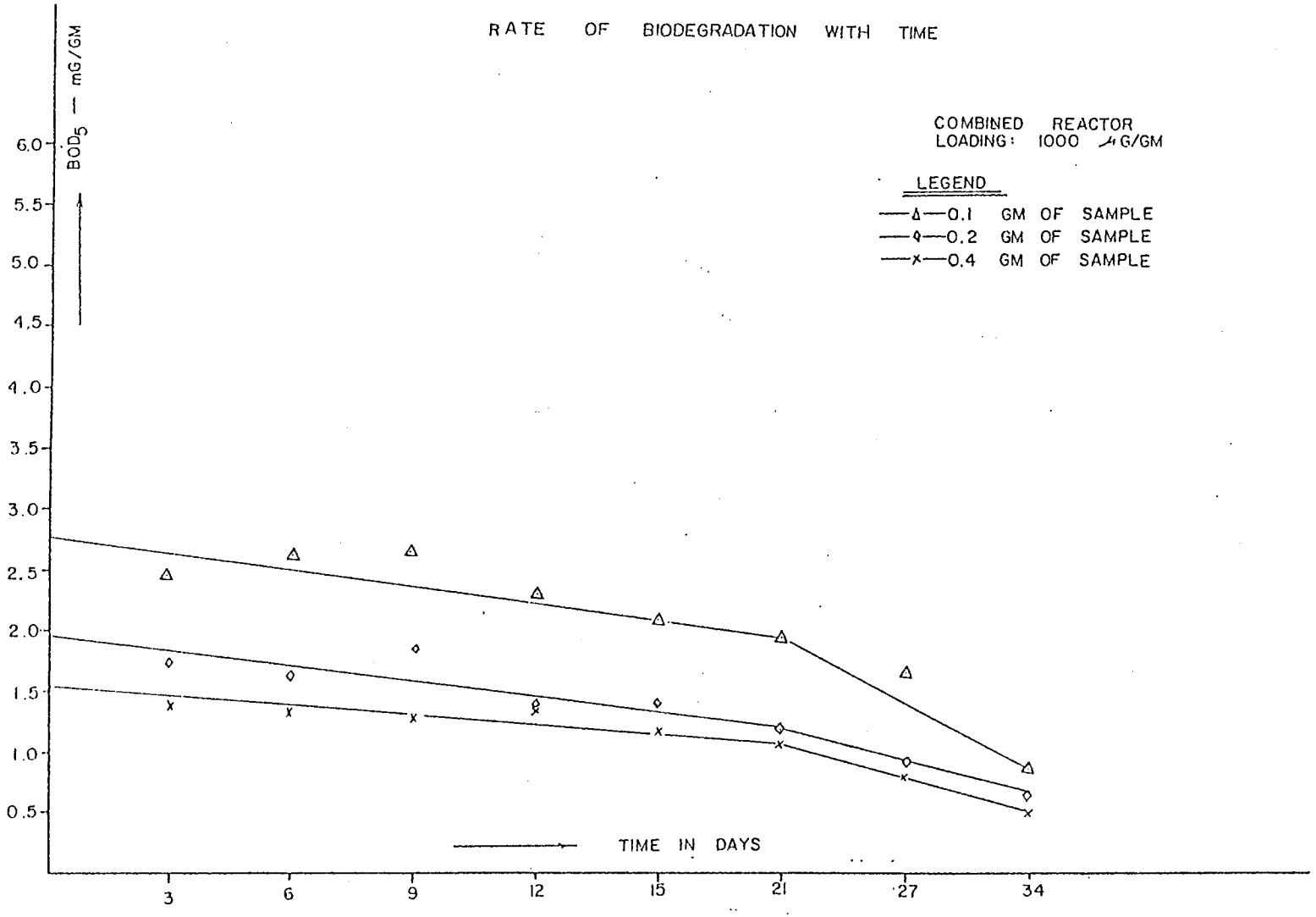

Figure 11. Rate of Biodegradation With Time Combined Reactor Loading 500 µg/gm

TABLE XVI

BOD_5 OF THE COMBINED SOIL SYSTEMS

Weight of the Sample	Biochemical Oxygen Demand - BOD_5 - Mg/Gm								
	0 Days	3 Days	6 Days	9 Days	12 Days	15 Days	20 Days	27 Days	34 Days
				Loading: 1000 µg/Gm					
0.1	4.82	3.761	3.851	4.067	3.11	3.567	2.519	1.577	1.018
0.2	3.47	2.621	2.25	2.502	2.405	2.129	1.49	1.049	0.648
0.4	2.30	1.95	1.952	1.962	2.237	1.92	0.995	0.946	0.596
				Loading: 500 µg/Gm					
0.1	2.71	2.42	2.61	2.677	2.305	2.098	1.954	1.696	0.857
0.2	1.92	1.72	1.635	1.83	1.394	1.428	1.188	0.928	0.664
0.4	1.54	1.366	1.305	1.273	1.33	1.243	1.083	0.809	0.504

CHAPTER V

DISCUSSION

The primary objective of this research was to explain the biodegradability of 2,4 Dinitrophenol, 2,4 Dichlorophenol and nitrobenzene in soil systems by the biochemical oxygen demand test.

The main reason for choosing the BOD as the surrogate parameter to explain the biodegradability of the organic compounds by land treatment is that the microorganisms in the soil used up the carbon present in the organic compounds for the synthesis of new cells; in other words produced organic material. This organic material showed up as the total organic carbon (TOC) and chemical oxygen demand (COD) and remained fairly constant throughout the period of analysis.

Due to this limitation the biochemical oxygen demand test seemed another alternative to measure the biodegradability of these organic compounds. Hence the BOD analysis was started with a little deviation from the standard methods by not adding any additional source of seed in the BOD bottle. At the same time some BOD tests were being run on these soil systems by Yia (36) with an additional source of seed. This additional source of seed initially being thoroughly acclimated to the organic compounds under consideration, there has been quite a difference in the results obtained.

By the addition of an additional source of acclimated seed, the oxygen depletion (in mg/lit) was higher for higher concentrations of the sample and lower for lower concentrations, which resulted in a pretty consistent

biochemical oxygen demand (expressed in mg/gm of dry soil) for all the dilutions considered.

Without the addition of any additional source of seed, the first part of the research (dealing with four different soil systems) resulted in approximately the same amount of oxygen depletion (in mg/lit) for all the dilutions considered. Thus the higher concentrations of the sample had lower BOD (in mg/gm) values and vice versa.

In the second part of the research, where the organic compounds were all combined, the higher concentrations of the sample had higher oxygen depletion (expressed in mg/lit), but was not sufficient to have approximately the same BOD_5 (expressed in mg/gm) values. This again resulted in higher concentration of the sample having lower BOD values and vice versa.

A repeatedly thorough analysis of the data suggested a possible inhibition taking place throughout the experiment.

As a first step of the analysis, the oxygen utilization in mg/gm of the three compounds, for all the three concentrations is plotted with time for all the data obtained.

Secondly, an attempt was made to calculate the specific oxygen uptake rate symbolized as "μ" in this discussion. For this purpose the data obtained was plotted on a semilog graph paper in order to find out the exponential log phase of the oxygen uptake.

The specific oxygen uptake rate was calculated for each concentration at the exponential log phase of the curve throughout the period of analysis. Using the formula

$$\mu = \frac{\ln O_t/O_o}{\Delta t}$$

where:

μ = specific oxygen uptake rate

O_t = oxygen depletion at time t

O_o = oxygen depletion at time 0

Δt = change in time

The specific oxygen uptake rates for the individual compound systems and the combined compound system are listed in Tables XVII and XVIII.

From Tables XVII and XVIII it can be seen that the lower concentration of the sample had a higher specific oxygen uptake rate, and it decreases with increasing concentration of the sample.

Figures 12, 13, 14, 15, 16 and 17 show the specific oxygen uptake rate vs concentration of the sample, for both individual compound systems and the combined compound system for samples taken at time of loading.

As can be seen from Figures 12-17 the curve obtained is pretty close to one that Haldane obtained in plotting specific growth rate and substrate concentration for heterogeneous populations growing on phenol.

Haldane describes the phenomenon saying that the specific growth rate of the species that can grow on toxic compounds appears to be subject to control by two competing substrate effects. The specific growth rate 'μ' tends to increase as substrate is increased, e.g., by a monod type relationship, but 'μ' also tends to decrease due to inhibitory effect of 'S' as its concentration is increased. The above mentioned phenomenon, the joint dependence of 'μ' on S as a substrate and S as a inhibitor is described by the Equation

$$\mu = \frac{\mu_{Max} \cdot S}{(K_s + S)\ [1 + (S/K_i)]}$$

This equation is similar to Michaelis Menten Equation or the Monod

TABLE XVII

SPECIFIC OXYGEN UPTAKE RATES OF THE FOUR INDIVIDUAL SOIL SYSTEMS

Base Mix			2,4 Dinitrophenol		
Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate ($Time^{-1}$)	Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate $Time^{-1}$)
	0.0972	2.04		0.1507	1.83
0 Days	0.2816	1.43	0 Days	0.2936	1.48
	0.5748	1.27		0.5507	1.27
	0.1062	1.73		0.0953	1.83
3 Days	0.3512	0.959	3 Days	0.2845	1.273
	0.5484	0.824		0.5921	0.729
	0.0984	1.9		0.0994	1.94
6 Days	0.2876	1.38	6 Days	0.2897	1.46
	0.5632	1.14		0.5589	0.65
	0.1003	1.45		0.0987	1.95
9 Days	0.2695	1.28	9 Days	0.2919	1.38
	0.5393	104		0.5311	1.38
	0.1113	1.52		0.1166	1.6
15 Days	0.2746	0.708	15 Days	0.2784	1.19
	0.5378	0.608		0.5220	0.91

TABLE XVII (Continued)

2,4 Dichlorophenol			Nitrobenzene		
Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate ($Time^{-1}$)	Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate ($Time^{-1}$)
	0.1056	2.02		0.1075	1.81
0 Days	0.2719	1.46	0 Days	0.2977	1.304
	0.5353	1.44		0.5644	1.12
	0.0957	1.99		0.1059	1.96
3 Days	0.2670	1.29	3 Days	0.2968	1.46
	0.5324	0.837		0.5410	0.744
	0.0969	1.76		0.0942	1.86
6 Days	0.2854	1.36	6 Days	0.2686	1.23
	0.5627	0.46		0.5237	0.924
	0.0913	1.84		0.0952	1.84
9 Days	0.2775	1.38	9 Days	0.2614	1.46
	0.5457	1.38		0.5418	0.91
	0.1187	1.69		0.0990	1.8
15 Days	0.2723	1.41	15 Days	0.2755	1.40
	0.5399	0.95		0.5248	0.95

TABLE XVIII

SPECIFIC OXYGEN UPTAKE RATES OF COMBINED SOIL SYSTEMS

Loading: 1000 µg/Gm			Loading: 500 µg/Gm		
Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate ($Time^{-1}$)	Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate ($Time^{-1}$)
0 Days	0.0840	1.81	0 Days	0.0996	1.84
	0.1639	1.34		0.1872	1.27
	0.3258	1.308		0.3391	1.26
3 Days	0.1005	1.74	3 Days	0.0964	2.01
	0.1808	1.48		0.1830	1.37
	0.3524	0.95		0.3556	0.836
6 Days	0.0997	1.88	6 Days	0.0951	2.04
	0.1973	1.29		0.1944	1.38
	0.3543	1.21		0.3701	0.753
9 Days	0.0981	1.84	9 Days	0.0930	1.875
	0.1858	1.38		0.1885	1.38
	0.3562	1.19		0.3652	1.23
12 Days	0.1061	1.85	12 Days	0.1015	1.80
	0.1883	1.61		0.1828	1.38
	0.3486	1.30		0.3599	1.29

TABLE XVIII (Continued)

Loading: 1000 µg/Gm			Loading: 500 µg/Gm		
Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate ($Time^{-1}$)	Time of Sampling	Wt. of the Sample (Gms)	Specific Oxygen Uptake Rate ($Time^{-1}$)
	0.0883	1.84		0.0972	1.84
15 Days	0.1775	1.4	15 Days	0.1890	1.38
	0.3509	1.3		0.3618	1.12
	0.0894	1.84		0.0921	1.84
21 Days	0.1910	1.38	21 Days	0.1843	1.46
	0.3648	1.38		0.3598	1.12
	0.0875	1.79		0.0902	1.88
27 Days	0.1744	1.38	27 Days	0.1778	1.35
	0.3486	1.36		0.3522	1.15
	0.0972	1.89		0.0910	1.84
34 Days	0.1850	1.74	34 Days	0.180	1.4
	0.3622	1.38		0.3614	1.12

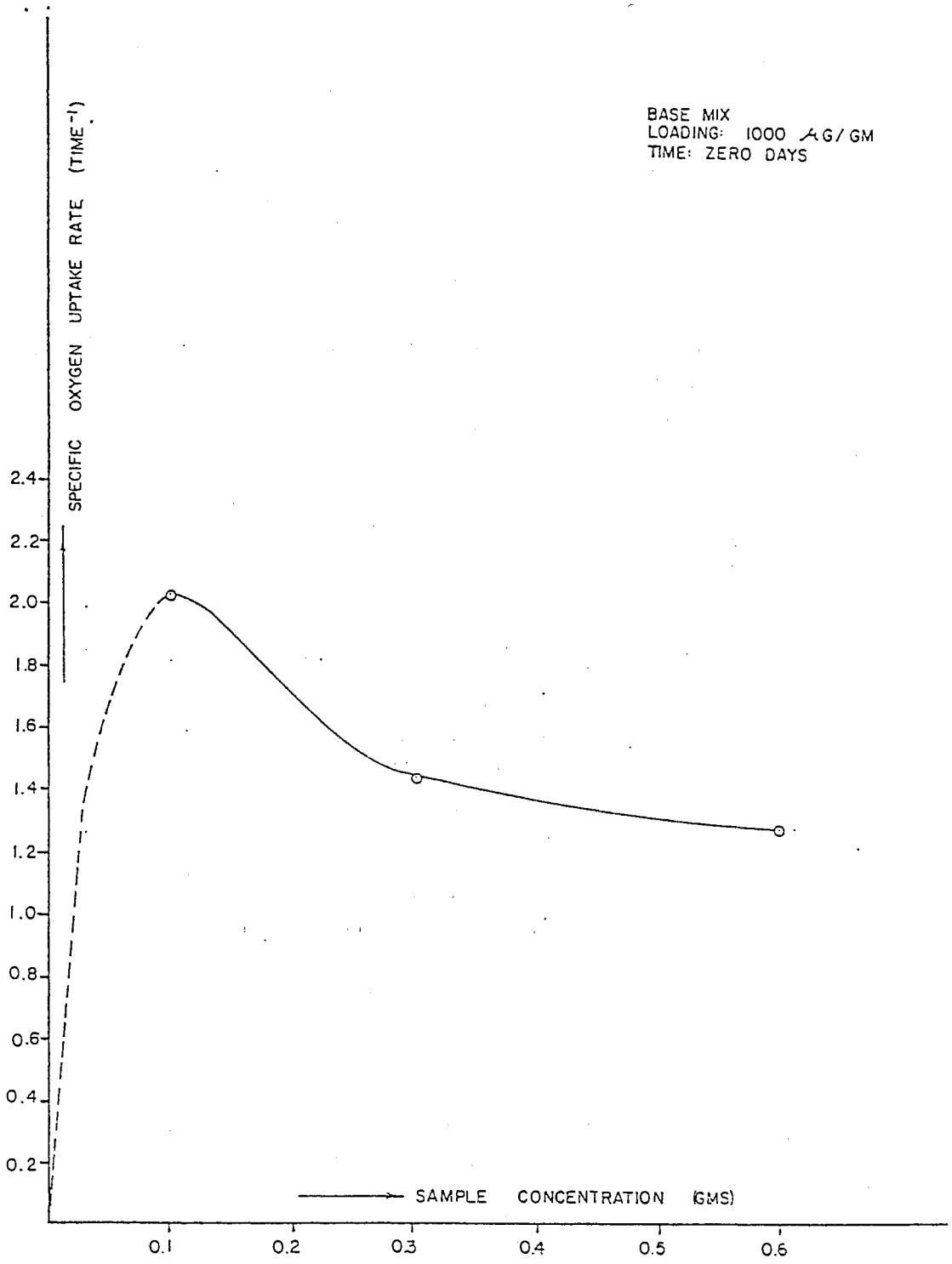

Figure 12. Relation Between Specific Oxygen Uptake Rate and Sample Concentration for Base Mix at Time Zero

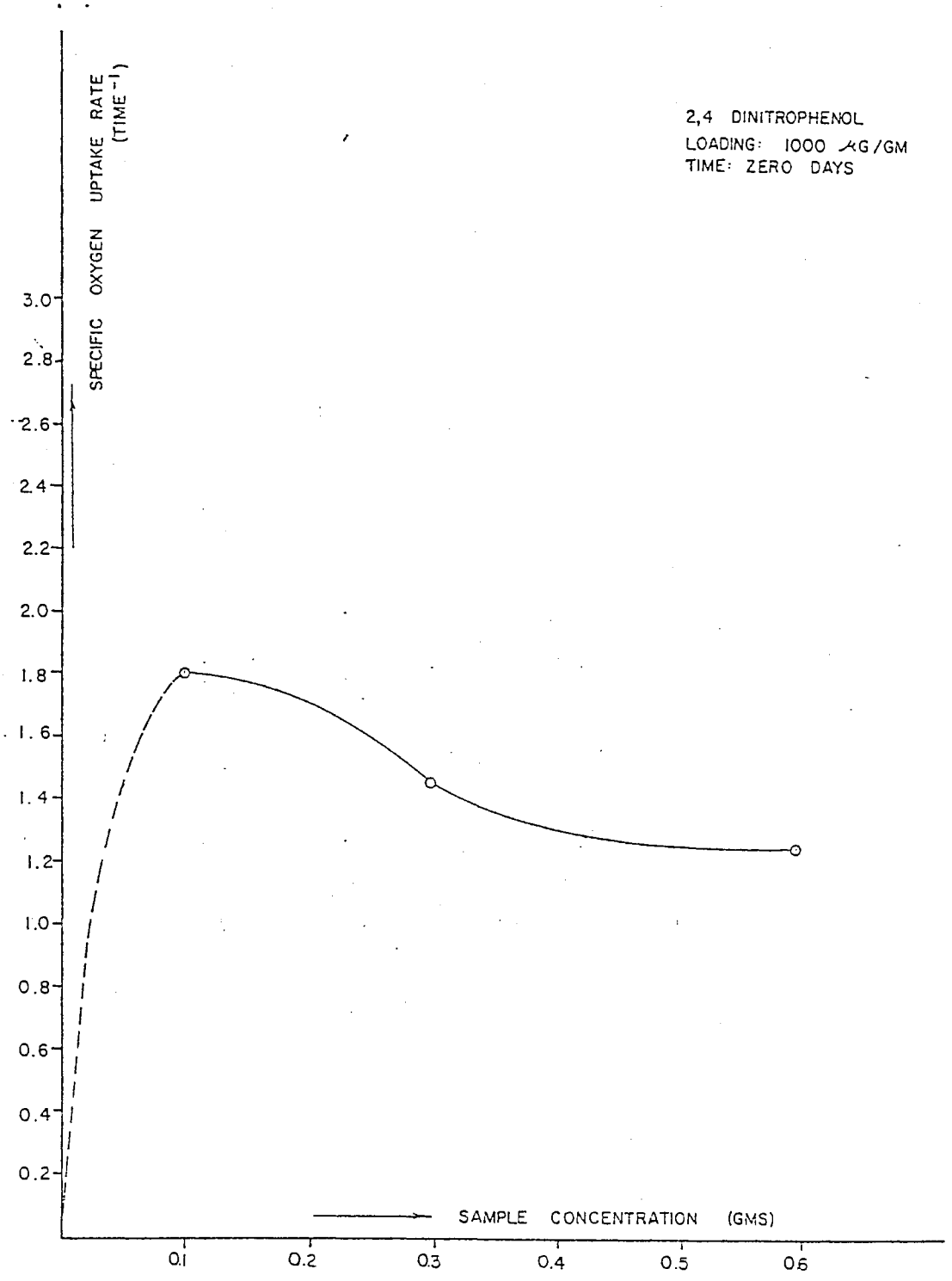

Figure 13. Relation Between Specific Oxygen Uptake Rate and Sample Concentration for 2,4 Dinitrophenol at Time Zero

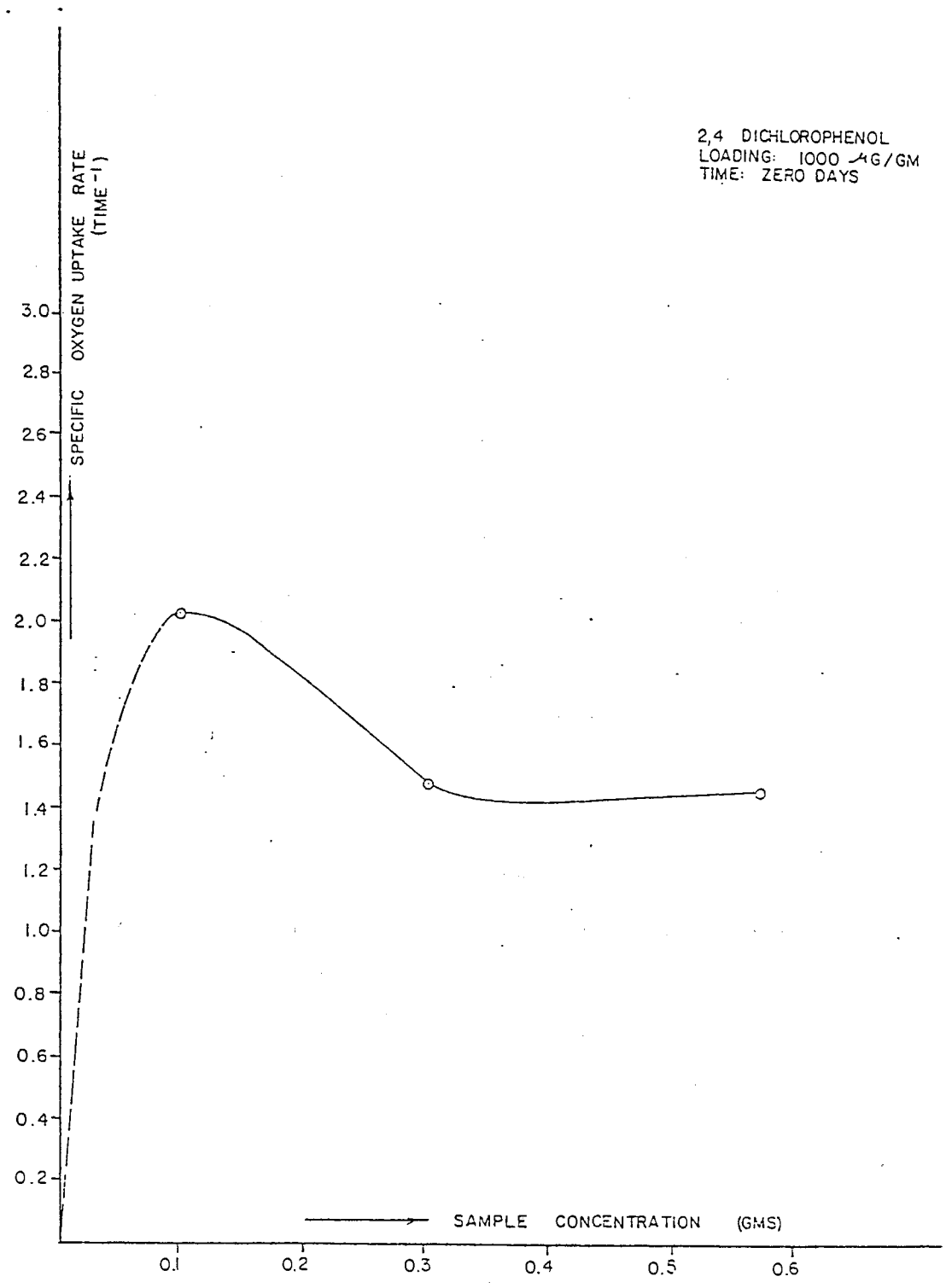

Figure 14. Relation Between Specific Oxygen Uptake Rate and Sample Concentration for 2,4 Dichlorophenol at Time Zero

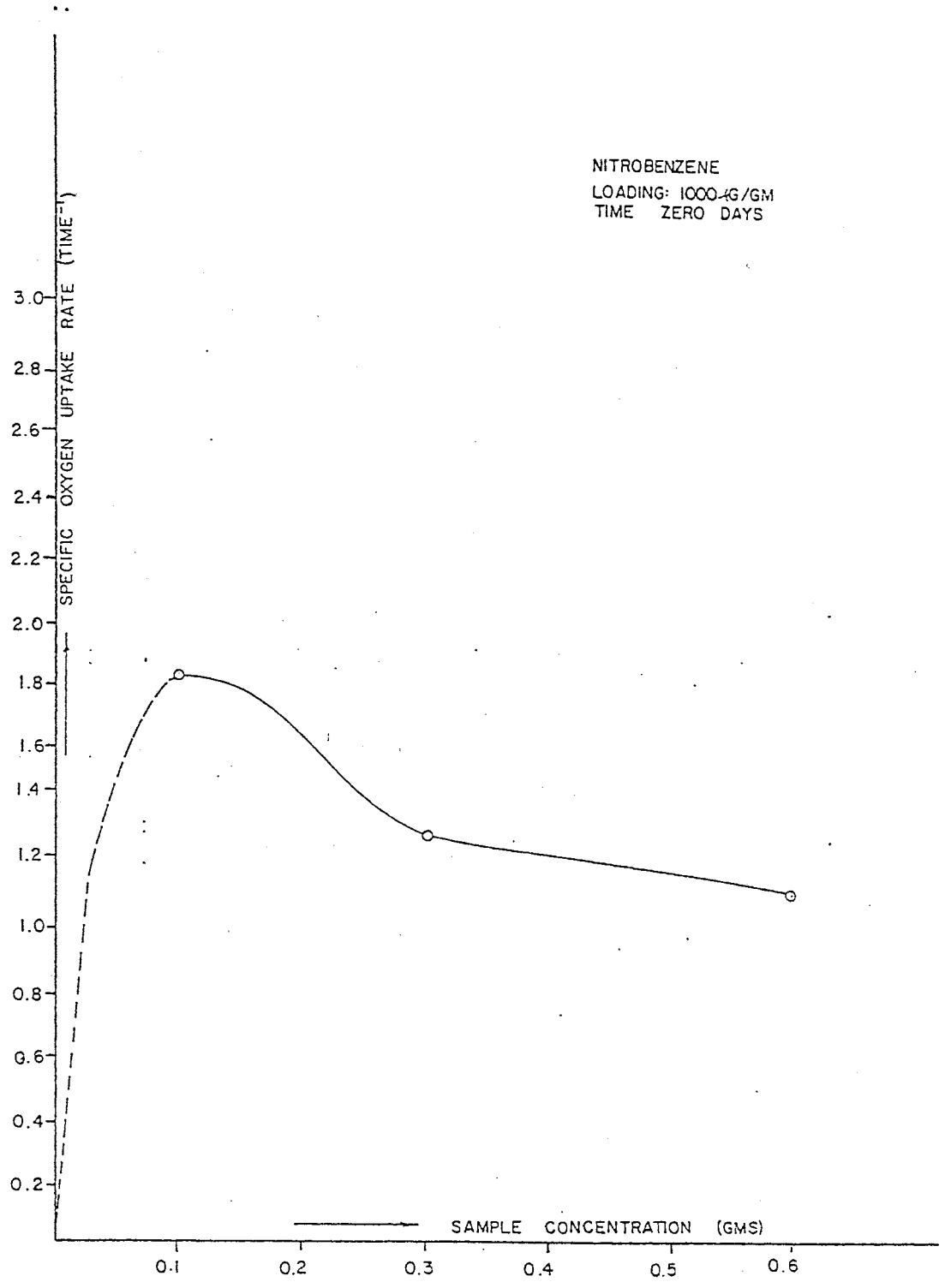

Figure 15. Relation Between Specific Oxygen Uptake Rate and Sample Concentration for Nitrobenzene at Time Zero

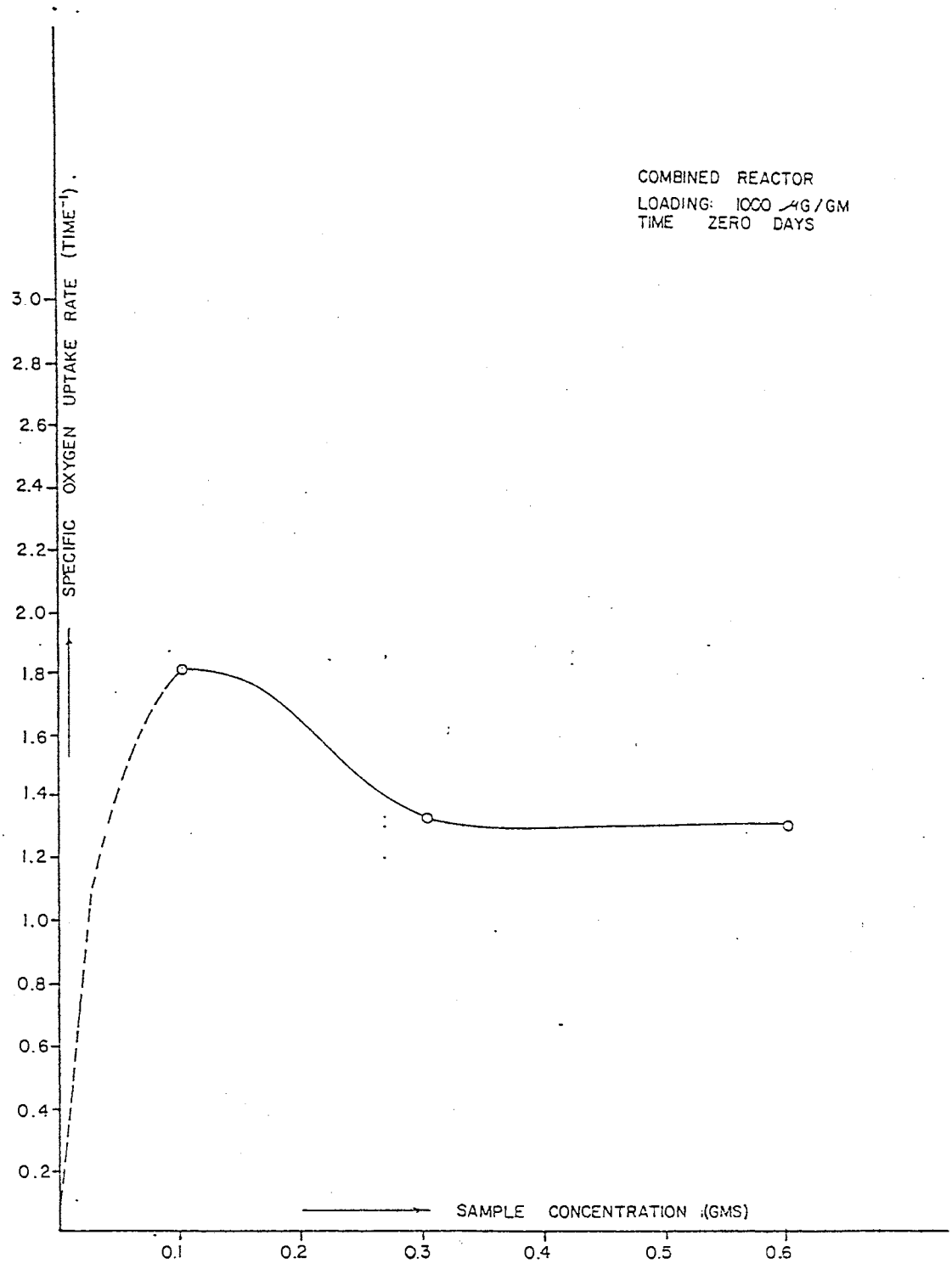

Figure 16. Relation Between Specific Oxygen Uptake Rate and Sample Concentration for the Combined Reactor for Time Zero at a Loading Rate of 1000 µg/Gm

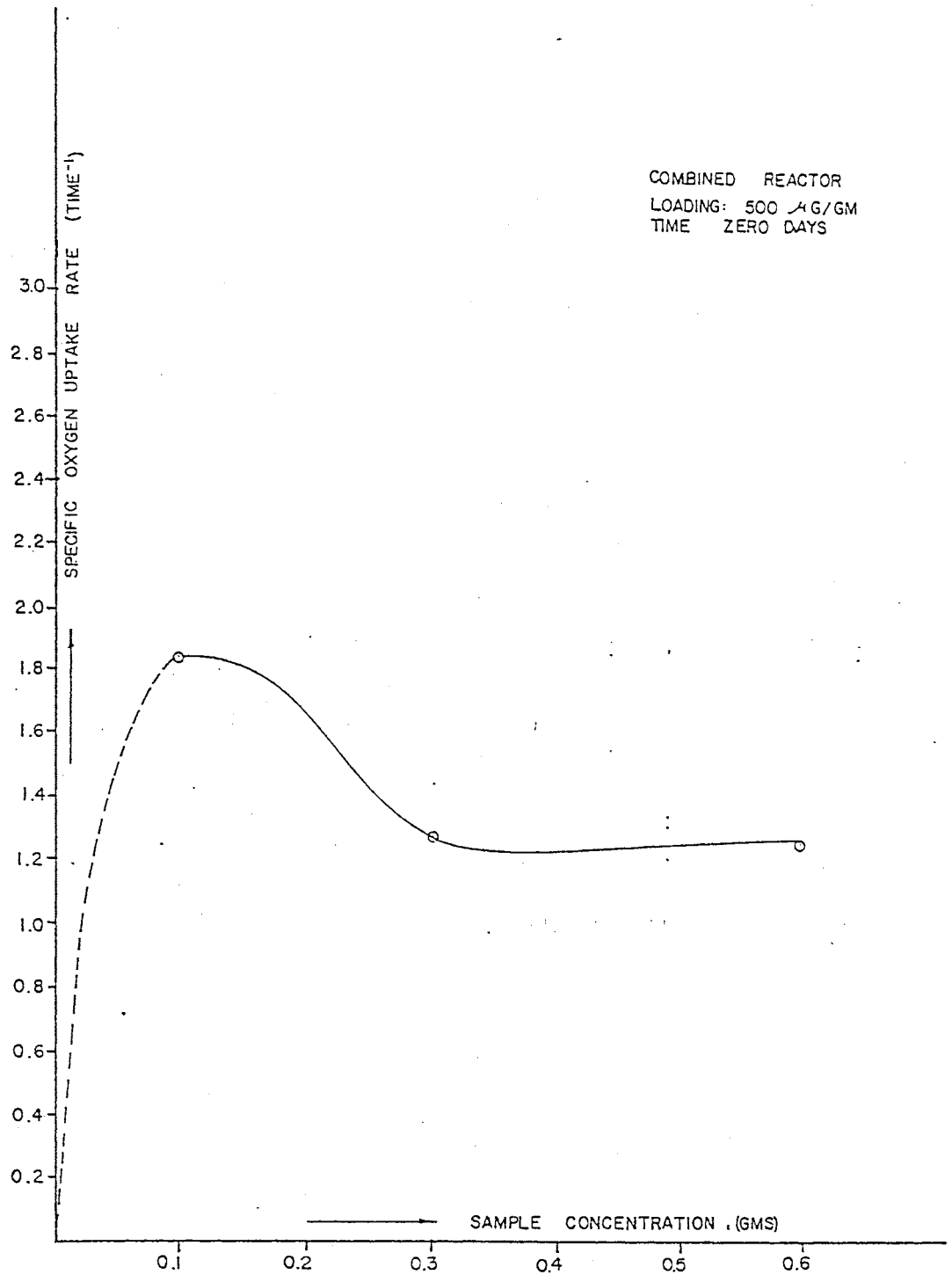

Figure 17. Relation Between Specific Oxygen Uptake Rate and Sample Concentration for Combined Reactors for Time Zero at a Loading Rate of 500 µg/Gm

Equation with the addition of the term containing the inhibition constant K_i. This equation predicts that as 'S' increases, 'μ' rises then peaks and finally decreases as the inhibitor term dominates.

From the data obtained on specific oxygen uptake rate and the plots of the specific oxygen uptake rate vs. concentration, and at the same time emphasizing that the three organic compounds used are toxic, there is a good possibility that inhibition was taking place throughout the experiment. This was the reason that higher concentration of the sample turned out to have low oxygen depletion, which in turn had low specific oxygen uptake rate and vice versa.

The biochemical oxygen demand test did show the removal of the organic compounds unlike the TOC or COD test.

It should be noted that the microorganisms must have or develop suitable enzyme systems if they are to metabolize particular organic compound. In most cases these microorganisms have the ability to modify their means of metabolism in order to utilize most organic compounds which may involve the adaption of the enzyme system to the new food source, or mutations of the microorganisms themselves can occur. This acclimation or adaption may proceed rapidly or may require a considerable period of time. However there are certain chemicals that will require a significant acclimation or adaption period before biological utilization occurs.

The three organic compounds considered for the analysis are known to be toxic. The microorganisms definitely require a significant acclimation period before the biological utilization of these compounds occurs. Without this additional source of the acclimated seed definitely leads to inhibitorial effects as seen throughout this research.

A definite way to avoid this inhibition effects is to grow some microorganisms with the organic compounds for a considerable period of time.

In other words acclimatize the seed to the toxic material and seed the BOD bottle with this acclimatized seed.

In all cases of BOD testing, irrespective of the purpose for testing, it is imperative that appropriate seed material be employed for determination of reliable BOD values. In some cases, definitely when dealing with hazardous or toxic compounds, the use of appropriate seed warrants the development of acclimated biological seed.

CHAPTER VI

CONCLUSIONS

1. Unlike the chemical oxygen demand test or the total organic carbon test, the biochemical oxygen demand test turned out to be a better surrogate parameter for measuring organic contaminates remaining in a land treatment system.

2. By not adding any additional source of seed in BOD bottle, the analysis led to inhibitorial effects.

3. Throughout the analysis, the higher concentration of the sample turned out to have low BOD_5 values (expressed in mg/gm) and low specific oxygen uptake rates and vice versa.

4. In the first part of the analysis, dealing with four individual soil systems mixed with base mix, the microorganisms were not utilizing the organic compounds for the first six days. The oxygen consumption exhibited was due to the base mix present in the system.

5. In the second part of the analysis, dealing with combined soil systems, a) no significant removal of the toxic waste was taking place until a period of nine days, for the system with a loading rate of 1000 µg of each compound per gram of soil. b) For the system with a loading rate of 500 µg of each compound per gram of soil, there was a slight decrease of BOD_5 from the beginning of the analysis, but the removal rate was slow until a time period of twenty-one days.

6. The specific oxygen uptake rate 'μ' tends to decrease due to inhibitory effect of 'S' as its concentration is increased.

7. In all cases of BOD testing, irrespective of the purpose for testing, it is imperative that appropriate seed material be employed for determination of reliable BOD values.

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