BIODEGRADATION OF ORGANIC COMPOUNDS FROM AN ABANDONED REFINERY SITE

by

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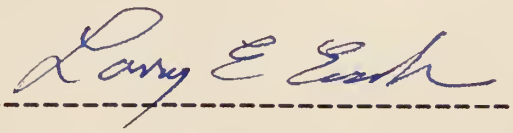

Larry E. Erickson
Major Professor

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CHAPTER I

INTRODUCTION

Polynuclear aromatic hydrocarbons (PNA's) are identified in wastes from industrial sources that represent a significant source of introduction of PNA's into the environment. Such wastes are generated from high temperature processes, municipal sewage treatment, and petroleum refining. This research investigates biodegradation of PNA's adsorbed in the soil from an abandoned refinery.

Polynuclear aromatic hydrocarbons are of critical environmental concern because of the following characteristics: chronic health effects, possible microbial recalcitrance, high bioaccumulation potential, and low removal efficiencies in traditional wastewater treatment processes (Herbes et al., 1976). Sixteen PNA's are on the U.S. Environmental Protection Agency (EPA) priority pollutant list. At the present time several different methods are being studied to treat waste streams cpntaining PNA's.

The background and a review of the literature concerning PNA's behavior is presented in Chapter II. The

review indicates that the majority of current research is directed toward delineating their fate in rivers, streams, and sediments. Research has been conducted in Europe and the USSR concerning PNA-soil interactions. In the United States there has been an emphasis on investigating metabolic pathways for microbial degradation and transformation of PNA's.

Chapter III presents the experiments conducted with the contaminated soil from the abandoned refinery to evaluate biodegradation as a potential remediation technology. The effect of saturated, unsaturated, and saturated plus surfactant conditions were investigated for the soils found at the abandoned site. Various models are employed with the data from these experiments, including first order kinetic model and Monod model. The maximum specific growth rate and biomass yields for each experiment are presented in this chapter.

The possibility of in situ treatment at the site is studied in the fourth chapter. Since the experiments demonstrated that the petroleum compounds in the soil can be biodegraded, the applications and limitations of this technique, and the operation and maintenance costs based on some past cases are discussed. Also a comparison between this treatment and other treatments already used is presented. Based on this information it is recommended that

the in situ biodegradation technique be considered for implementation at the site under investigation.

The last chapter presents some recommendations to improve the analytical techniques used in the experiments. The high pressure liquid chromatography and biomass measurement are examined.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

INTRODUCTION

Gale (1952) suggested that microorganisms exist which are capable of oxidizing all naturally produced organic compounds under suitable conditions. The problem is that many environments, such as anaerobic or acidic ones, do not represent suitable conditions, and in this type of situation many organic molecules may remain more or less unchanged over very long periods of time. This is exactly what has happened in Arkansas City.

The Milliken Oil Refinery operated in the vicinity of Arkansas City during the early part of the 20th century. Following a fire in the mid 1920's the property was used for disposal of various waste materials from unknown sources. The Kansas Department of Health and Environment detected the presence of carcinogenic polynuclear aromatic compounds (PNA's) in soil and ground water samples. Large concentrations of lead, chloride, sulfate, and nitrate also were observed in water from some wells (Spruill, 1987).

This site, along with six others in the state of Kansas, is included in the Superfund list and is been studied at the present time for clean up. Biodegradation is a good possibility for remedial action in this site. This is a review of the literature that will provide the necessary background in biodegradation of PNA's to evaluate this possibility.

The samples collected at the site showed that many aliphatic hydrocarbons and polynuclear aromatic compounds are present (Spruill, 1987). The PNA's have higher toxicity and many are known to be carcinogenic.

In this work, the chemical and physical properties of PNA's (giving emphasis to the solubility aspect) are presented, and the organisms capable of degradation of PNA's and their metabolic pathways are reviewed. The environmental factors affecting biodegradation of these compounds are discussed, and a summary of kinetic data from different investigators is presented.

STRUCTURE AND PHYSICAL-CHEMICAL DATA OF PNAS

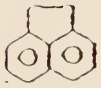
Polynuclear aromatic compounds are composed of several connected aromatic ring structures in linear, angular, or cluster arrangements. The linear arrangement is the one

that has the lowest stability, the cluster arrangement has the intermediate stability, and the angular arrangement has the highest stability (Blumer, 1976). Figure II-1 shows the structure of some of these PNA's (Sims, 1982). Substitution of carbon in the benzene ring with sulfur, nitrogen, oxygen or other elements creates heterocyclic aromatic compounds. One important source of polynuclear aromatic compounds in the environment is the combustion of fossil fuels of various kinds. In the Arkansas City case and other cases of petroleum refining, the process of treating oil products with sulfuric acid in effect concentrates and removes PNA's from salable products (Sims,1983).

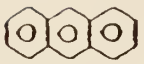
It has been further determined (Blumer and Youngblood,1975) that the temperatures associated with pyrolysis of organic compounds determine the compounds that are produced. For petroleum as source at 100 to 150 C, there are numerous alkyl substitutions, and for internal combustion engines at 2000 C, there are few substitutions (Sims,1983).

There have been observations of cancer related to environmental agents since 1775, when Pott observed cancer in chimney sweepers. Today there is extensive literature on the acute toxicity of PNA's.

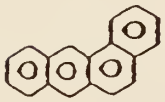
POLYNUCLEAR AROMATICS



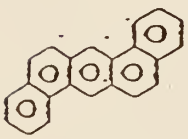
ACENAPHTHYLENE



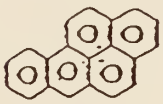
ANTHRACENE



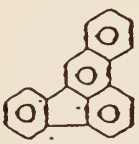
BENZ(a)ANTHRACENE



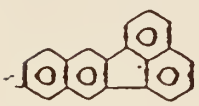
DIBENZ(a,h)ANTHRACENE



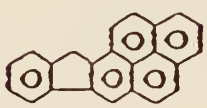
BENZ(a)PYRENE



BENZO(b)FLUORANTHENE



BENZO(k)FLUORANTHENE



INDENO(1,2,3-cd)PYRENE

Figure II-1. Structures of some polynuclear aromatic hydrocarbon compounds. Sims, 1982.

Sixteen PNA's are on the U.S. Environmental Protection Agency (EPA) priority pollutant list. These compounds are listed in Table II-I along with some physical data and their carcinogenicity ratings.

Carcinogenicity designations shown in Table II-I are based on International Agency for Research on Cancer (IARC) analysis of available information as follows: (IARC,1982)

(i) Sufficient evidence - causal relationship shown;

(ii) Limited evidence - credible but alternative explanations possible;

(iii) Inadequate evidence - either insufficient or confounding data; and

(iv) No evidence - several adequate studies show no evidence.

In Table II-I, ST means short term test, A animal test, mp melting point, bp boiling point, and log K the logarithm of the octanol:water partition coefficient.

Polynuclear aromatic hydrocarbons can be taken into the body by inhalation, skin contact, or ingestion, although they are poorly adsorbed from the gastrointestinal tract. Because PNA's are hydrophobic, they can travel across cell lipid membranes and may concentrate in fatty tissues (Enzminger and Ahlert, 1987). Animal studies indicate that PNA's are metabolized by liver mixed function oxidases to

epoxides, dihydrodiols, phenols, and quinones. These reactions represent an intoxication of the parent PNA as the metabolites have been identified as mutagenic, carcinogenic and teratogenic agents. The epoxides have been demonstrated to bind to genetic material which starts the carcinogenic tumor (Simmon and Baden, 1980).

McKee and Wolf (1963) suggested that solubility may play a critical role in toxicity to fish as anthracene (20 times less soluble than phenanthrene) is not toxic even in supersaturated solutions.

Many of the PNA's have small solubility values: six of those in Table II-I have values below 0.01 mg/liter. Five values in Table II-I are above 1 mg/l; these include naphthalene, 30 mg/l, fluorene, 2.0 mg/l, and phenanthrene, 1.3 mg/l. These compounds are potentially mobile in water; however, studies had shown that they are strongly adsorbed in soil.

Adsorption causes the transfer of contaminants from the aqueous to the solid phase of the aquifer, whereas contaminants are returned to the aqueous phase by desorption. Therefore, adsorption serves to retard the rate of migration of contaminants. When the containment of the zone of contamination is desirable, it would help to enhance adsorption of the contaminants. However, during remediation

Table II-I

Structures and Physical-chemical Properties of PNAs

PNA	Mol. Wt.	mp (C)	bp (c)	sol. (mg/l)	log k	Carcinogen Rating
Naphthalene	128	80	218	30	3.37	NR
Acenaphthene	154	96	279	3.47	4.33	NR
Acenaphthylene	152	92	265	3.93	4.07	NR
Anthracene	178	216	340	0.07	4.45	ivST,ivA
Phenanthrene	178	101	340	1.29	4.46	iiST,iiiA
Fluorene	166	116	293	1.98	4.18	NR
Fluoranthene	202	111	-	0.26	5.33	iiST,ivA
Benz(a)-anthracene	228	158	400	0.014	5.61	iST,iA
Chrysene	228	255	-	0.002	5.61	iiST,iiA
Pyrene	202	149	360	0.14	5.32	iiST,ivA
Benzo(a)pyrene	252	179	496	0.004	6.04	iST,iA
Benzo(b)-fluoranthene	252	167	-	0.001	6.57	iiiST,iA
Benzo(k)-fluoranthene	252	174	480	0.001	6.84	iiiST,iA
Dibenz(a,h)-anthracene	278	262	-	0.001	5.98	iST,iA
Benzo(g,h,i)-perylene	276	222	-	0.0003	7.23	iiiST,iiiA
Indeno(1,2,3,-cd)pyrene	276	163	-	0.062	7.66	iiiST,iiA

Where i= sufficient evidence; ii= limited evidence; iii= inadequate evidence; iv= no evidence; ST= short term test; A= animal test; K= octanol:water partition coefficient; NR= no report. Sims,(1982);Versar,(1979);IARC Monographs,(1982).

it may be necessary to flush the contaminants from the aquifer system, in which case enhancing the desorption of the contaminants would become important.

Dunn (1968) has proposed an interfacial kinetic model that predicts the growth kinetics for an organism growing at the interface. The model assumes that absorption and desorption rates from the interface are fast compared to growth. For small population, growing at an interface (utilizing solid directly), the model predicts dependence of the growth rate on interfacial surface area. If the population is large and the interfacial surface area is small, the model predicts that the rate of growth will be linear.

Wodzinski and Bertolini (1977) found that for growth of the naphthalene isolate in the presence of solid naphthalene, the rate of growth is exponential and independent of the surface area, which indicates utilization of the dissolved naphthalene and not the solid directly. However, Hosler (1963) found that when using naphthalene an exponential curve for the bacterial cell accumulation was followed by linear accumulation (Figure II-2) indicating a limiting factor. He studied the nutrient and additional trace elements at different concentrations and found no change in fermentation performance.

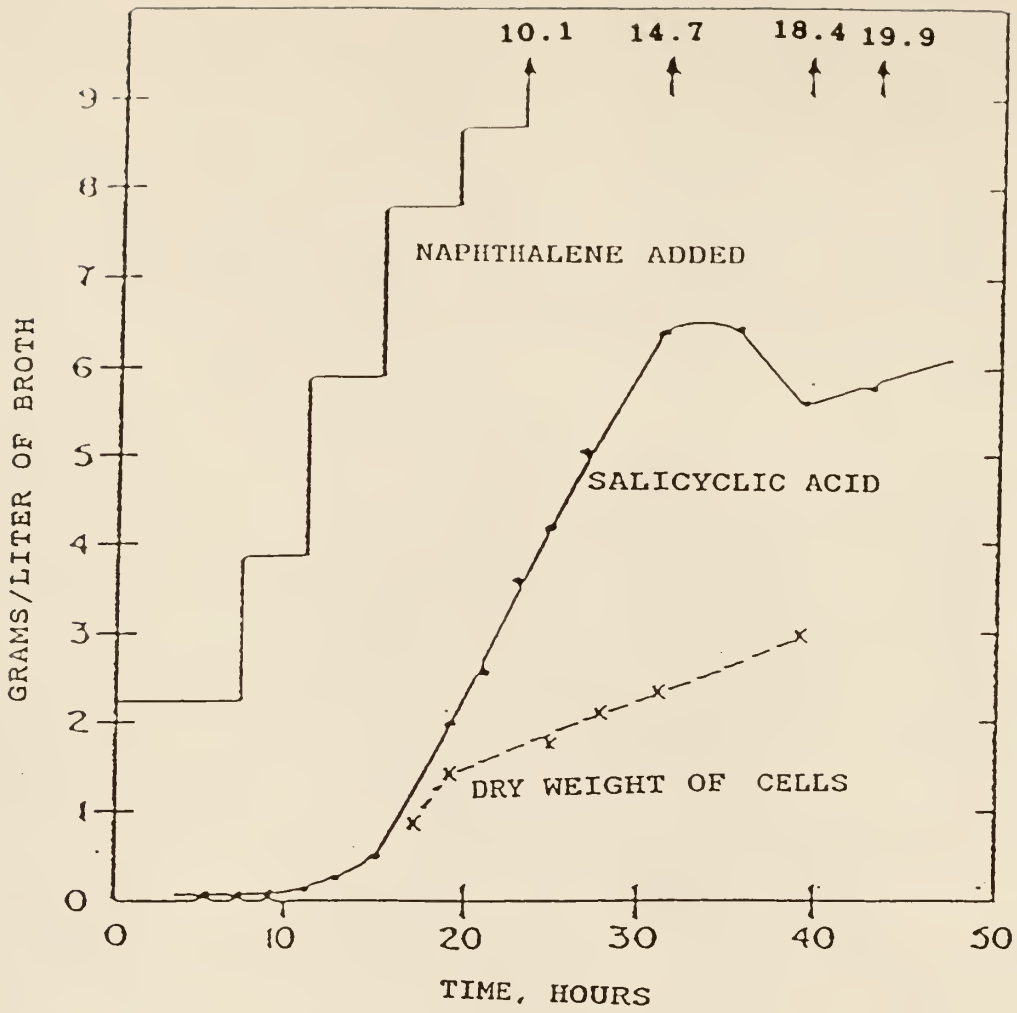


Figure II-2. Production of salicyclic acid from naphthalene. Hosler, 1963.

In another study, Wodzinski and La Rocca (1977) explored the hypothesis that aromatic hydrocarbons are utilized in the dissolved state by using diphenylmethane and naphthalene dissolved in heptamethylnonane as substrate. Table II-II shows that as more heptamethylnonane is added to the system, the concentration of naphthalene in the aqueous and in the heptamethylnonane phases decreases and that growth rates decreased as more heptamethylnonane is added to the system. One possible explanation is that the cells can obtain naphthalene at the water-hydrocarbon interface, and the concentration of naphthalene in the heptamethylnonane phase decreases with increasing amounts of added heptamethylnonane, which results in slower growth.

When adding the heptamethylnonane-naphthalene solution, the concentration of naphthalene in the heptamethylnonane phase did not vary significantly, but the surface area of heptamethylnonane increased. The growth rate increased in this experiment indicating that the cells can utilize the naphthalene dissolved in the heptamethylnonane phase at the interface between the heptamethylnonane and water phases. The results obtained are consistent with Dunn's kinetic model.

Table II-II

Generation Times of *Pseudomonas sp.* on Naphthalene

Heptamethylnonane added to 500 ml of naphthalene solution.		Heptamethylnonane containing 0.015 g of naphthalene per ml added to 500 ml of solution.	
Heptamethylnonane (ml)	Generation time (h)	Heptamethylnonane (ml)	Generation time (h)
0	1.2	1	1.7
1	1.7	3	1.2
3	1.8	12	1.1
12	2.7		

Wodzinski and La Rocca (1977).

Some investigators have explored the adsorption of PNA's in aquifers. Abdul and Gibson (1986) investigated the adsorption of six polycyclic aromatic hydrocarbons by two aquifer materials. They found that the adsorption of these PNA's is adequately described by the linear isotherm model. Regression equations relating the slope of the linear isotherm to the coefficient of partition between octanol and water of the compounds and the fraction of organic carbon of the material predicted the order of adsorption of the six PNA's, but the predicted and measured values of the partition coefficients differ by a factor of up to four. To present the adsorption of these PNA's as a straight line does not take into account the fact that there is a saturation line. Figure II-3 shows the results obtained from this study for naphthalene and its saturation line. Abdul *et al*. (1987) found that the adsorption of non-polar organic contaminants by aquifer materials can be correlated with either the octanol-water partition coefficients or with the aqueous solubilities of these contaminants. These correlations can be used in estimating the extent to which adsorption would retard the movement of non-polar organic contaminants through aquifers.

A more recent contribution from Kamlet *et al*. (1988) presents a set of parameter estimation rules that in

combination with an equation recently reported by Leahy (1986):

$$\log K_{OW} = 0.45 + 5.15V_I/100 - 1.29(\pi^* - 0.40\delta) - 3.60\beta \quad \text{(II-1)}$$

allow facile and accurate estimation of octanol:water partition coefficients of large numbers of PNA's of environmental interest. The parameter estimation methods are as follows. For naphthalene, $\pi^* = 0.70$ and $\beta = 0.15$. For each additional fused aromatic ring, add 0.10 to π^* and 0.05 to β, e.g., for naphthalene to dibenzanthracene, $\Delta\pi^* = 0.30$ and $\Delta\beta = 0.15$. For chloro-PNA's and alkyl PNA's derivatives use the same increments as for the corresponding benzene derivatives. For HBA substituents on PNA's, start with corresponding substituted benzene; add nothing to π^* or β for first fused ring; add 0.10 to π^* and 0.05 to β for additional fused rings.

Nakamara, Erickson and Gutierrez (1977) found that more than one half of the cells may be attached to large oil drops. They concluded that direct contact of cells with large oil drops and utilization of submicron droplets may be important for hydrocarbon uptake by cells. In another study, Gutierrez and Erickson (1978) proposed the following mechanism for hydrocarbon utilization for C. Lipolytica. Cells produce surface active materials; these surfactants

reduce the interfacial tension between the oil and the aqueous phase. As the interfacial tension decreases, the Sauter mean diameter also decreased and the interfacial area increases. Some cells attach to the large oil drops and feed upon them. Pseudosolubilization increases as the interfacial tension decreases and the interfacial area increases. Submicron drops can be formed by the explosion of air bubbles surrounded by oil films at the liquid surface. At the time of the explosion, the hydrocarbon film may be broken into many small drops. Another possibility is that submicron drops are carried into the aqueous phase by cells desorbed from larger drops.

Polynuclear aromatic compounds vary in biodegradation potential. Light molecular weight PNA's, such as naphthalene, have been shown to be biodegradable (Atlas, 1981). Heavier molecular weight PNA's, like benzo(a)pyrene, are very resistant to biodegradation. The reduction in microbial degradability of PNA's with increasing number of rings is mainly due to their lower solubility in water (Pan et al.,1984).

METABOLIC PATHWAYS

There is extensive literature that treats the microbial metabolism of benzene and various PNA's, like phenanthrene and naphthalene. The mechanisms of biodegradation of PNA's will be discussed, and some examples from the literature will be presented.

The unsubstituted saturated ring structure of PNA's is difficult to degrade biologically. Various investigators agree that there is a need for the introduction of enzymes to get higher metabolism of PNA's. Dihydroxylation of the aromatic nucleus is a prerequisite for enzymatic cleavage of the benzene ring by oxygenases (Dagley,1975), making the molecule biologically reactive.

There are differences in the metabolism of the benzene ring by fungi and by bacteria. Figure II-4 illustrates the initial reaction of oxygenases on PNA's and the initial products for eucaryotes (fungi) and procaryotes (bacteria). Ames et al. (1972) demonstrated that the epoxides (like arene oxide in Figure II-4) formed by fungi metabolism of PNA's can alter the reading frame during the transcription of genetic information from DNA to RNA. This fact has as a consequence mutagen formations.

There are two types of oxygenases (Dart and Streton, 1980). One type is called mono-oxygenases, hydroxygenases, or mixed-function oxidases. These enzymes add one atom of oxygen to a substrate molecule and reduce the second atom to

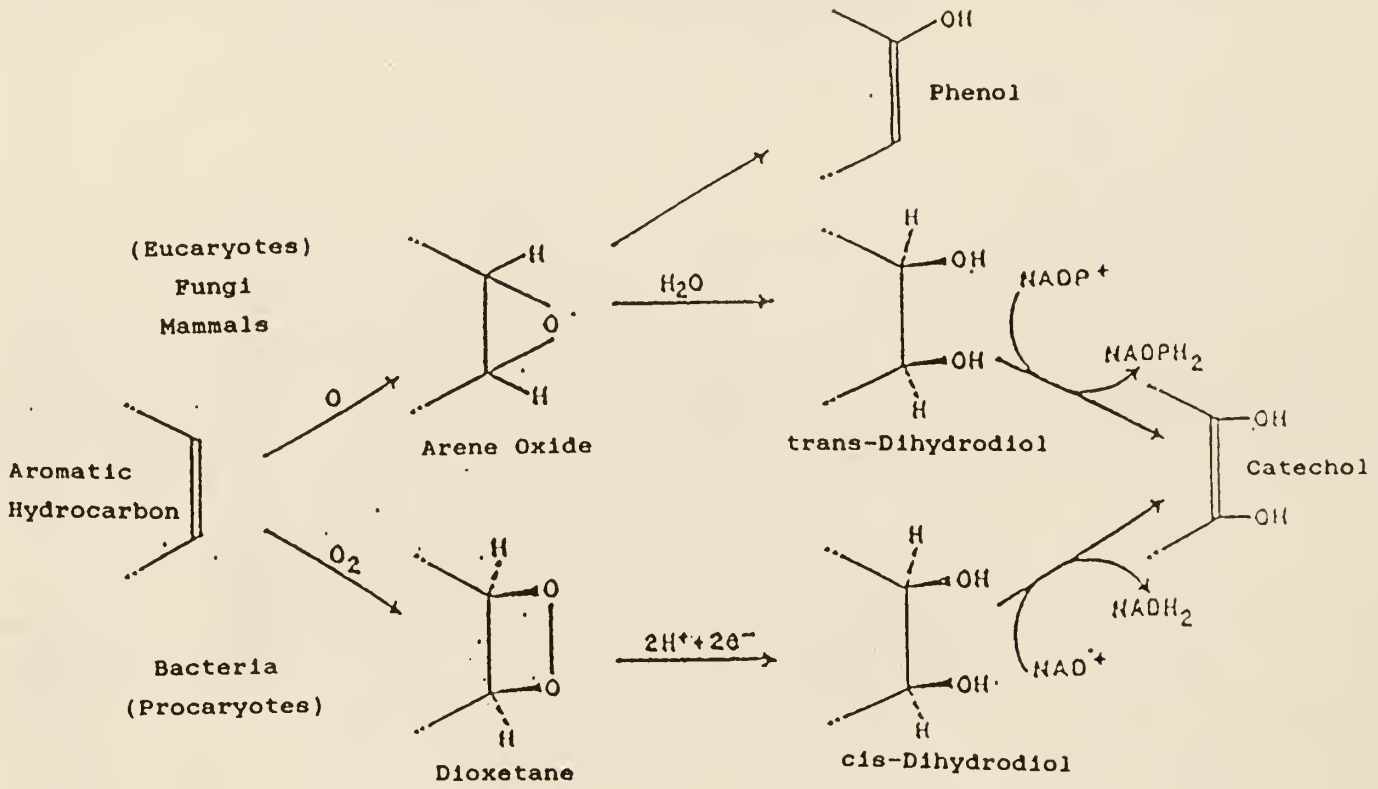


Figure II-4. Initial reaction of fungi and bacteria to oxidize polynuclear aromatic hydrocarbons. Sims, 1982; Cerniglia et al. 1979; and Gibson, 1976.

water by means of a reduced enzymatic cofactor. Mono-oxygenases are characteristic of eucaryotes including mammals and fungi. This is the pathway that produces the epoxides that cause cancer.

The second group of oxygenases are the dioxygenases, which add two atoms of oxygen to a substrate molecule. Although a reduced coenzyme may be used, it is not with the concomitant formation of water. These are characteristic of procaryotes, including bacteria.

Following the initial hydroxylation and ring fission of the aromatic nucleus, the microorganisms transform the phenols and carboxylic acids produced through the Krebs cycle. Figure II-5 shows this pathway for naphthalene by soil bacteria (Dean-Raymond and Bartha, 1975; Gibson, 1976; and Gibson, 1968).

Figure II-6 presents a catabolic pathway of naphthalene and naphthalene-2sulfonic acid by P. testosteroni A3 and by Pseudomonas sp. C22 proposed by Brilion et al. (1981). They have concluded that naphthalene and naphthalene sulfonates are degraded via salicylate and gentisate as key metabolites. These results differ significantly from those found in other naphthalene-utilizing bacteria, where catechol as the only metabolite of salicylate is subject to ortho- or meta-cleavage.

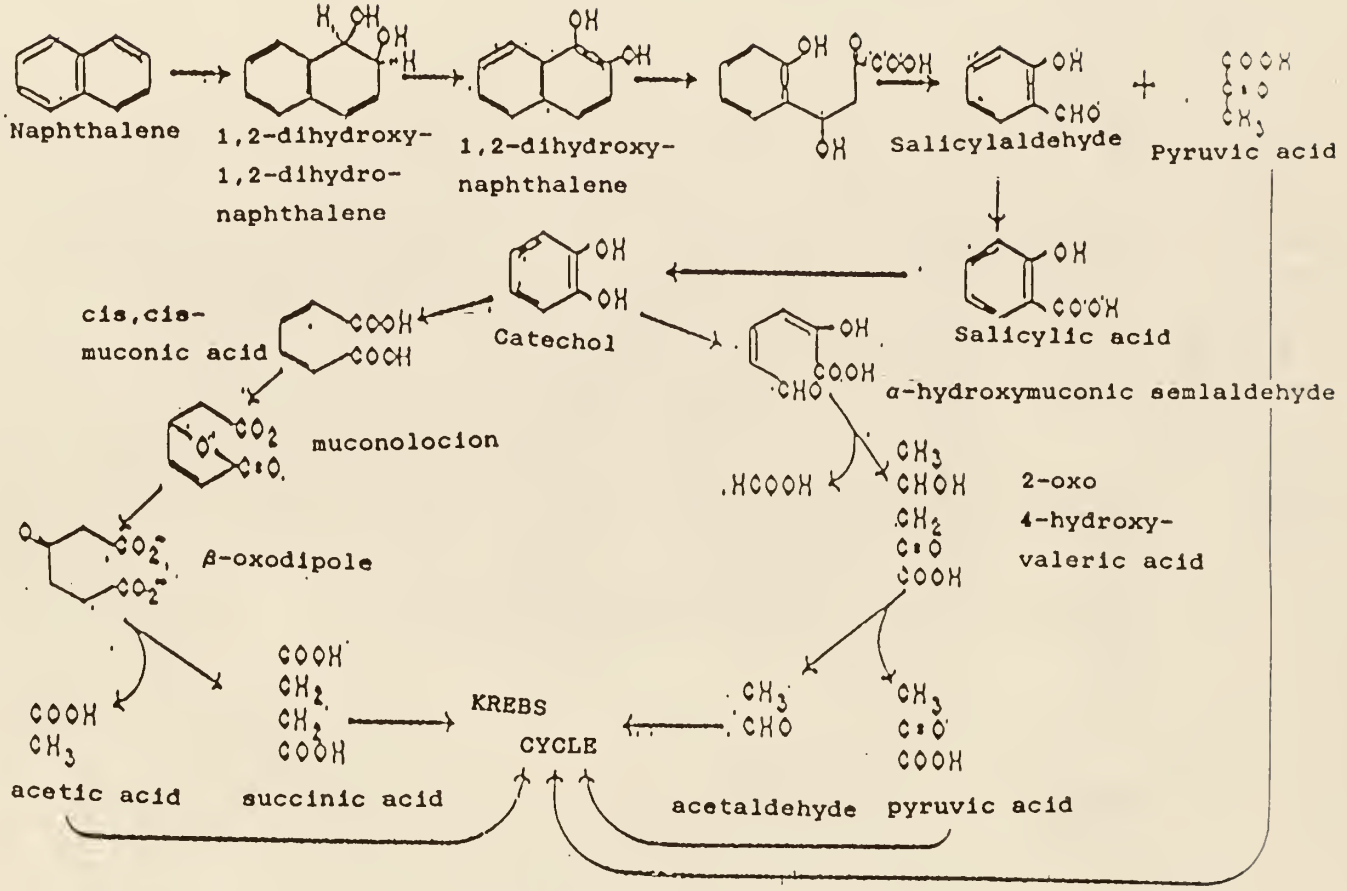


Figure II-5. Metabolism of naphthalene by soil bacteria. Sims, 1982; Dean-Raymond and Bartha, 1975; Gibson, 1976; and Gibson, 1968.

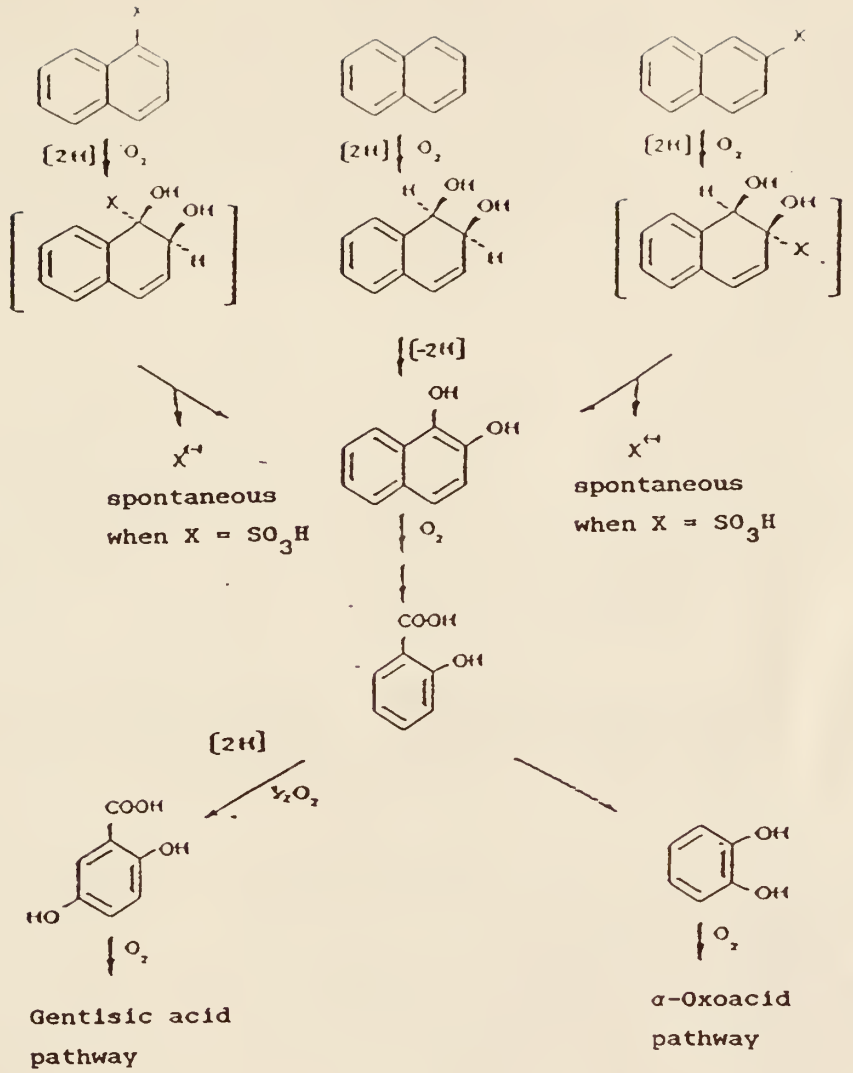


Figure II-6. Proposed catabolic pathway of naphthalene and naphthalene-2-sulfonic acid by P.testosteroni A3 (X in position 2 = SO_3H) and of naphthalene-1-sulfonic acid by Pseudomonas sp. C22 (X in position 1 = SO_3H). Brilion et al., 1981.

Polycyclic aromatic hydrocarbons, such as anthracene and phenanthrene, are also degraded by the ortho-cleavage pathway. Initial reactions are very similar to those used for simpler aromatic compounds with an initial hydroxylation by a mono or dioxygenase to form a 1,2-dihydroxy substituted PNA.

When an alkyl chain is present on a PNA, it is removed by beta-oxidation if it is larger than an ethyl group. Ring cleavage usually can occur when methyl side chains are present. However, when certain locations on the ring are substituted, the resulting compound is very resistant to degradation (McKenna and Heath, 1976).

ORGANISMS CAPABLE OF DEGRADING PNAS

Organisms capable of degrading the aromatic rings include a number of bacteria, such as Chromobacter, Bacillus, and Arthrobacter. Other soil organisms, such as Xorynebacterium, Nocardia, and Mycobacterium have been also mentioned (Dart and Streton, 1980). Table II-III shows organisms that have been reported to show growth for some PNA's.

Aerobic bacteria possess the unique biochemical ability to catalyze the oxidation of compounds. This is

Table II-III

Organisms Capable of Oxidizing PNA's

Soil Microorganism	Reference
I Bacteria	
Pseudomonas putida	Gibson, 1968
Pseudomonas putida	Gibson et al., 1968
Pseudomonas putida	Jeffrey et al., 1975
Pseudomonas putida	Jeffrey et al., 1975
Pseudomonas sp.	Dagley et al., 1960
Pseudomonas sp	Jerina et al., 1971
Pseudomonas arvilla	Nozaki et al., 1963
Pseudomonas fluorescens	Jeffrey et al., 1975
Pseudomonas aeruginosa	Rogoff & Wender, 1957
Beijerinckia sp	Gibson et al., 1975
Beijerinckia sp	Selander et al., 1976
Beijerinckia sp	Akhtar et al., 1975
Rhizobium sp	Hussein et al., 1974
II Actinomycetes	
Nocardia sp	Webley et al., 1962
Nocardia sp	Jamison et al., 1969
III Fungi	
Cunninghamella elegans	Cerniglia & Gibson, 1978
Cunninghamella elegans	Cerniglia & Gibson, 1979
Cunninghamella elegans	Cerniglia et al., 1979

Sims, (1982).

done using molecular oxygen to initiate reaction sequences that enter central pathways of metabolism including the Krebs cycle and the fatty acid spiral (Dagley, 1975).

The ability of bacteria to utilize aromatic hydrocarbons for growth was first demonstrated by Stormer (1908) who isolated Bacillus hexacarbovorum by virtue of its ability to grow with toluene and xylene. Sohngen (1913) reported the utilization of benzene by microorganisms, and one year later Wagner (1914) isolated two organisms, Bacterium benzoli a and b, both capable of growth with benzene, toluene, and xylene.

Bacteria from water and sediment of an oil-polluted harbor were examined for ability to degrade petroleum by Walker et al. (1975). It was found that bacteria present in the water samples produced significantly greater degradation of 2-,3-,4-, and 5-ring cycloalkanes and mono-, di-, tri-, tetra-, and penta-aromatics compared with bacteria in sediment samples.

Yeast and fungi have been also found to be able to degrade PNA's. In a study by Fedorak et al. (1984), seventy four yeast and two hundred and twenty four fungi were isolated from marine water and sediment samples taken from the Strait of Juan de Fuca. Only three yeast and sixty three fungi were able to degrade some or all of the n-alkanes present in Prudhoe Bay crude oil. Twelve organisms

which degraded n-alkanes were tested for their ability to mineralize naphthalene and phenanthrene which had been added to the crude oil. No CO_2 was detected from any of the cultures indicating that they were unable to completely or partially oxidize any of the resolvable compounds in this fraction. Cerniglia and Crow (1981) found that the six yeast they examined for the ability to metabolize naphthalene, biphenyl and benzo(a)pyrene were able to oxidize these aromatic hydrocarbons.

Very little work has been published on the possibility of algae being involved in aromatic biodegradation. Although Dart (1980) has shown that a wide range of green and blue-green algae can grow in the presence of relative high levels of aromatic compounds. Cerniglia et al. (1980) showed that members of the cyanophyceae and green algae can metabolize naphthalene to produce 1-naphthanol.

ENVIRONMENTAL FACTORS AFFECTING BIODEGRADATION

La Riviere (1955) pointed out that the production of surface active agents by some bacteria would aid in the dispersal of increased amounts of hydrocarbons into the aqueous phase for improved growth. Wodzinski and Johnson

(1968) found that there is an apparent relationship between the water solubility of the aromatic hydrocarbons and the bacterial growth rate. They studied four bacteria that were able to utilize naphthalene, phenanthrene, and anthracene. A strain of Pseudomonas sp. isolated from phenanthrene gave a cell yield (grams of cell produced per gram of hydrocarbon used) of 40% with a generation time of 10 hours. The one isolated from naphthalene gave a cell yield of 51% and a generation time of 1.5 hours. They concluded that insoluble aromatic hydrocarbons would not be degraded rapidly by bacteria because the substrate would be less available to cells due to its low concentration in the medium. Wodzinski and La Rocca (1977) used a Pseudomonas sp. that was isolated on naphthalene (solid) but could not utilize heptamethylnonane. It was grown in the presence of various amounts of naphthalene and heptamethylnonane (liquid). The growth rates indicated that the bacterium could utilize naphthalene at the aqueous hydrocarbon interface, which is not the case in the absence of heptamethylnonane.

In another study by Mulkins-Phillips and Steward (1974), four chemical dispersants, Corexit 8666, Gamlen Sea Clean, G.H. Woods Degreaser-Formula 11470, and Sugee 2 were examined singly and in individual combinations with Arabian crude oil. They were studied at 10 and 25 C for their effects on the growth of bacteria indigenous to local marine

waters. None of these dispersants were toxic even at relatively high concentrations. Sugee 2, which had the poorest emulsifying capacity, promoted n-alkane degradation compared with the values using the crude oil alone.

In the mixed microbial population of soil, one type of microorganism may partially metabolize a compound without deriving carbon or energy for cell synthesis, but it may provide a substrate for another organism (Shabad, 1975). McKenna (1977) used a mixed microbial culture in a cooxidation experiment. He found that multi-ring PNA's are degraded by mixed cultures. But he also stated that no organisms have been isolated which can grow on PNA's possessing more than three fused rings. Structures with four or more condensed rings have been shown to be attacked by co-oxidation as a result of commensalism (Atlas, 1981). The conclusion of these experiments is that multi ring PNA's can be degraded by microorganisms when the PNA's are present as cosubstrates.

There is considerable agreement in the literature about the relationship between initial concentration and rate of degradation. Sherrill and Syler (1980) showed that the rate of degradation of phenanthrene in natural aquatic systems is related to the degree of pollution of the site and pH concentration. By increasing the concentration 100 times the rate of degradation increased three times.

Gardner et al. (1979) used anthracene, fluoranthene, benz(a)anthracene, and benz(a)pyrene and arrived to the same conclusion. Poglazova et al. (1967, 1968) found that bacteria isolated from soil containing large amounts of benzo(a)pyrene were much more active in degrading it than bacteria from uncontaminated soil. Shabad (1975) has reported that there is a need for previously acclimated microorganisms to be used to degrade newly added PNA's to soil systems. Herbes (1981) has arrived at the conclusion that continuous inputs of PNA's result in an increased ability within the microbial community to utilize certain polycyclic aromatic hydrocarbons. Turnover times of 13 hours (naphthalene), 62 hours (anthracene), and 300 hours (benz(a)anthracene) were found for the sediments collected in this study. Only naphthalene and anthracene transformations were observed in water samples, but rate constants were found to be consistently 5- and 20- fold lower, respectively.

Colaruotolo et al. (1985) have found that microorganisms present in the locus of concentrated deposits of halo-organic chemicals have not only managed to stay alive but also have adapted themselves to grow and multiply on the organic material in the land field as their sole source of carbon and energy. Prior to chemical wastes being accumulated in a given landfill the soil and surrounding

environment was populated with bacteria that used the normal hydrocarbon material in the soil as their source of carbon and energy. As the wastes were deposited on them, all but the strongest were killed off. These bacteria evolved so that they adapted their metabolism to break the carbon to chlorine bond causing the formation of more easily metabolized materials similar to the substrates the bacteria have been accustomed to metabolizing.

In another study by Heitkamp et al. (1987), mineralization half lives for naphthalene in microcosms ranged from 2.4 weeks in sediment chronically exposed to petroleum hydrocarbons to 4.4 weeks in sediment from a pristine environment. Microbial analysis of sediments indicated that hydrocarbon utilizing microbial population also varied among ecosystems and were 5 to 12 times greater in sediment after chronic petrogenic chemical exposure than in sediments from an uncontaminated ecosystem.

Degradation rates are affected by different factors. We have already discussed acclimation, microbial flora, and the presence of other substances, such as surfactants and cosubstrates. It has been demonstrated that the molecular weight, temperature, pH, and oxygen status also influence the rates of decomposition.

Coover and Sims (1987) found that increasing the temperature significantly (they studied 10, 20, 30 C)

improved the rate and extent of apparent loss of low molecular weight PNA's. They did not observe any effect of temperature on rates for PNA's of five to six rings. Another investigation done by Sherrill and Syler (1980) showed that although biodegradation was directly related to incubation temperature (degradation of phenanthrene increased by approximately 20% for each 5 C raise in temperature over the range 15 to 37 C), it was not correlated with ambient site temperature. Samples taken during winter had the same degradation potential as those taken during warmer weather.

The pH of the PNA's is very important for the study of biodegradation. At a low pH (4.5), bacterial activity is inhibited but fungi activity is not. As discussed earlier, it is the fungi metabolism that causes mutations on the parent DNA. By increasing the pH to neutrality, the bacteria will dominate choosing the desired metabolism (Sims, 1983).

In acid soils, pH appears to be a primary limiting factor in microbial degradation of oil. Verstraete et al. (1975) found that break down of gas oil proceeded slowly under acidic conditions (pH 4.5). Podzol soil and alkanes were more susceptible to microbial degradation than the aromatic compounds. However, the adjustment of the soil to a pH of 7.4 about doubled the rate of breakdown of alkanes

and aromatics. Further increase in the soil pH to 8.5 resulted in less hydrocarbon degradation than at pH 7.4.

Dibble and Bartha (1979) believe that the optimum soil pH for oil degradation is about 7.8. They monitored CO_2 evolution in oil sludge-treated soil adjusted to pH of 5.0, 6.0, 7.0, and 7.8. The highest pH tested resulted in the highest CO_2 evolution rate. Sims (1982) reported that pH affects some PNA's but not all. He found that pH adjustment affected significantly reduction on indeno(1,2,3,-cd)pyrene, but dibenz(a,j)acridine degradation was not affected by pH adjustment.

The size of the PNA's also influences the rates of transformation. Lee (1977) found that low molecular weight PNA's (one to two rings) were biodegraded to CO_2. But higher molecular weight compounds were resistant to biodegradation. Herbes and Schwall (1978) reported turnover times in petroleum contaminated sediment increased from 7.1 hours for naphthalene to 400 hours for anthracene, 10,000 hours for benz(a)anthracene, and more than 30,000 hours for benz(a)pyrene. Polynuclear aromatic compounds tended to be only partially degraded to CO_2.

Oxygen availability influences biodegradation. Excessive moisture causes suppression of microbial proliferation because the oversupply limits gaseous exchange

and lowers available oxygen supply, creating thereby an anaerobic environment (Gibson, 1984). If ground water is taken from an anaerobic gasoline or fuel oil contaminated area with no significant degradation activity, and oxygen and nutrients are then added, rapid degradation down to one μg/l or less starts with little lag time (Jensen et al., 1986). Cerniglia et al. (1980) found that naphthalene was oxidized in the light but not in the dark, presenting another factor that influences the biodegradation rates.

KINETICS OF BIODEGRADATION

Biodegradation rates of polynuclear aromatic hydrocarbons have been studied in the past by several investigators. Table II-IV summarizes the degradation of PNA's in soil systems. Information from a variety of sources is presented to describe PNA degradation as a function of soil concentration. The kinetic parameters used are the initial concentrations, rate constants, and rates of transformation. The kinetic parameter, k, for substrate is calculated assuming a first order equation for biodegradation in soil:

$$dC/dt = - kC \qquad \text{(II-2)}$$

$$dC/C = -kdt \qquad \text{(II-3)}$$

$$\ln(C/C_0) = -kt \qquad \text{(II-4)}$$

where C_0 is the initial concentration of the organic waste. A semilogarithmic plot of C/C_0 against time provides the value of the rate constant, which is the slope of the line. The rate constant is expressed in $days^{-1}$. The rate of transformation, r, is calculated as the product of the initial concentration of the waste, C_0, and the rate constant, k. The rate of transformation is expressed as mg substrate/ kg soil-day.

The conclusions from the results in Table II-IV are that for a given PNA compound the initial rate of transformation increases with increasing initial soil concentration, and that the initial rate of transformation decreases with increasing number of fused benzene rings (molecular weight).

The environmental effects of adding certain selected petroleum products to field soils at widely separated geographical locations under optimum conditions for biodegradation were studied by Raymond et al. (1976). They applied six different types of oils at a rate of 11.9

Table II-IV

Kinetic Parameters Describing Rates of Degradation of Aromatic Compounds in Soil Systems.

PNA	C_0 (mg/kg)	k (day^{-1})	r (mg/kg-day)	Reference
Pyrocatechol	500	3.47	1735	Medvedev & Davidov,1972
Phenol	500	0.69	364	Medvedev & Davidov,1972
Fluorene	0.9	0.02	0.016	Groenewegen & Stolp,1976
Fluorene	500	0.35	173	Medvedev & Davidov,1972
Indole	500	0.69	364	Medvedev & Davidov,1972
Naphtol	500	0.77	385	Medvedev & Davidov,1972
Naphthalene	7.0	5.78	40.4	Herbes & Schwall,1978
Naphthalene	7.0	0.005	0.035	Herbes & Schwall,1976
Naphthalene	25,000	0.173	4331	Sisler & Zobell,1947
1,4-Naphtho-quinone	500	0.578	288.8	Medvedev & Davidov,1972
Acenaphthene	500	0.173	86.6	Medvedev & Davidov,1972
Anthracene	3.4	0.21	0.714	Gardner et al.,1979
Anthracene	36.4	0.005	0.196	Gardner et al.,1979
Anthracene	25,000	0.198	4950	Sisler & Zobell,1947
Phenanthrene	2.1	0.027	0.056	Groenewegen & Stolp,1976
Phenanthrene	25,000	0.277	6930	Sisler & Zobell,1947
Carbazole	500	0.067	33	Medvedev & Davidov,1972
Acridine	5	0.281	1.16	Medvedev & Davidov,1972
Benz(a)-anthracene	3.5	0.007	0.024	Groenewegen & Stolp,1976
Benz(a)-anthracene	.12	0.046	0.005	Herbes & Schwall,1978

Table II-IV. Continued.

PNA	C_0 (mg/kg)	k (day^{-1})	r (mg/kg-day)	Reference
Benenz(a)-anthracene	20.8	0.003	0.062	Gardner et al.,1979
Benz(a)-anthracene	72.8	0.004	0.257	Gardner et al.,1979
Benz(a)-anthracene	25,000	0.173	4331	Sisler & Zobell,1947
Fluoranthene	3.9	0.016	0.061	Groenewegen & Stolp,1976
Fluoranthene	18.8	0.004	0.072	Gardner et al.,1979
Fluoranthene	72.8	0.005	0.379	Gardner et al.,1979
Pyrene	3.1	0.020	0.061	Groenewegen & Stolp,1976
Pyrene	500	0.067	33	Medvedev & Davidov,1972
Pyrene	5	0.231	1.16	Medvedev & Davidov,1972
Chrysene	4.4	0	0	Groenewegen & Stolp,1976
Chrysene	500	0.067	33	Medvedev & Davidov,1972
Chrysene	5	0.126	0.63	Medvedev & Davidov,1972
Benz(a)-pyrene	0.048	0.014	0.0007	Herbes & Schwall,1976
Benz(a)-pyrene	0.01	0.001	0.00001	Herbes & Schwall,1976
Benz(a)-pyrene	3.4	0.012	0.041	Groenewegen & Stolp,1976
Benz(a)-pyrene	9.5	0.002	0.022	Gardner et al.,1979
Benz(a)-pyrene	32.6	0.004	0.129	Gardner et al.,1979

Table II-IV. Continued.

PNA	C_0 (mg/kg)	k (day)	r (mg/kg-day)	Reference
Benz(a)-pyrene	1.0	0.347	0.347	Shabad et al.,1971
Benz(a)-pyrene	28.5	0.019	0.533	Shabad et al.,1971
Benz(a)-pyrene	29.2	0	0	Shabad et al.,1971
Benz(a)-pyrene	9,100	0.018	161.7	Lijinski & Quastel,1956
Benz(a)-pyrene	19.5	0.231	4.5	Poglazova et al.,1967b
Benz(a)-pyrene	130.6	0.116	15.08	Poglazova et al.,1968
Dibenz(a,h)-anthracene	9,700	0.033	320.1	Lijinski & Quastel,1956
Dibenz(a,h)-anthracene	25,000	0.039	962.5	Sisler & Zobell,1947

Where C_0 = initial concentration, mg/kg; k = rate constant, $days^{-1}$; and r = rate of transformation, mg/kg-day. Sims,(1982).

m^3/4000 m^2 in a single application. Rates of degradation did not exceed 2.4 m^3/4000 m^2 per month.

In Table II-V the specific turnover rates of substituted naphthalene by naphthalene grown cells of P. testosteroni A3 and Pseudomonas sp. C22 are presented. Cells were grown at 30 C in mineral salts medium by use of a 2-liter fermentor. Air (1 l/min) containing naphthalene was introduced to the culture. The initial concentrations were 1 mM for naphthylsulfonates and naphthylcarboxylate and 0.5 mM for all other substituted naphthalene. Absolute specific turnover rates were expressed as μmoles/min-g protein (Brilion et al. 1981).

Reddy et al. (1980) have developed a conceptual model to describe organic carbon loss from land areas receiving organic wastes. In this model, the overall organic carbon composition is divided into several fractions which exhibit different characteristics with respect to the ease with which the carbon can be decomposed. In the Reddy et al. (1980) model, carbon loss is modeled by first order kinetics with the more resistant carbon fractions having much slower decomposition rates. A somewhat analogous approach has been employed by Molina et al. (1983), who have developed a model which computes the short term dynamics in soil of ammonia, nitrate, carbon, and nitrogen substituted organics. This

Table II-V

Specific Turnover Rates of Substituted Naphthalene by *P. testosteroni* A3 and *Pseudomonas sp.* C22.

Substrate	Specific turnover rates (μmoles/min-g protein)	
	Strain A3	Strain C22
Naphthalene	330	150
1-Naphthalenesulfonates	<.01	12
2-Naphthalenesulfonates	34	25
2-Naphthalenescarboxylate	12	12
1-Hydroxynaphthalene	7	5
2-Hydroxynaphthalene	24	18
2-Nitronaphthalene	ND	13
2-Chloronaphthalene	ND	10
2-Methylnaphthalene	ND	14
2-Methoxynaphthalene	<.01	10

ND= low, not precisely determinable rates.

Brilion *et al*. (1981).

model has classified the active organic carbon phases into different phases which have specific kinetic constants.

Sims (1982) used a first order kinetic model and calculated the half life times for some PNA's in his study. Table II-VI presents these results after six months from the start of the experiment.

A first order kinetic model was found to adequately characterize the loss of benzo(a)pyrene in a study done by Coover and Sims (1987), but it tended to overestimate degradation during an extended incubation period. Figure II-7 shows the results obtained in this study.

CONCLUSIONS

Polynuclear aromatic compounds are a class of organics including hydrocarbon and heterocycle species. Different PNA's vary in their susceptibility to biodegradation. The main factor is the ring number. The biodegradation rate is affected by solubility and temperature, both dependent on molecular weight.

PNA's do decompose in soil systems, as shown by this review. Research is needed to establish conclusively rates of decomposition and soil levels above which application of PNA's adversely affects the food chain. Investigation of

enhancement factors that increase degradation rates of PNA's, especially those with more than three rings, is another area that needs attention.

The information presented suggests that a substantial degradation rate could be achieve for the PNA's if bacterial seeding or in situ acclimation of a land is conducted.

Storage and land-filling of PNA's wastes are not ultimate solutions but simply postpone the problem of disposal. Concentrations of PNA's need to be lowered to below threshold levels so that they are non-toxic and non hazardous to the water supplies or to the food chain.

Table II-VI

Kinetic Results for Degradation of PNA's in Soil.

(180-270 days)

PNA	C_0 (mg/kg)	k ($days^{-1}$)	r (mg/kg/day)	t (days)
Acenaphthylene	63	0.032	2.0	22
Dibenzofuran	58	0.038	2.2	19
Dibenzothiophene	71	0.033	2.3	21
Anthracene	62	0.018	1.1	39
Acridine	134	0.009	1.3	73
Benz(a)anthracene	9.7	0.017	0.17	41
Dibenz(a,h)-anthracene	147	0.007	0.96	119
Benzo(b)-fluoranthene	53	0.01	0.50	73
Benzo(k)-fluoranthene	2.7	0.007	0.018	100
Benzo(a)pyrene	69	0.008	0.52	92
Dibenz(a,j)-acridine	73	0.015	1.1	46
Indeno(1,2,3,-cd)-pyrene	169	<.001	<.057	>600

Where C = initial concentration, mg/kg; k = rate constant, $days^{-1}$; r = (k)(C), biodegradation rate, mg/kg-day.

Sims, (1982).

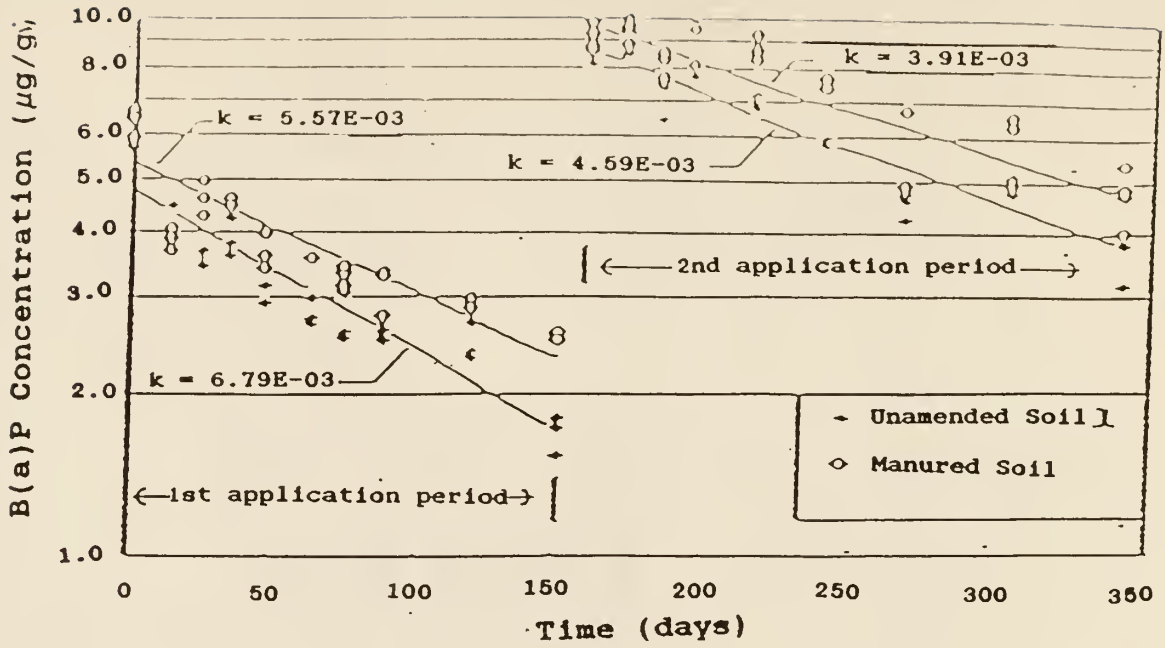


Figure II-7. Apparent loss of Benzo(a)pyrene over the course of two application periods in manure amended and unamended Durant Clay Loam and the application of a first order kinetic model to the data. Coover and Sims, 1987b.

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CHAPTER III

BIODEGRADATION OF ORGANIC COMPOUNDS FROM A REFINERY SITE

This chapter describes biodegradation experiments for samples from an abandoned refinery site. The purpose was to determine if the compounds were biodegradable and if the addition of surfactant enhanced degradation. This information is necessary to evaluate the possibility of remedial action at the site.

THEORY

The batch growth process has four distinctive phases: the lag phase, the exponential phase, the stationary phase, and the death phase. In the lag phase there is no increase in the initial number of cells since they have not acclimated yet. The next phase is the exponential growth phase where the cell number increases exponentially. Following this exponential phase is the stationary phase. At this point there is a shortage of nutrients; thus, some

cells are dividing and others die. The number of cells stays constant since they use the nutrients freed from the dead cells. But the nutrient depletion goes on and the death phase begins. Usually this phase follows exponential decay.

The specific growth rate during the exponential phase is called the maximum specific growth rate. The exponential phase is easily determined from the linear section of a plot of the logarithm of biomass concentration against time. If the linear section is not clear in this plot, there are more quantitative statistical methods to define it. The maximum specific growth rate is the slope of this line:

$$\ln X = \mu t \qquad \text{(III-1)}$$

where X is biomass concentration, μ is the maximum specific growth rate, and t is time.

Equation (III-1) is the result of the integration of

$$\mu = \frac{1}{X}\frac{dX}{dt} \qquad \text{(III-2)}$$

which assumes a constant specific growth rate during the exponential growth phase.

Biomass yield (biomass produced/substrate consumed) can be calculated from biomass and substrate data in the exponential growth phase. Thus,

$$Y_s = (X_2-X_1)/(S_1-S_2) \qquad \text{(III-3)}$$

where subscript 1 refers to the starting time and subscript 2 refers to the ending of the exponential growth phase.

Kinetic parameters of interest with respect to biodegradation include the rate of transformation (g substrate/g soil-day) and the rate constant, k ($days^{-1}$). The kinetic parameter, k, for substrate is calculated assuming a first order equation for biodegradation in soil:

$$dC/dt = - kC \qquad \text{(III-4)}$$

$$dC/C = -kdt \qquad \text{(III-5)}$$

$$\ln(C/C_0) = -kt \qquad \text{(III-6)}$$

where C_0 is the initial concentration of the organic waste. a semilogarithmic plot of C/C_0 against time provides the value for the rate constant, which is the slope of the line.

The rate constant is expressed in $days^{-1}$. The rate of transformation is calculated using the initial concentration of the waste, C_0, and k :

$$dC/dt = -kC_0 \qquad \text{(III-7)}$$

The rate of transformation is expressed as g substrate/g soil-day.

Another kinetic model that is widely used is the Monod model. In this model the specific growth rate is related to the substrate concentration by the following equation;

$$\mu = \frac{\mu_{max} S}{K_s + S} \qquad \text{(III-8)}$$

A plot of $1/\mu$ versus 1/S will have as its y intercept $1/\mu_{max}$, and K_s/μ_{max} as its slope.

Surfactants

The worldwide consumption of surfactants, even excluding soaps, is now measured in billions of pounds. In the United States alone, surfactants are a multibillion dollar industry, with hundreds of different types used in industrial and consumer products for a multitude of different purposes. In this study the surfactant Tween 20 (polyoxyethylene sorbitan monolaurate) was chosen for its availability and properties.

Surfactants are a class of natural and synthetic chemicals whose abilities to promote the wetting, solubilization, and emulsification of various types of organic chemicals have found widespread application. These properties make surfactants potentially useful in the in situ treatment of certain organic fractions in waste deposits. Used in conjunction with various groundwater flooding and dewatering techniques, surfactants may offer a means of improving the removal efficiency of these organics over the results likely to be obtained with water alone.

Adsorption causes the transfer of contaminants from the aqueous to the solid phase of the aquifer, whereas contaminants are returned to the aqueous phase by desorption. Therefore, adsorption serves to retard the rate of migration of contaminants. When the containment of the

zone of contamination is desirable, it would help to enhance adsorption of the contaminants. However, during remediation, it may be necessary to flush the contaminants from the aquifer system, in which case enhancing the desorption of the contaminants would become important.

MATERIAL AND METHODS

The soil used for this experiment was from the Arkansas City dumpsite, where the Milliken Oil Refinery was located. Samples were classified into three different types according to location; acid sludge, soil found 10 feet below the surface and soil found 20 feet below the surface, which was also below the water table. A mixed culture was harvested from the surrounding soil of the acid sludge pond and used as inocula.

The experiment consisted of three different treatments and three different types of soil. The treatments were saturated, unsaturated, and saturated with the addition of surfactant. The surfactant used was Tween 20 due to its properties and availability. For the saturated cases 800 ml beakers were used, and for unsaturated cases flower pots were used.

The acid sludge received all three treatments; experiment AT for acid sludge plus surfactant (saturated), experiment AS for acid sludge saturated, experiment AU for acid sludge unsaturated. The samples taken at 20 feet were investigated under saturation (experiment 20S) and saturation with surfactant (experiment 20T) conditions, and the sample from 10 feet below the surface received unsaturated treatment (experiment 10U).

The experiment lasted for 175 days with measurements taken every 14 days. The acid sludge was neutralized to a pH of 6.5 with calcium carbonate and water. It was maintained at this pH with the nutrient medium which included a phosphate buffer. This media contained the following: 7.5 grams/liter (g/l) of $(NH_4)_2SO_4$; 1.5 g/l of Na_2HPO_4; 3.5 g/l of KH_2PO_4; 0.5 g/l of $MgSO_4.7H_2O$; 30 X 10^{-3} g/l of $FeSO_4.7H_2O$; 60 X 10^{-3} g/l of $CaCl_2.2H_2O$; 15 X 10^{-6} g/l of $CuSO_4.5H_2O$; 30 X 10^{-6} g/l of $Na_2B_4O_7.10H_2O$; 60 X 10^{-6} g/l of $MnCl_2.4H_2O$; 150 X 10^{-6} g/l of $ZnSO_4.7H_2O$; distilled water and soil as the only source of carbon.

The experiments were conducted at room temperature. Since the samples contained polynuclear aromatic hydrocarbons, they were placed under a hood throughout the

experiment. Each sample contained 650 grams of soil. To start the experiment the acid sludge material was neutralized. The original material had a pH of approximately 1.0 . Close to 500 grams of calcium carbonate were needed to bring 650 grams of soil to a pH of 6.5.

The samples under saturated conditions received the nutrient solution every two days so that they were at all times under one centimeter of solution. The unsaturated samples received the nutrient solution every other day, which was the average time it took the soil to get dry.

Samples were analyzed for the organic concentration in terms of chemical oxygen demand (COD), and high pressure liquid chromatography (HPLC). Chemical elements were measured with elemental analysis. Samples were also analyzed for cell concentration using Standard Plate Count Methods. Temperature and pH were also monitored.

The chromatography system consisted of a Varian Model 5000 liquid chromatograph equipped with a Varian Model 9176 strip chart recorder,an ISCO ISIS Auto-sampler and auto-injector, and a Varian Vari-Chrom UV-VIS Detector. Separation was accomplished by reverse-phase HPLC utilizing a Varian MCH-10 column. The column is packed with octadecylsilane ($C_{18}H_{37}$ covalently bonded to silica). In reverse-phase chromatography the separation is governed by

the hydrophobic character of the solute compounds; the more hydrophobic the compound, the longer it is retained in the column. Isocratic elution for one minute using 40% acetonitrile/ 60% water, then linear gradient elution to 100% acetonitrile over 15 minutes at a flow rate of 2.0 ml/minute is the recommended method. Due to technical difficulties, this method could not be used and 100% acetonitrile was used. The HPLC program and instrument settings are described in Tables III-II through III-V.

Detection of the substrate was accomplished by UV absorption at 254 nm. Concentrations were recorded in integrater units (I.U.) by the 9176 recorder. The standard curve used for converting the integrater units into grams of substrate were derived from standard curves using naphthalene and phenanthrene at known concentrations. The procedure and data for these conversion equations are given in the Appendix.

This HPLC procedure required extraction and dilution of the sample for preparation procedures. Ten grams of soil were placed in an 125 ml beaker or flask. Methylene chloride (50 ml) was added to the sample container. The solvent-soil system was covered with laboratory film paper and allowed to sit for thirty minutes. Then, it was homogenized for one minute with VIETis Homogenizer. The methylene chloride extract was filtered and the flask rinsed

twice with methylene chloride. The filtrate was evaporated to near-dryness in a rotary evaporator (Buchi Rotavapor R110) with a 90-100 C water bath. The final volume was brought quantitatively to ten ml with methylene chloride. This extract was diluted 1:20 with acetonitrile and filtered using glass fiber filter with Millipore vacuum filtration. Then it was injected into the HPLC. All samples were extracted the same day and completely analyzed within seven days of extraction . If the peak area exceeded the linear range of the system, the extract was diluted and reanalyzed.

The COD of the samples was determined by EPA approved Hach system using premixed reagents in ready to use,screw capped vials and compact reactor heater. The samples were diluted so that the range of COD was from 10-1400 mg/l. After two hours in the reactor heater the digestion was completed, and the vials were cooled at room temperature for fifteen minutes. Then the percent of transmittance at 620 nm was measured using Baush & Lomb Spectronic 20 spectrophotometer, and the calibration table provided by Hach was used to determine the COD of each sample.

Determination of the biomass concentration was accomplished by the Standard Plate Count Method (SPCM). This method was chosen over optical density and dry weight because the samples treated were solids. The method for

determination of the biomass concentration is based on Microbiological Methods for Monitoring the Environment,EPA 600/8-78-017. Each sample consisted of eleven grams of soil diluted so that the final plate count was between 30-300 colonies. The dilution water was sterilized in 100 ml dilution bottles for twenty minutes at 20 psia in the autoclave. Since the microbial population in the sample was unknown, a series of dilutions was prepared and plated in duplicates to obtain a plate count within the desired range. Sterile plate count agar was prepared and poured into sterile petridishes. Duplicate plates were prepared for each dilution. Figure III-1 shows the method used to dilute each sample. The plates were incubated at 32 C for 48 ± 3 hours. Each plate was counted immediately after incubation using an Accu-lite Colony Counter, model 133-8002.

Inocula for the experiment were obtained from soil around the dumpsite and were maintained with medium and nutrient broth. This was done in order to reduce the lag phase. To establish the initial biomass concentration, 50 ml of this inocula were placed in each sample. The initial biomass concentration was measured with the dry weight method. This method consists of determining the biomass concentration from the dry weight of the sample. In this experiment it was performed by filtering samples through

0.45μm nitrocellulose filters. The filters are placed in an oven at 105 C for 24 hours of drying before weighing.

RESULTS AND DISCUSSION

The purpose of the present experiments was to determine if biodegradation was a possible remedial solution for the Arkansas City dumpsite. There are two conditions present in the site; saturated and unsaturated soil. These, along with the addition of a surfactant, were the treatments studied. The results show that it is possible to biodegrade the materials and that the addition of surfactant increases the rate of transformation for the materials treated with it.

The data for these experiments are given in Tables III-VI through III-XII. Figures III-2 through III-7 illustrate the concentration profiles of biomass and substrate based on HPLC data as well as biomass and substrate profiles as predicted by the Monod model. Table III-XII shows the specific growth rates for the experiments using the definition of specific growth (biomass data only). Table III-XIII summarizes the results for the different materials and treatments using the Monod model and the data from the HPLC analysis. Table III-XIV presents the same results using the data from the COD analysis. Tables III-XV and III-XVI

show the values of the kinetic parameter of the first order kinetic model for the substrate and the agreement of a simple exponential model for the biomass using the HPLC and COD data respectively. Figures III-14 to III-25 demonstrate the agreement of these two models for biomass and substrate concentrations.

In Table III-XII the sample that shows the highest specific growth rate is for the acid sludge saturated (0.086 $days^{-1}$); the sample with the lowest specific growth rate is the 10 feet below the surface with 0.032 $days^{-1}$. The average is 0.055 $days^{-1}$.

The Monod model has been used to relate the biomass data to the substrate data for both HPLC and COD methods of measuring the substrate concentration. Figures III-2 through III-13 show that the specific growth rates based on this model are consistently one order of magnitude lower than the ones obtained from the biomass data alone.

The HPLC data indicate that the material from twenty feet below the surface with the surfactant treatment shows the highest specific growth rate, 0.0081 $days^{-1}$; and the acid sludge saturated shows the lowest rate, 0.0009 $days^{-1}$. The treatment aided by the surfactant, experiment AT, exhibits higher specific growth rate than either the

unsaturated (AU) or the saturated (AS) treatment. The acid sludge material shows lower rates than either the twenty or ten feet below the surface materials. This is expected since the acid sludge contains more toxic compounds than the others.

From the COD data, the highest specific growth rate is for the material ten feet below the surface (unsaturated), 0.018 $days^{-1}$; and the lowest is for the material twenty feet below the surface saturated, 0.00087 $days^{-1}$. This set of data demonstrates that the addition of surfactant improves the specific growth rate for the twenty feet under the surface material, but not for the acid sludge. In this case the unsaturated treatment gives a higher rate.

In Table III-XV the biomass yield, first order rate constant, and rate of transformation are presented for the experiments. These are the results of applying the first order kinetic model to the HPLC data. In Figures III-2 through III-7 the fit of this model is presented. For all the different materials this model presents a good fit for the exponential growth phase. The highest rate constant is for the twenty feet below the surface material (saturated). The average for this analysis is 0.0089 $days^{-1}$. This average compares favorably with the rate constants observed by other investigators at similar initial concentrations (see

Table II-IV). When calculating the rate of transformation (rate constant times the initial concentration), the highest one is the material 20 feet below the surface (saturated), followed closely by the same material with the surfactant added. The other four samples are one order of magnitude smaller. Between the acid sludge samples, the acid sludge plus surfactant has the highest rate of transformation, followed by the saturated case, and the slowest is the unsaturated case. Using this model the addition of surfactant does improve the rate of degradation.

Figures III-8 throught III-13 show the agreement of the first order kinetic model for the different materials using the data from the COD analysis. Table III-XVI presents the same information as Table III-XV but using the data collected from the COD analysis. The rate constant of the acid sludge material is one order of magnitude less than the others. For the acid sludge, the average is 0.0064 $days^{-1}$; and for the 20 feet below the surface and the 10 feet below the surface, the average is 0.011 $days^{-1}$. The highest rate constant for the acid sludge has been observed for the saturated plus surfactant case, while the lowest has been observed for the unsaturated treatment. When the rate of transformation is examined, the acid sludge plus the

surfactant shows the highest, and the unsaturated treatment shows the lowest rate.

The data in Figures III-2 through III-13 show no indication of substrate inhibition at the beginning of the experiments. Figures III-2 through III-7 indicate that the biomass concentration fluctuates for the first half of the experiments and then starts to decline sharply in the second half, possibly due to inhibitory effects of the polynuclear aromatic hydrocarbons metabolites. For the material twenty feet below the surface the addition of surfactant extended the exponential growth phase. These figures also show the substrate data decrease over the course of the experiments.

An elemental analysis was performed for all the materials found at the site under investigation. The results exhibit that the acid sludge contains 50% carbon, the material twenty feet below the surface contains 2% carbon, and the material ten feet below the surface contains 1% carbon. After the experiments concluded this analysis was repeated. It showed that the acid sludge contains 36% carbon, the material twenty feet below the surface contains 0.75% carbon, and the material ten feet below the surface contains 0.47% carbon.

The first order kinetic model shows agreement between the two different methods used for measuring the substrate concentration for the acid sludge material. The rate

constant for the COD data is consistently higher than the ones for the HPLC. This could be explained by the nature of the COD analysis which does not distinguish between the biomass and the chemical substrates.

CONCLUSIONS

The experiments conducted with the material from the dumpsite indicate that these materials can be biodegraded by a mixed soil culture. For some samples the biodegradation is as high as 85%. The average rate of degradation for the acid sludge material is 0.0044 $days^{-1}$. For the material ten and twenty feet below the surface, the average rate is higher, 0.011 $days^{-1}$. The difference is possibly due to the higher content of polynuclear aromatic hydrocarbons in the acid sludge material. The average rate of transformation is 2.4 mg/g soil-day, making biodegradation a feasible solution to the present problem.

The addition of surfactant enhanced the rate of degradation almost seven times for the acid sludge material, and 8% for the material twenty feet below the surface. The addition of surfactant also increased the exponential growth phase for the material twenty feet below the surface. The

unsaturated conditions did not show an improvement over the saturated conditions in the treatment. The rate for the acid sludge unsaturated was slower than for the saturated treatment, and for the other material the difference between treatments was not noticeable.

The substrate concentration measured by COD method gave consistently higher values than the HPLC method. The COD is an easier technique but the results are not as accurate, specially for the measuring of soil and biomass together. The Standard Plate Count method to determine the biomass concentration showed good agreement and consistency. Due to the solid nature of the samples the dry weight method can not be used.

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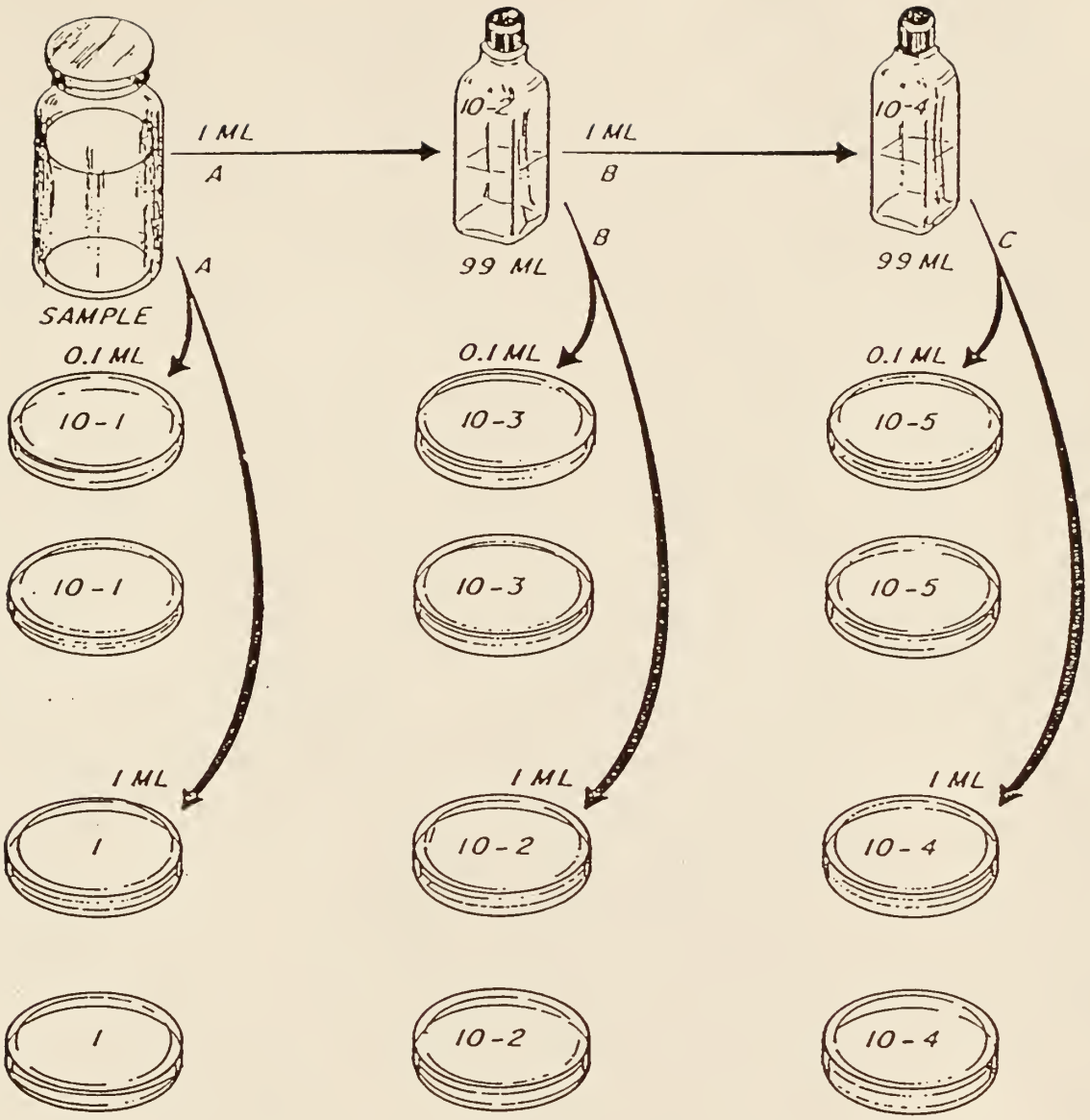

Figure III-1. Typical dilution series for standard plate count EPA,1978.

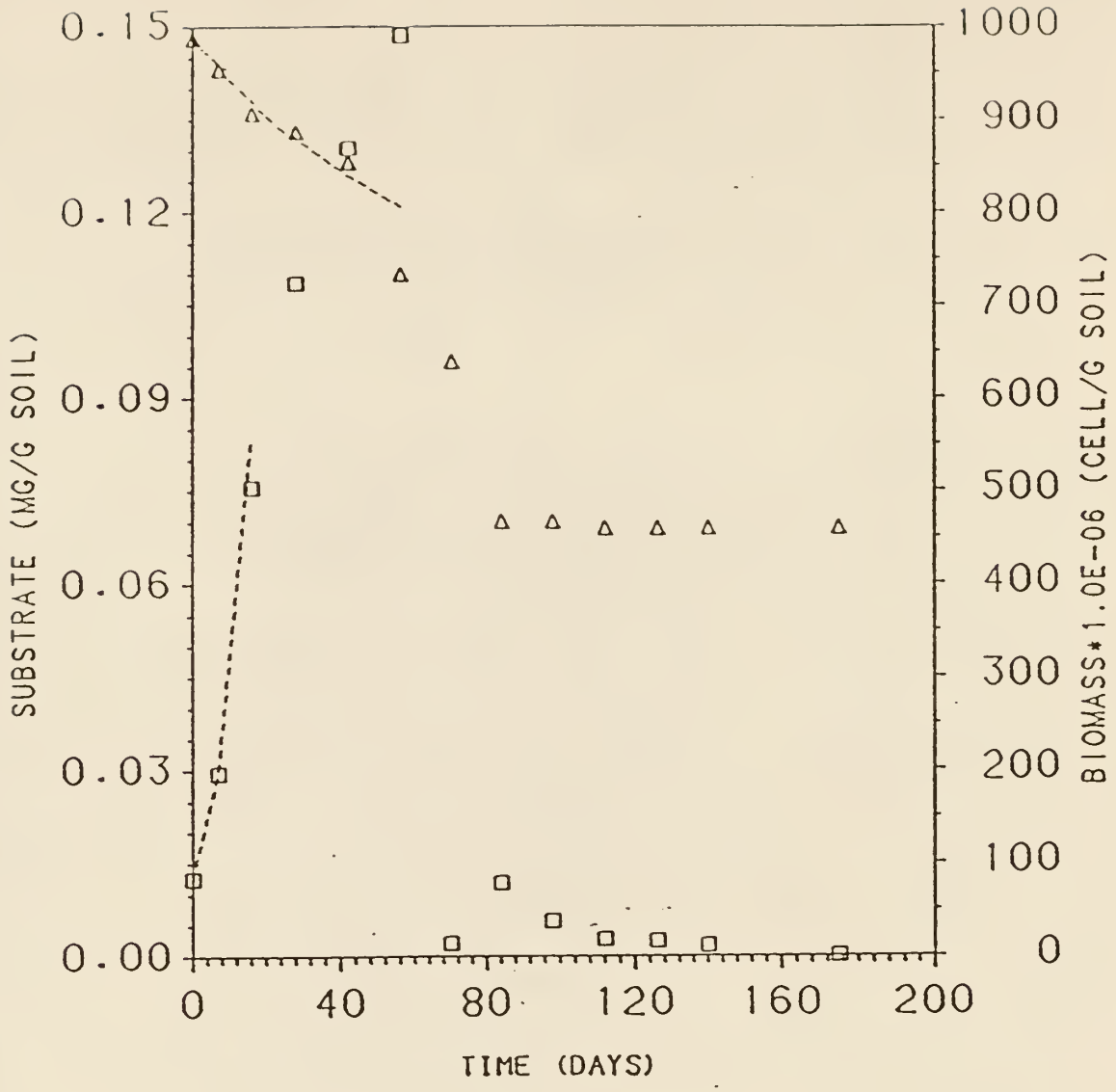

Figure III-2. Substrate as measured by HPLC, and biomass concentrations for acid sludge plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} = 0.0022 days^{-1}, and K_s= 0.149 g/g soil, Y_s= 2.4E+10 cell/g substrate; -----. Data from Table III-VI. Parameter estimates from Tables III-XIII and III-XV, for experiment AT.

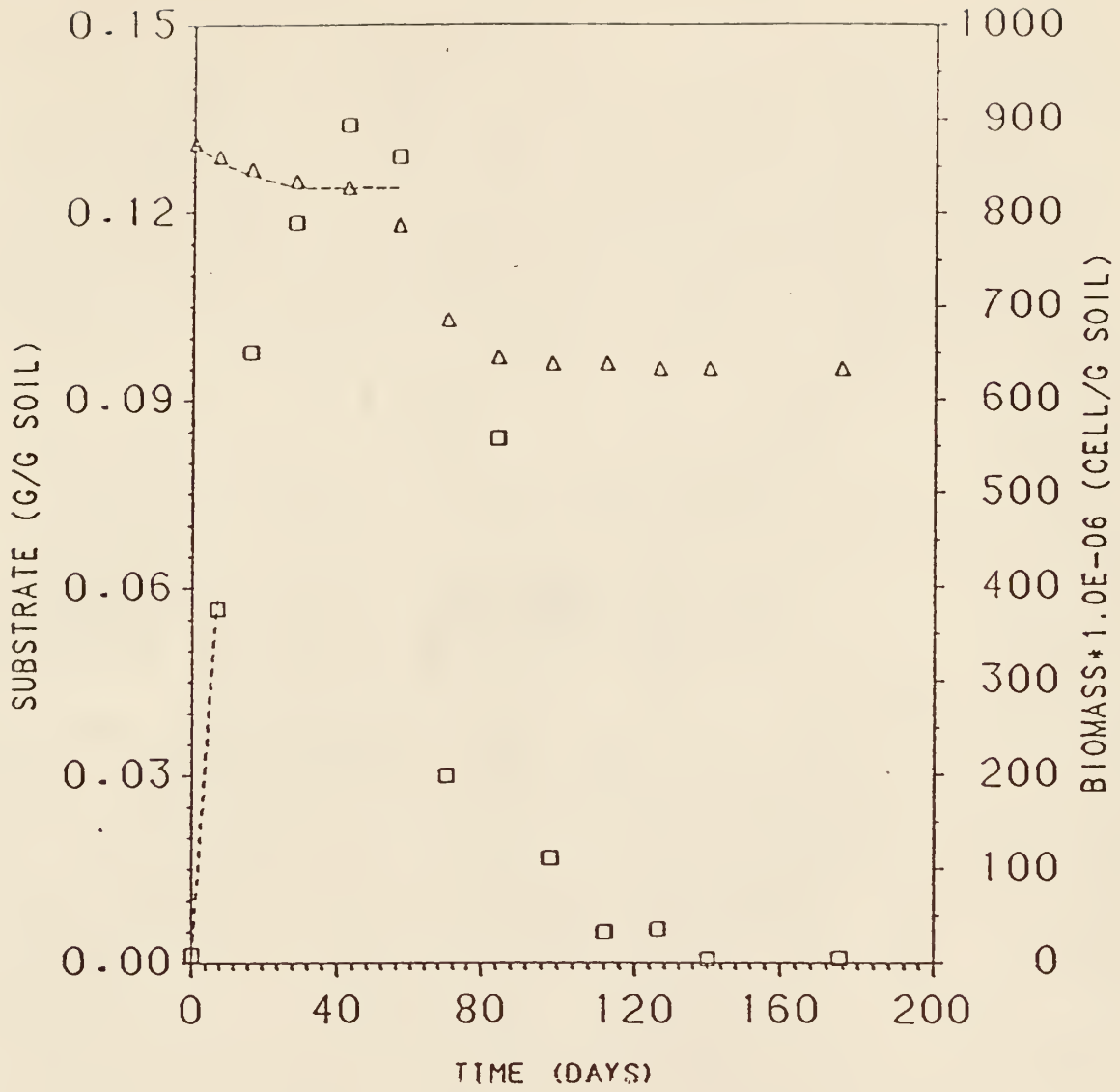

Figure III-3. Substrate as measured by HPLC, and biomass concentrations for acid sludge saturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} = 0.00087 days^{-1}, and K_s= 0.130 g/g soil, Y_s= 1.2E+11 cell/g substrate; -----. Data from Table III-VII. Parameter estimates from Tables III-XIII and III-XV, for experiment AS.

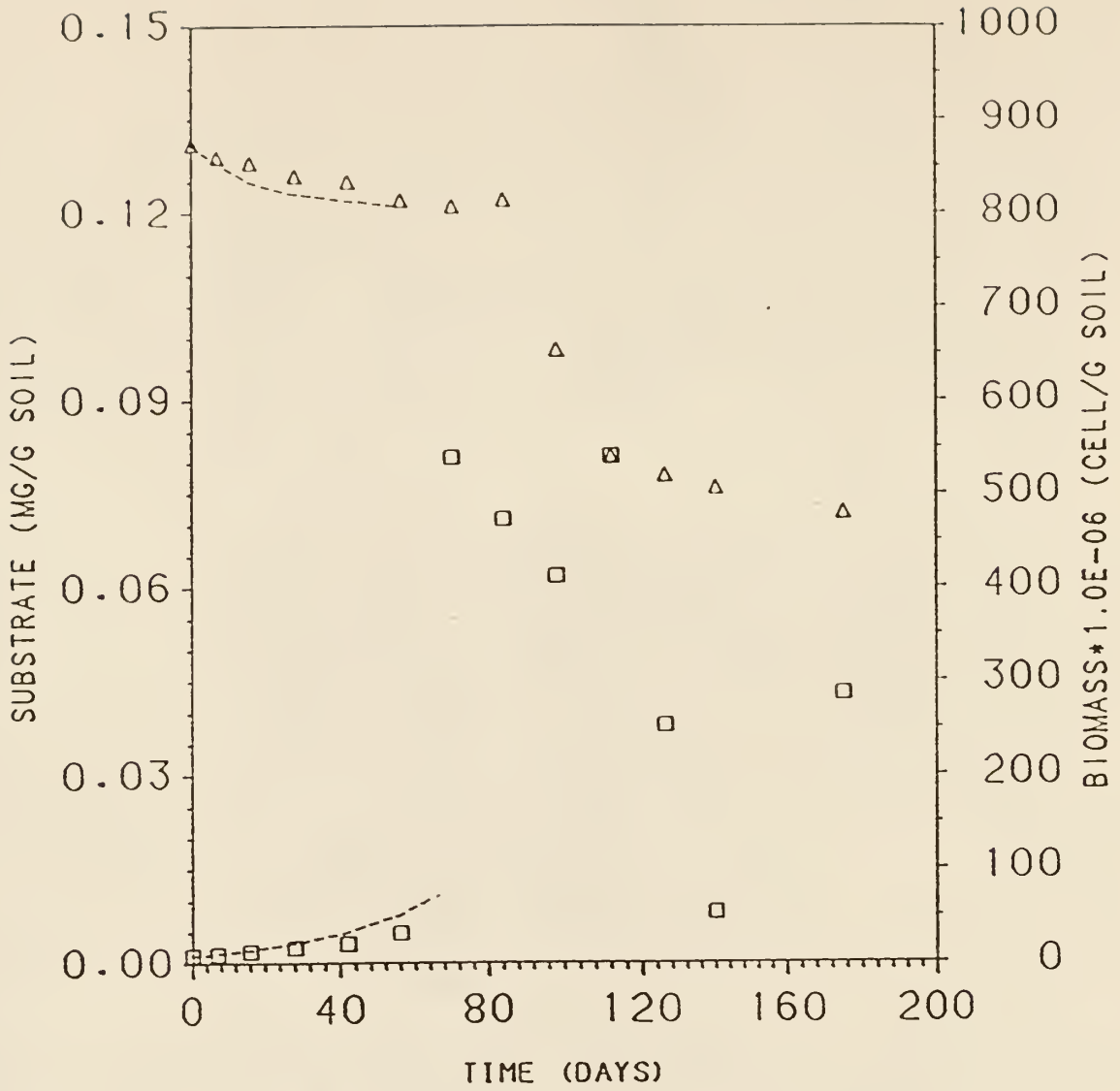

Figure III-4. Substrate as measured by HPLC, and biomass concentrations for acid sludge unsaturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} = 0.0016 days^{-1}, and K_s= 0.135 g/g soil, Y_s= 5.0E+10 cell/g substrate; -----. Data from Table III-VIII. Parameter estimates from Tables III-XIII and III-XV, for experiment AU.

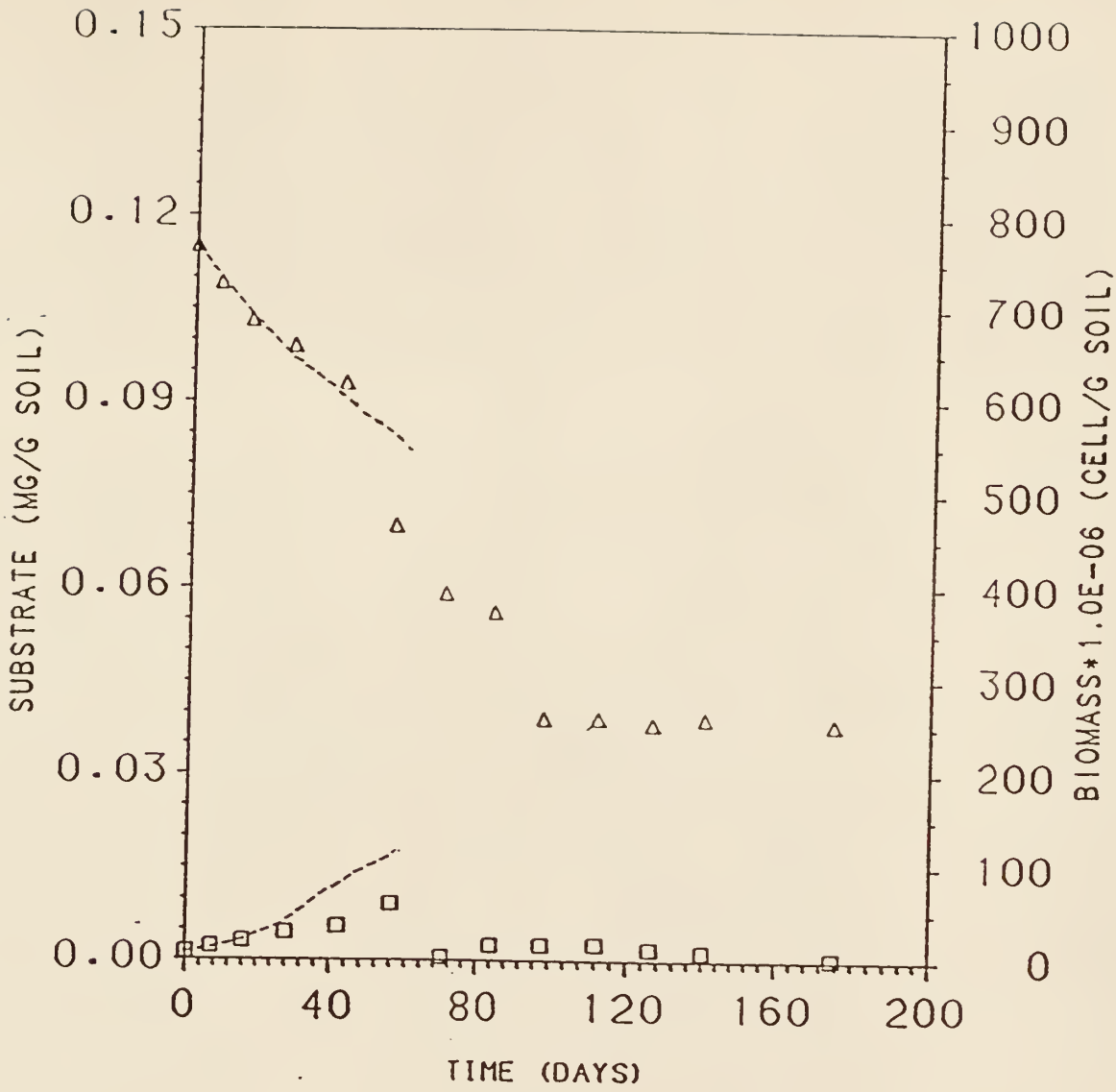

Figure III-5. Substrate as measured by HPLC, and biomass concentrations for ten feet unsaturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} = 0.0042 days^{-1}, and K_s= 0.120 g/g soil, Y_s= 1.2E+09 cell/g substrate; -----. Data from Table III-IX. Parameter estimates from Tables III-XIII and III-XV, for experiment 10U.

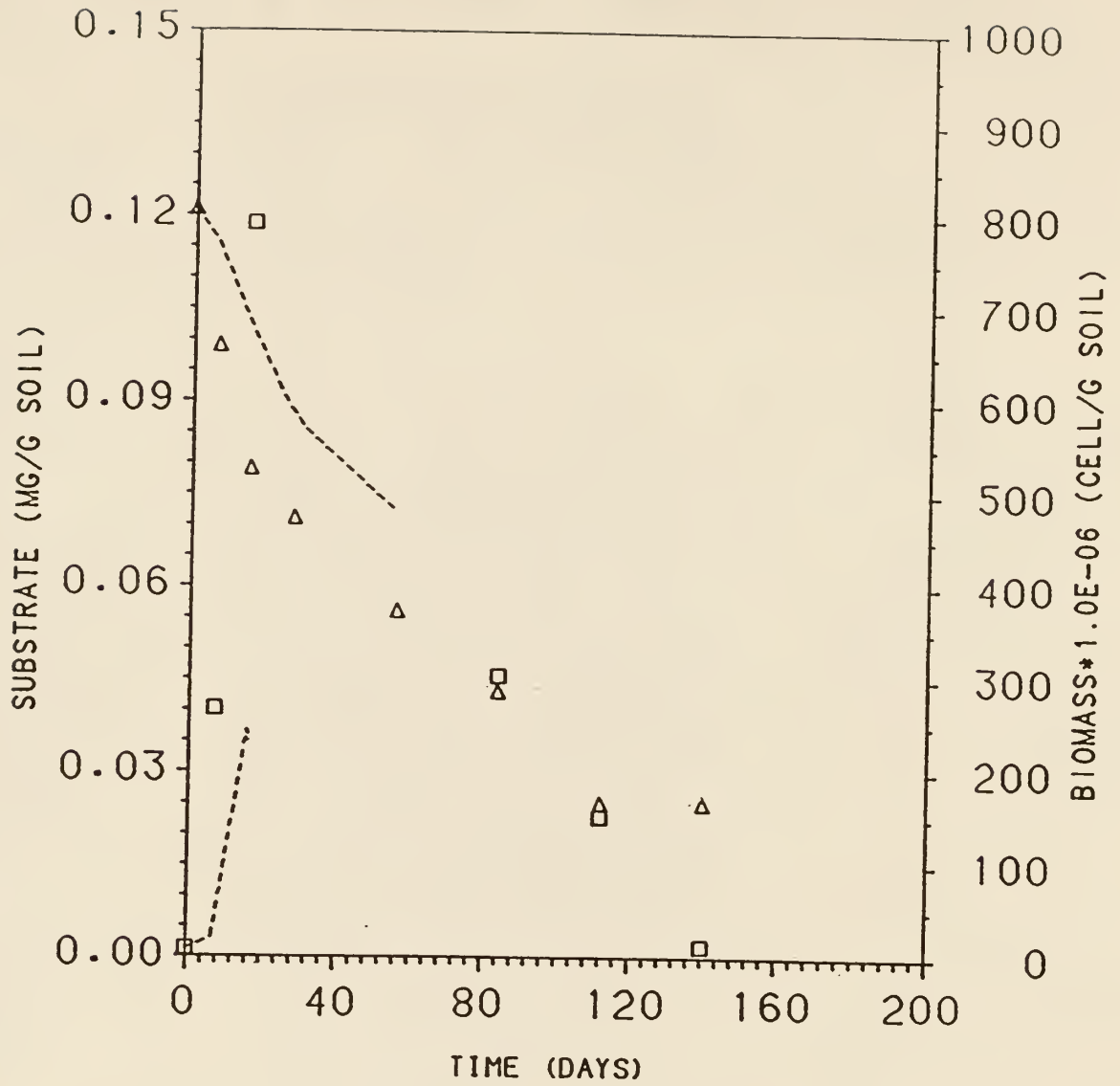

Figure III-6. Substrate as measured by HPLC, and biomass concentrations for twenty feet saturated plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} = 0.0081 days^{-1}, and K_s = 0.093 g/g soil, Y_s = 4.1E+10 cell/g substrate; -----. Data from Table III-X. Parameter estimates from Tables III-XIII and III-XV, for experiment 20T.

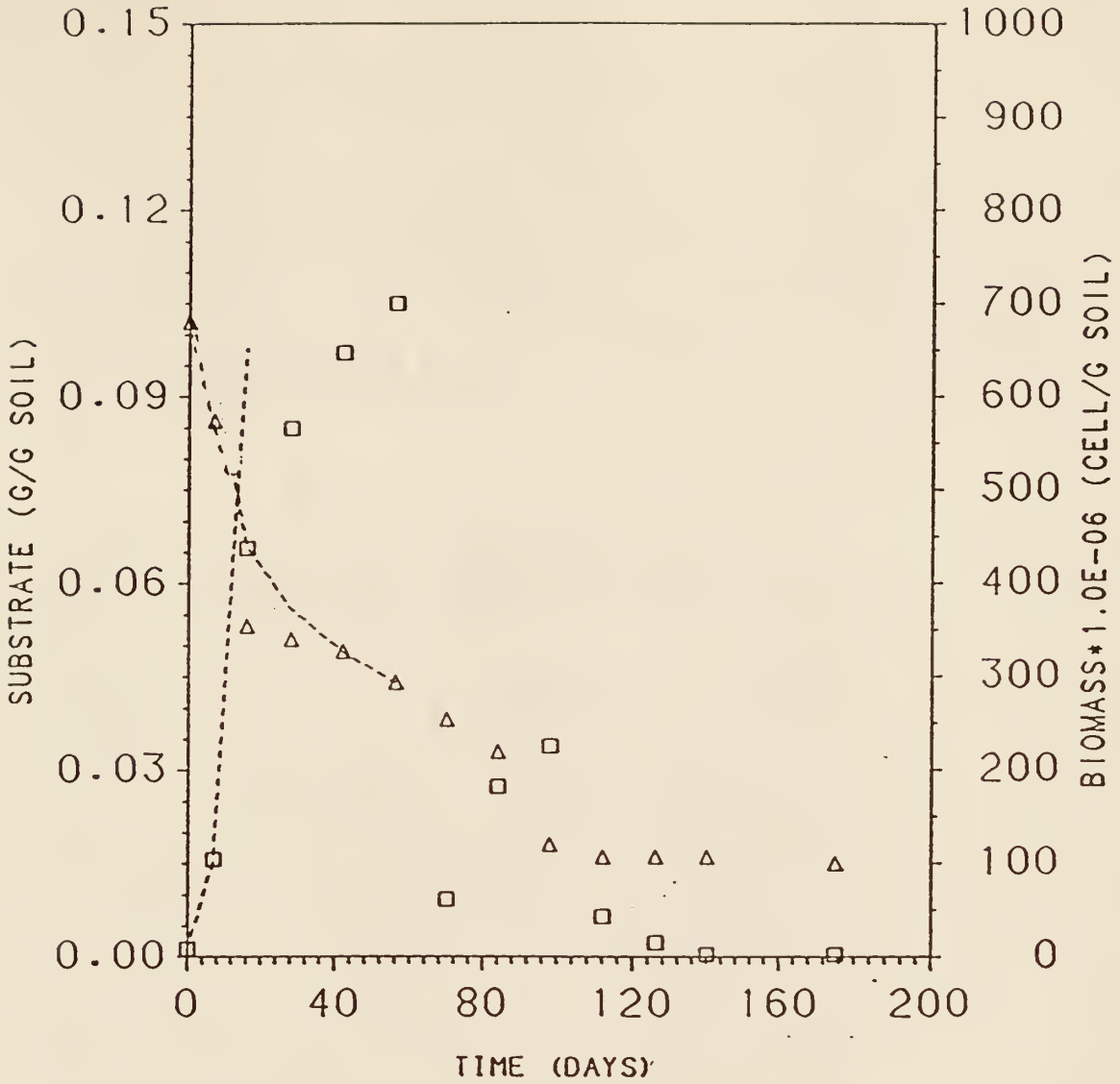

Figure III-7. Substrate as measured by HPLC, and biomass concentrations for twenty feet saturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} =0.0064 days^{-1}, and K_s=0.088 g/g soil, Y_s= 1.2E+10 cell/g substrate; -----. Data from Table III-XI. Parameter estimates from Tables III-XIII and III-XV, for experiment 20S.

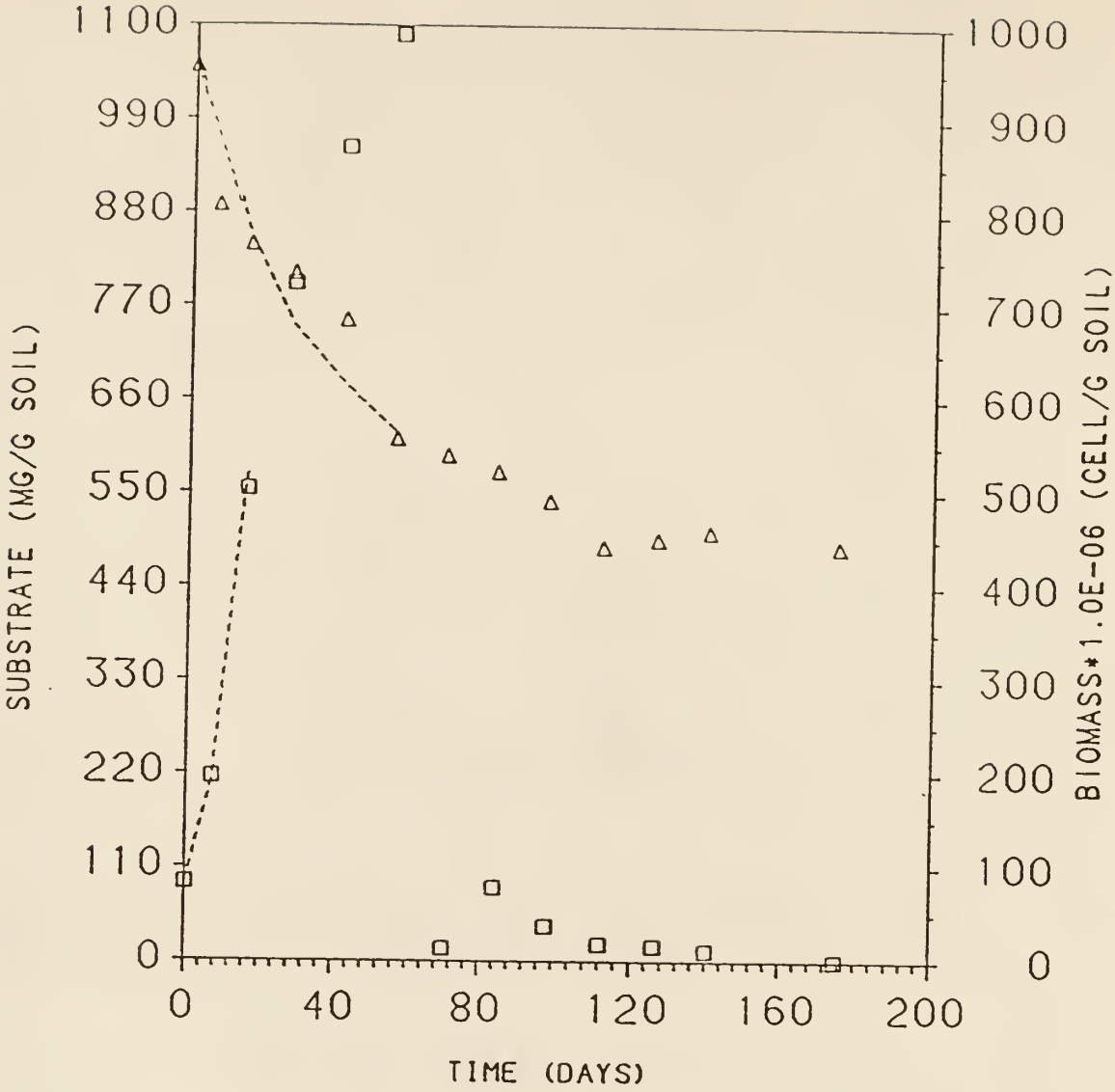

Figure III-8. Substrate as measured by COD, and biomass concentrations for acid sludge plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} = 0.0039 days^{-1}, and K_s= 0.970 g/g soil, Y_s= 2.1E+10 cell/g COD; ----. Data from Table III-VI. Parameter estimates from Tables III-XIV and III-XVI, for experiment AT.

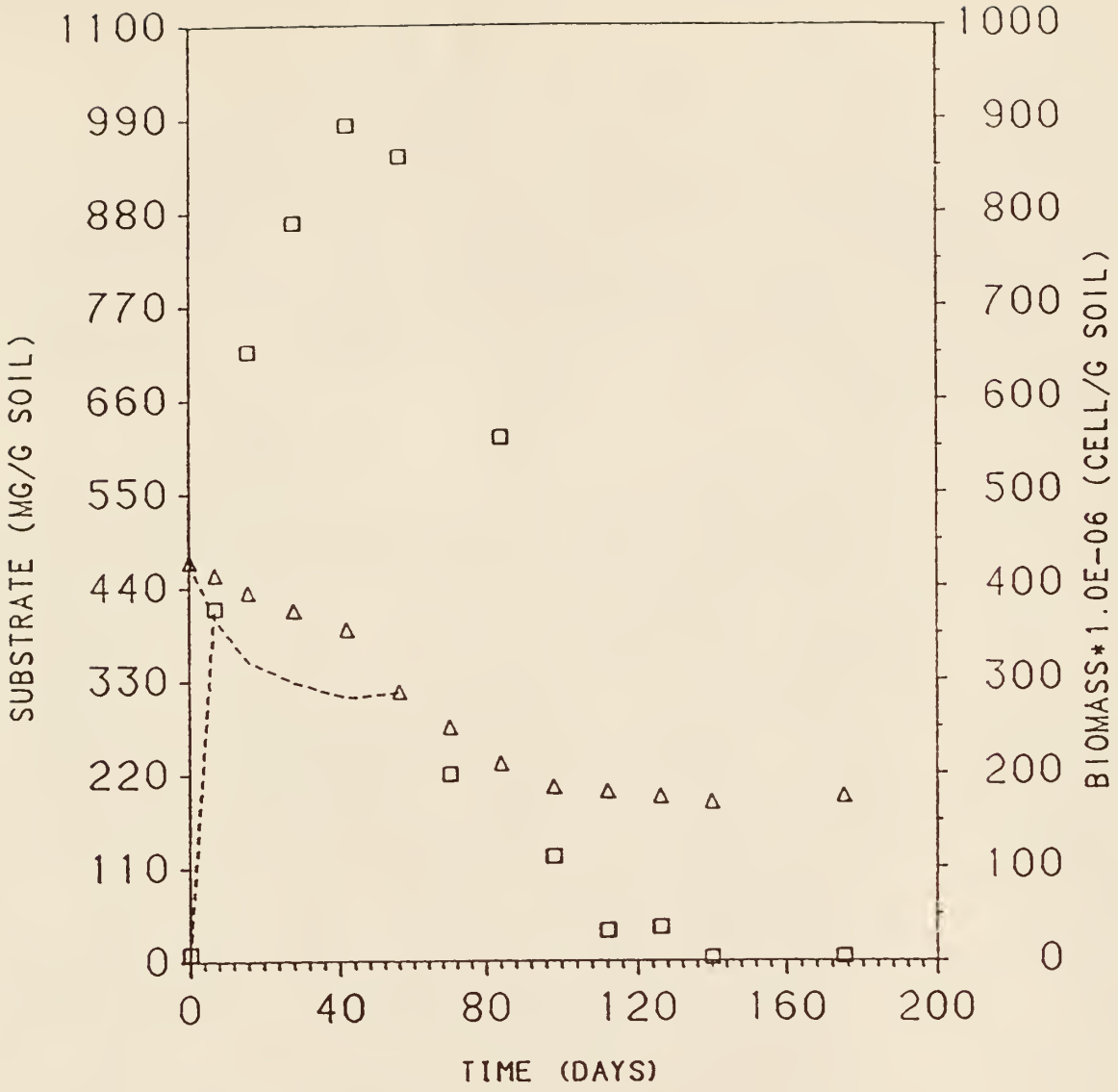

Figure III-9. Substrate as measured by COD, and biomass concentrations for acid sludge saturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} =0.0014 days^{-1}, and K_s=0.465 g/g soil, Y_s= 5.5E+10 cell/g COD; -----. Data from Table III-VII. Parameter estimates from Tables III-XIV and III-XVI, for experiment AS.

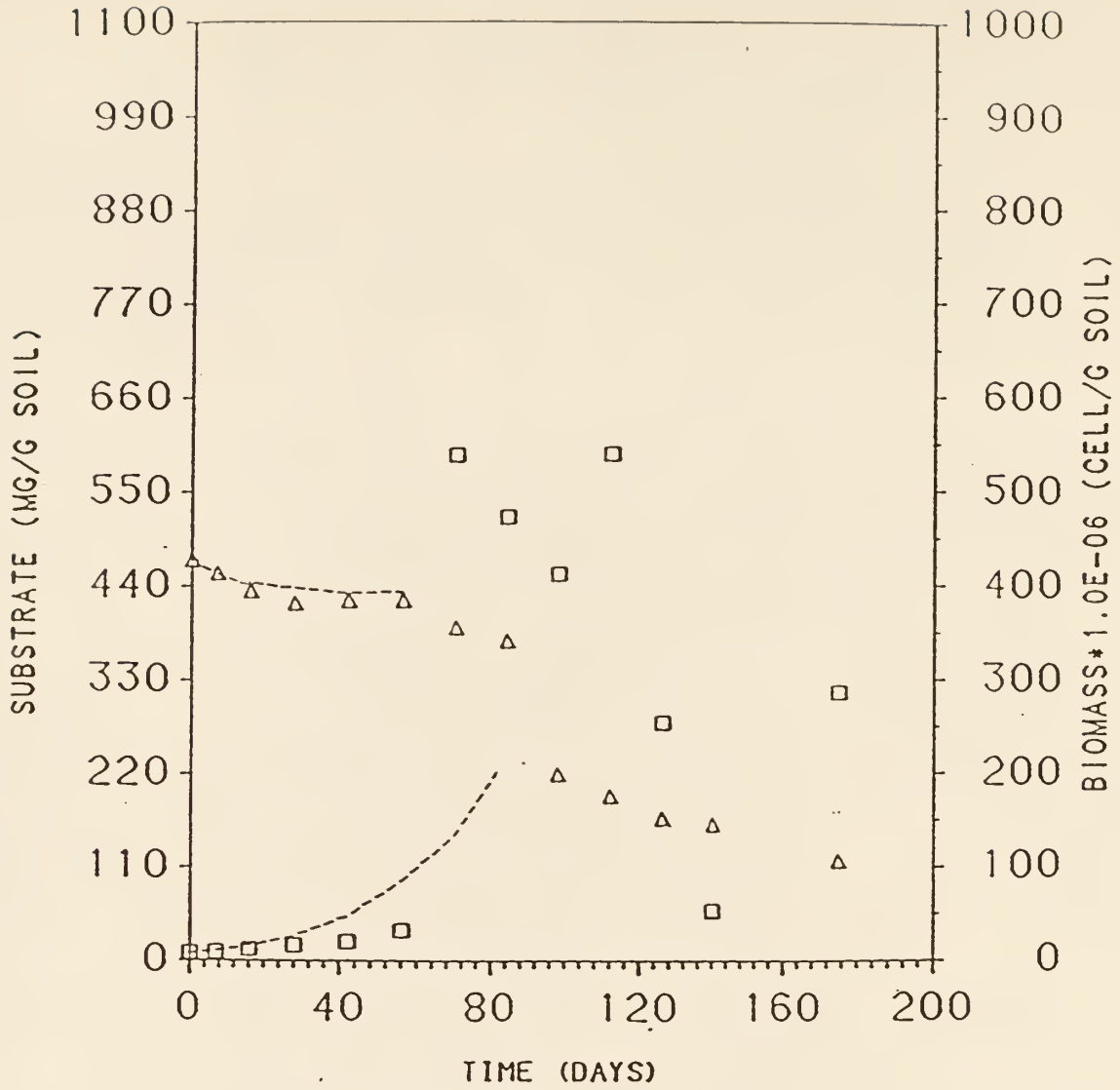

Figure III-10. Substrate as measured by COD, and biomass concentrations for acid sludge unsaturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} =0.0053 days^{-1}, and K_s=0.544 g/g soil, Y_s= 6.7E+10 cell/g COD; -----. Data from Table III-VIII. Parameter estimates from Tables III-XIV and III-XVI, for experiment AU.

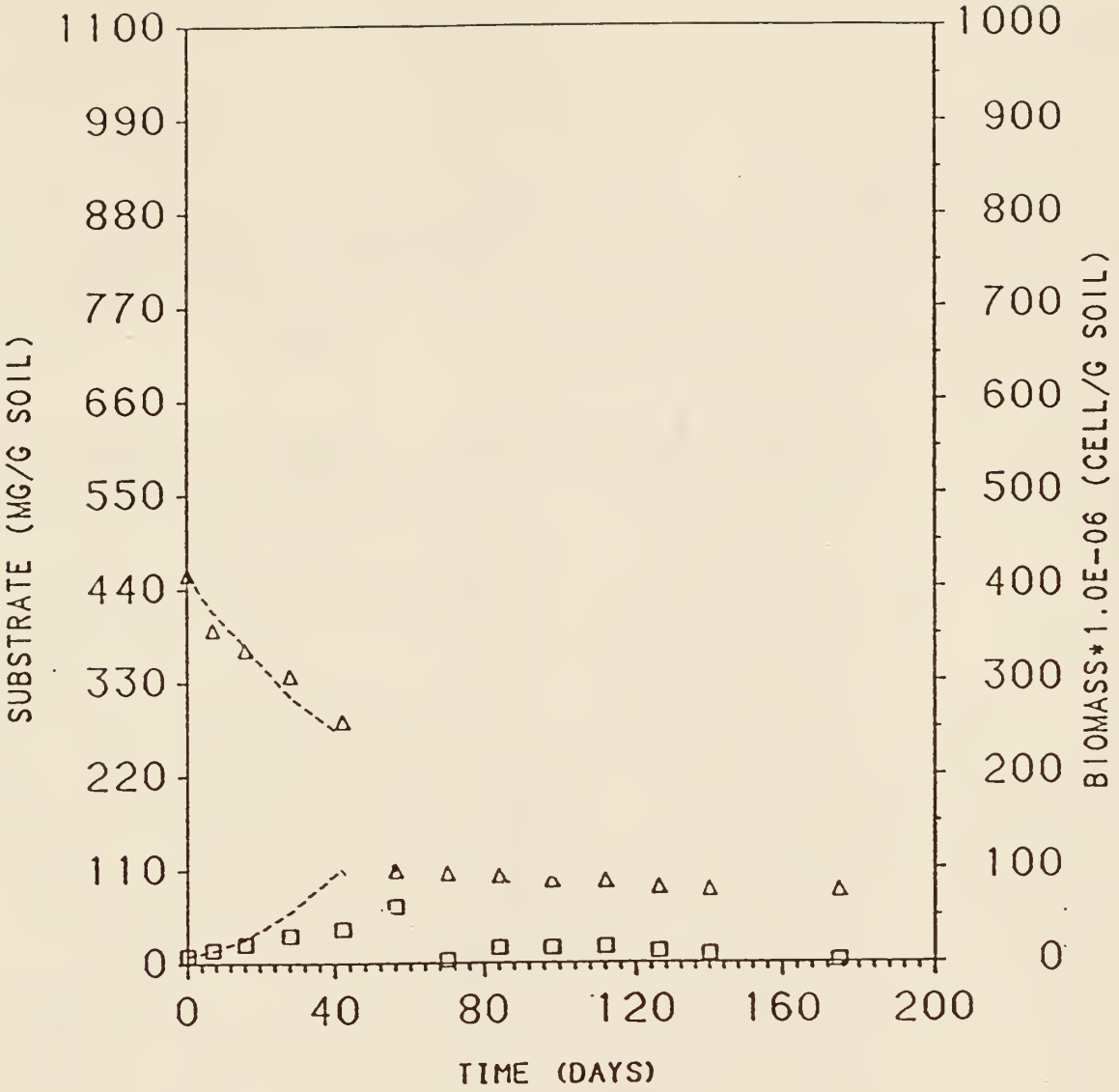

Figure III-11. Substrate as measured by COD, and biomass concentrations for ten feet unsaturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} =0.018 days^{-1}, and K_S=0.587 g/g soil, Y_s= 1.5E+09 cell/g COD; -----. Data from Table III-IX. Parameter estimates from Tables III-XIV and III-XVI, for experiment 10U.

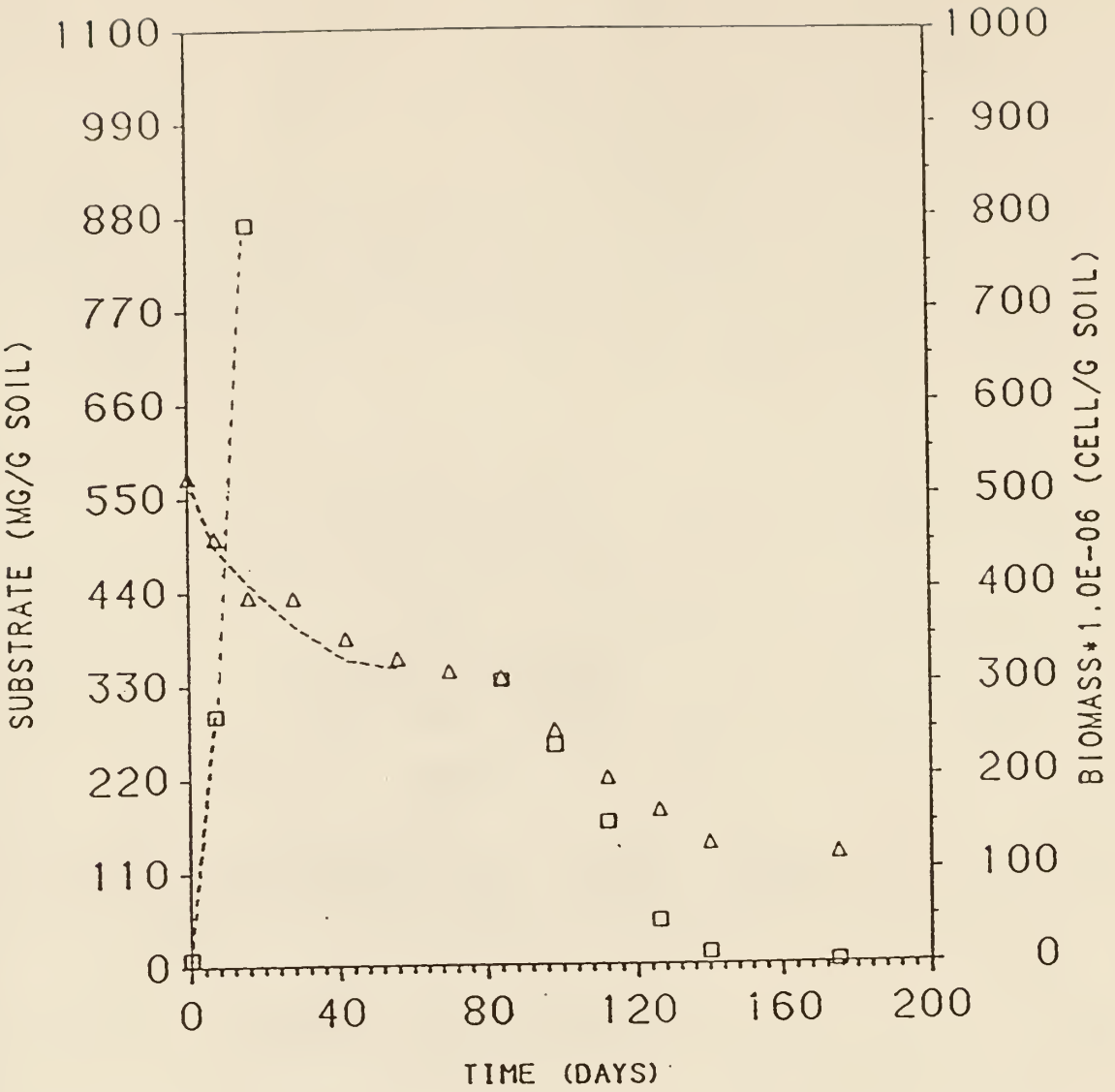

Figure III-12. Substrate as measured by COD, and biomass concentrations for twenty feet saturated plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} = 0.0066 days^{-1}, and K_s= 0.536 g/g soil, Y_s= 1.2E+11 cell/g COD; ----. Data from Table III-X. Parameter estimates from Tables III-XIV and III-XVI, for experiment 20T.

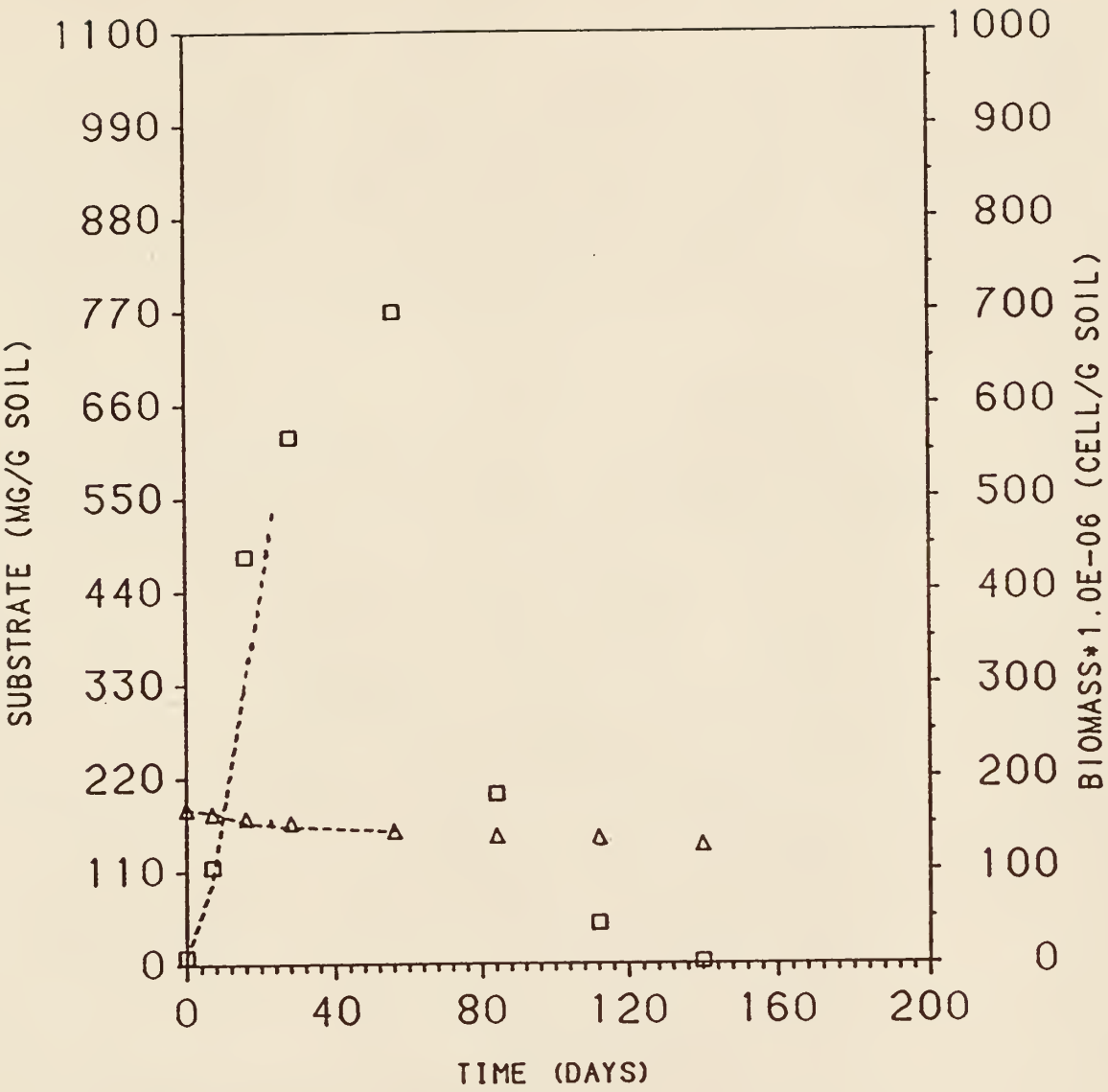

Figure III-13. Substrate as measured by COD, and biomass concentrations for twenty feet saturated; biomass concentration, □; substrate concentration, Δ; biomass and substrate concentrations predicted by the Monod model with μ_{max} =0.00087 days^{-1}, and K_s=0.18 g/g soil, Y_s= 2.6E+11 cell/g COD; -----. Data from Table III-XI. Parameter estimates from Tables III-XIV and III-XVI, for experiment 20S.

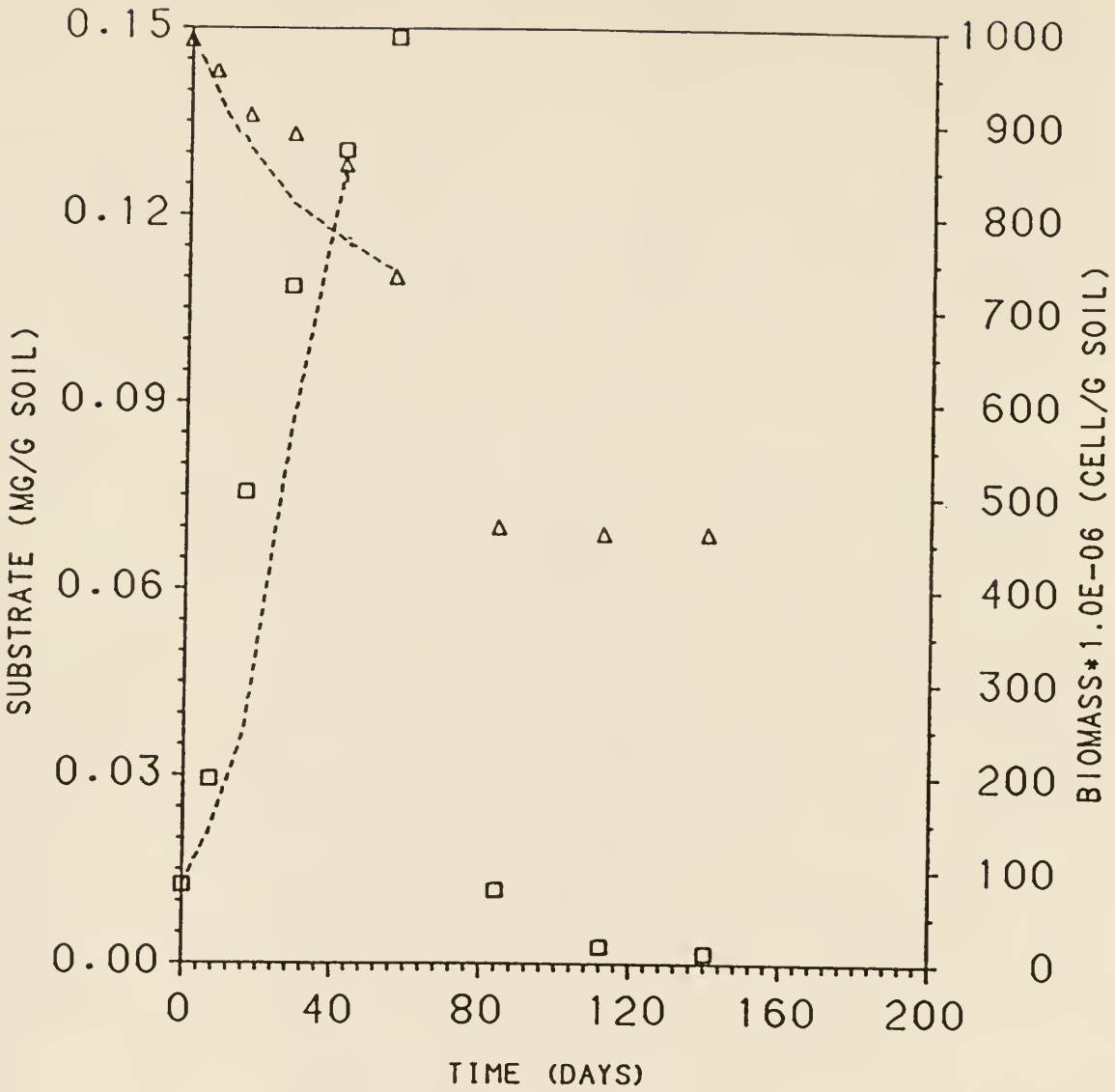

Figure III-14. Substrate as measured by HPLC, and biomass concentrations for acid sluge plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.04 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0048 days^{-1}. Data from Table III-VI. Parameter estimates from Tables III-XII and III-XV, for experiment AT.

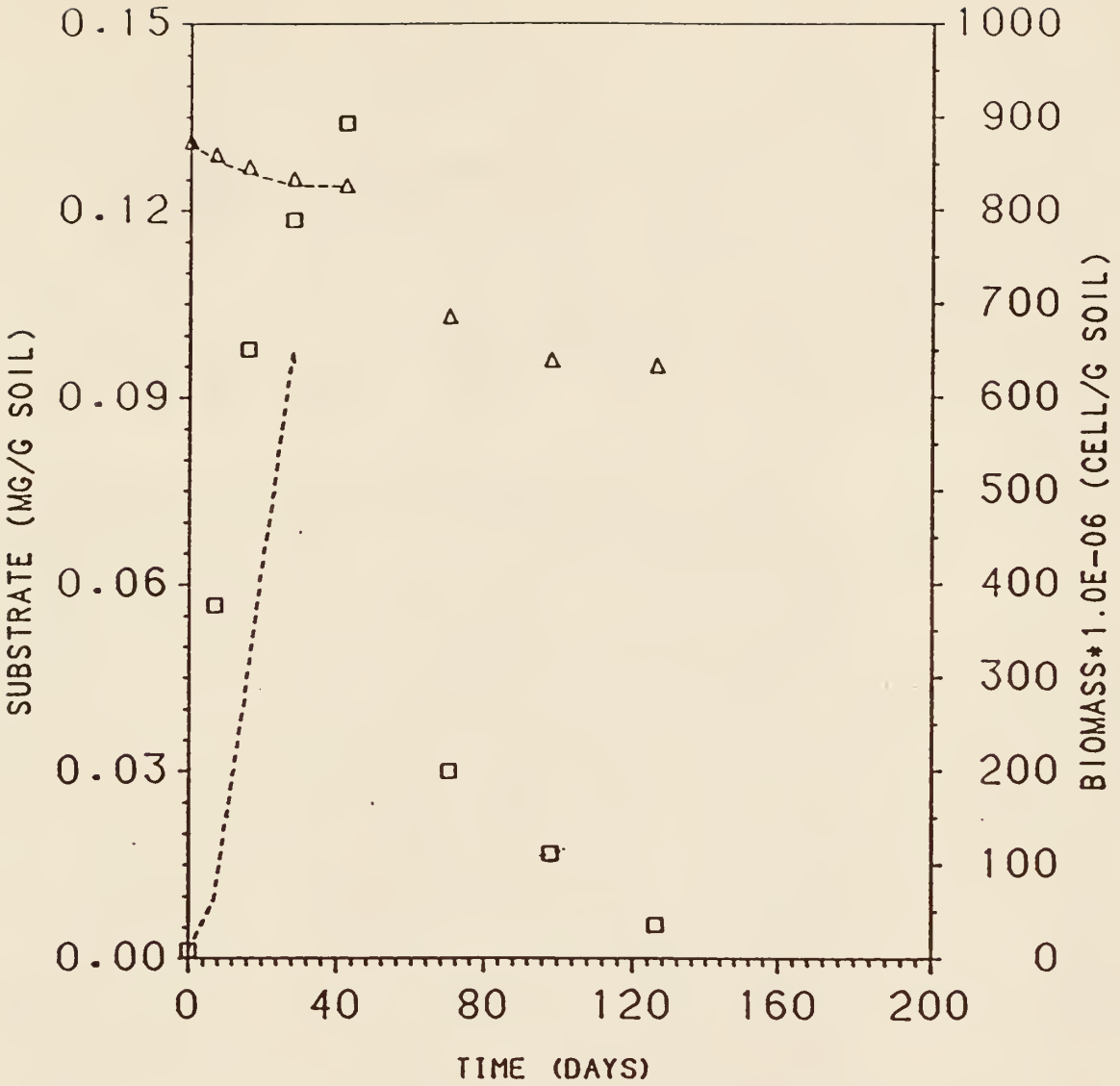

Figure III-15. Substrate as measured by HPLC, and biomass concentrations for acid sluge saturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.086 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0013 days^{-1}. Data from Table III-VII. Parameter estimates from Tables III-XII and III-XV, for experiment AS.

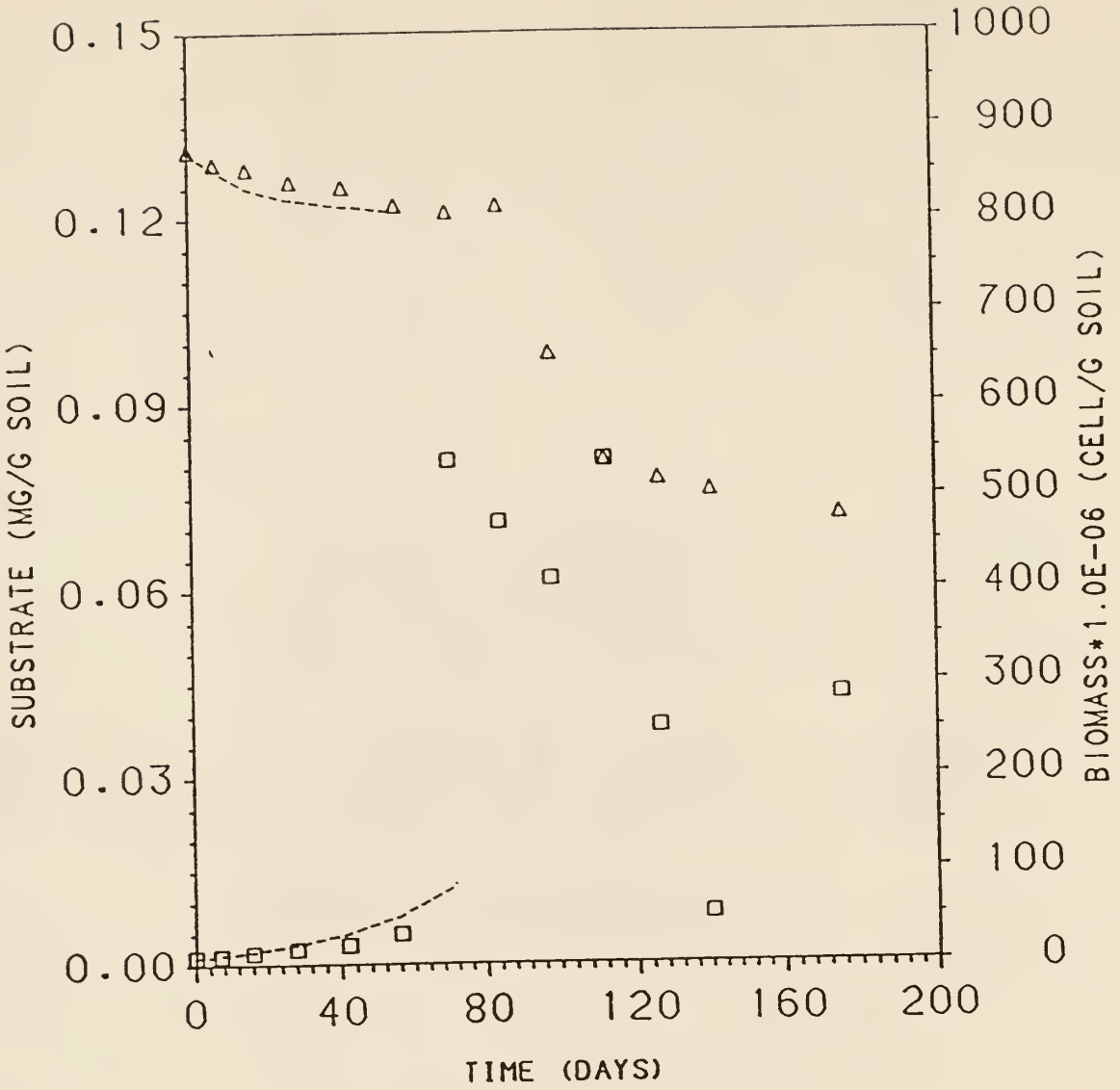

Figure III-16. Substrate as measured by HPLC, and biomass concentrations for acid sluge unsaturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.047 $days^{-1}$; substrate concentration predicted by first order kinetic model with k = 0.0012 $days^{-1}$. Data from Table III-VIII. Parameter estimates from Tables III-XII and III-XV, for experiment AU.

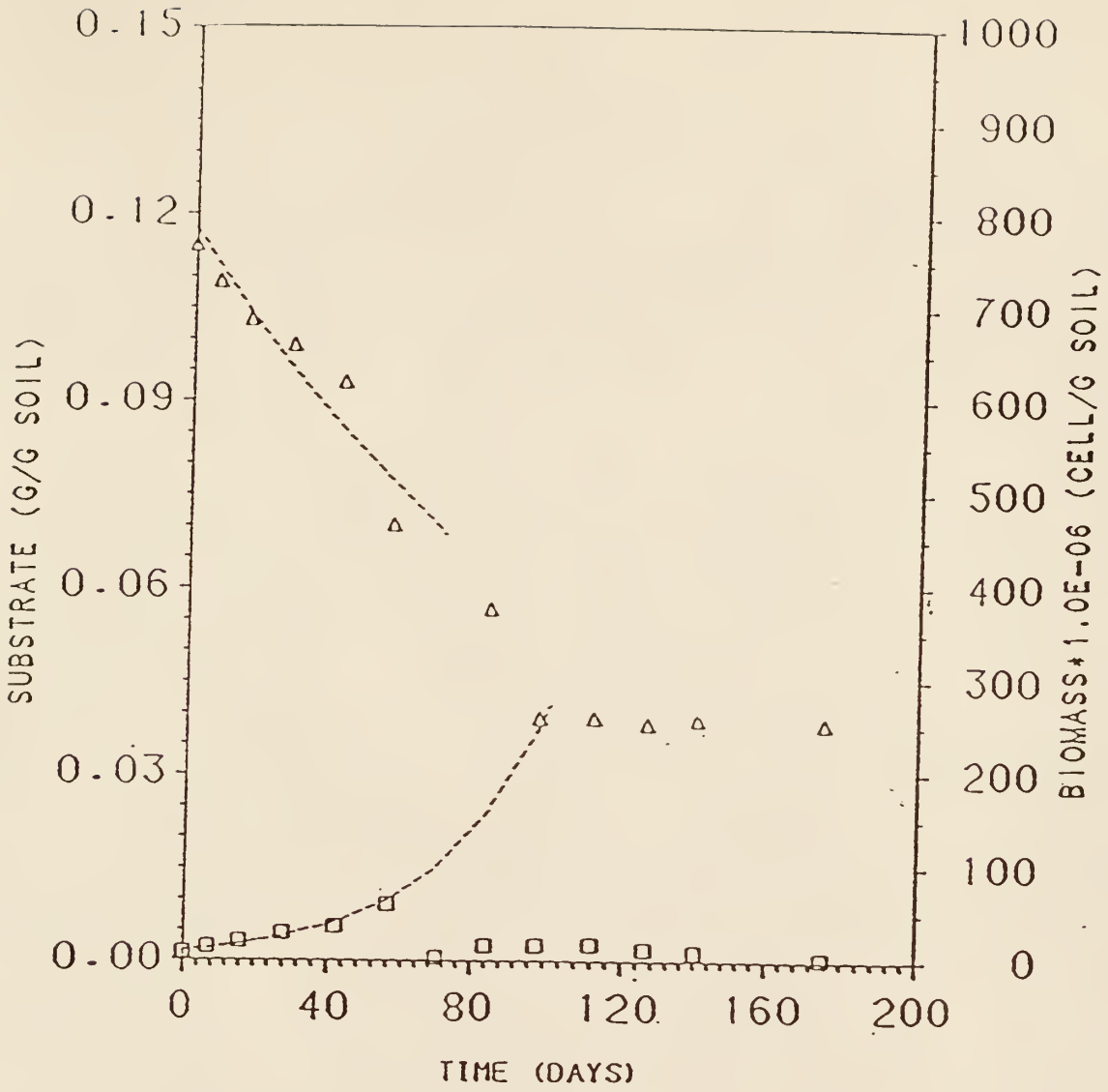

Figure III-17. Substrate as measured by HPLC, and biomass concentrations for ten feet unsaturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.032 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0076 days^{-1}. Data from Table III-IX. Parameter estimates from Tables III-XII and III-XV, for experiment 10U.

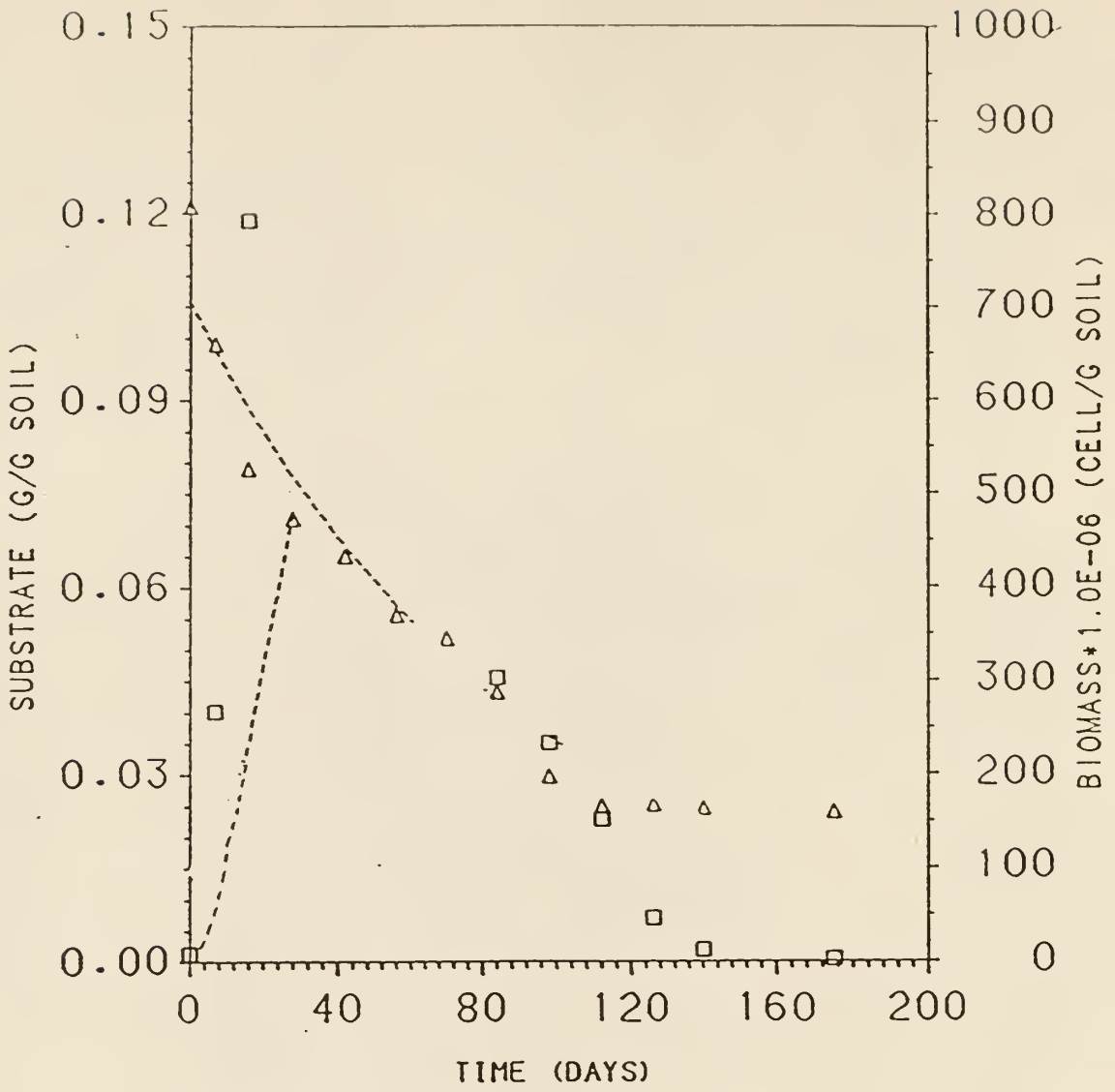

Figure III-18. Substrate as measured by HPLC, and biomass concentrations for twenty feet saturated plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.061 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.011 days^{-1}. Data from Table III-X. Parameter estimates from Tables III-XII and III-XV, for experiment 20T.

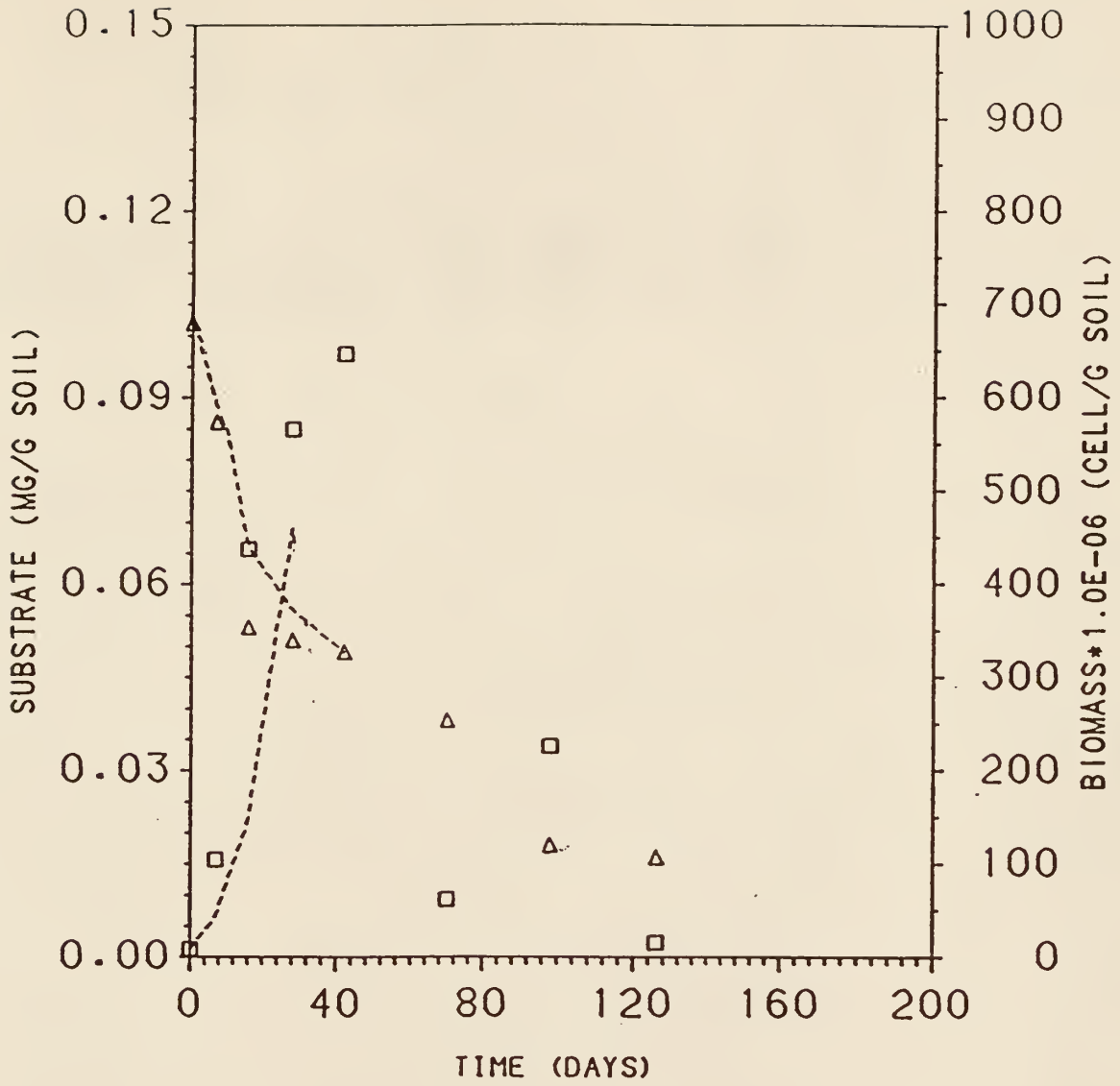

Figure III-19. Substrate as measured by HPLC, and biomass concentrations for twenty feet saturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.064 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.014 days^{-1}. Data from Table III-XI. Parameter estimates from Tables III-XII and III-XV, for experiment 20S.

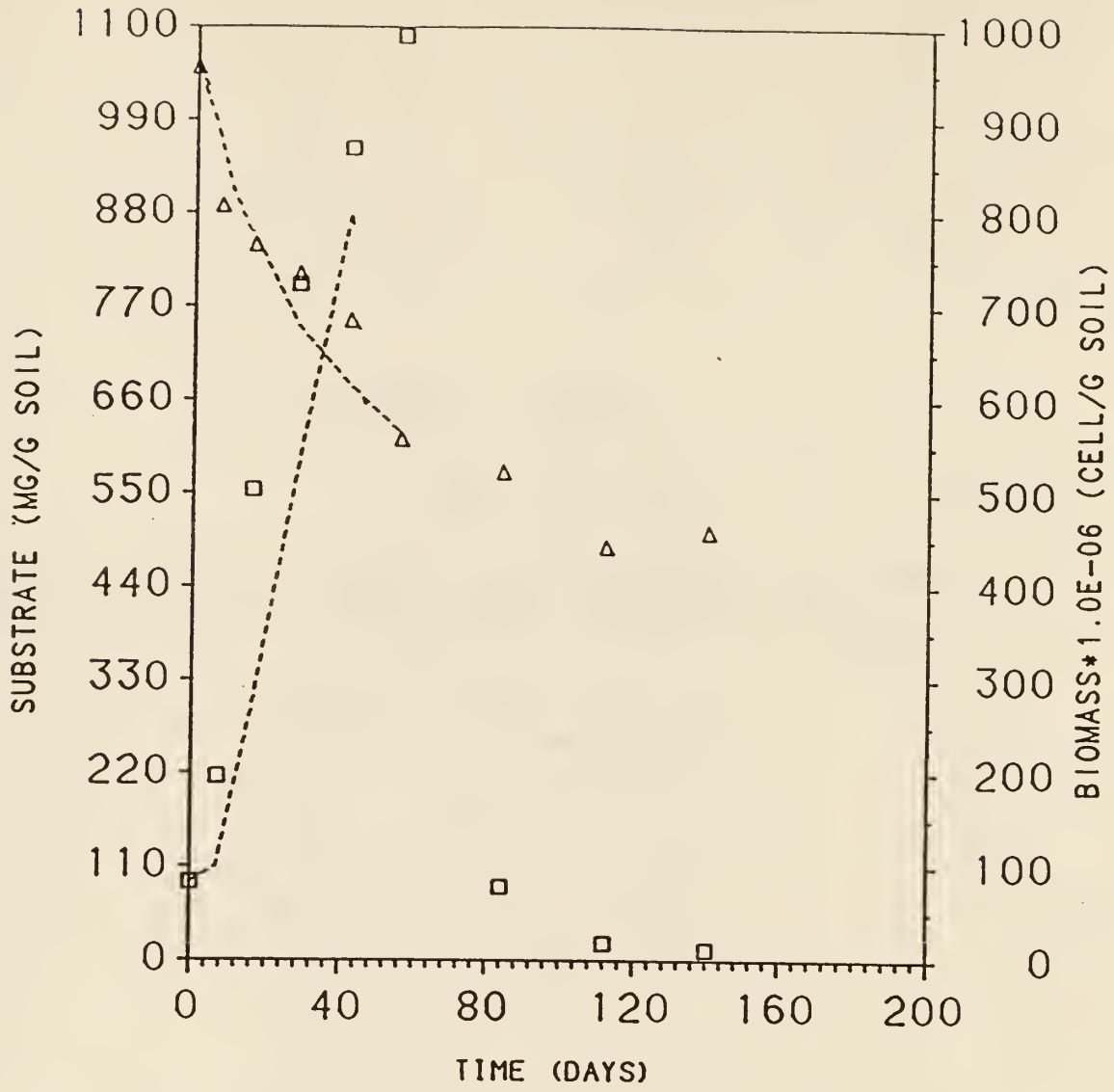

Figure III-20. Substrate as measured by COD, and biomass concentrations for acid sluge plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.04 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0080 days^{-1}. Data from Table III-VI. Parameter estimates from Tables III-XII and III-XVI, for experiment AT.

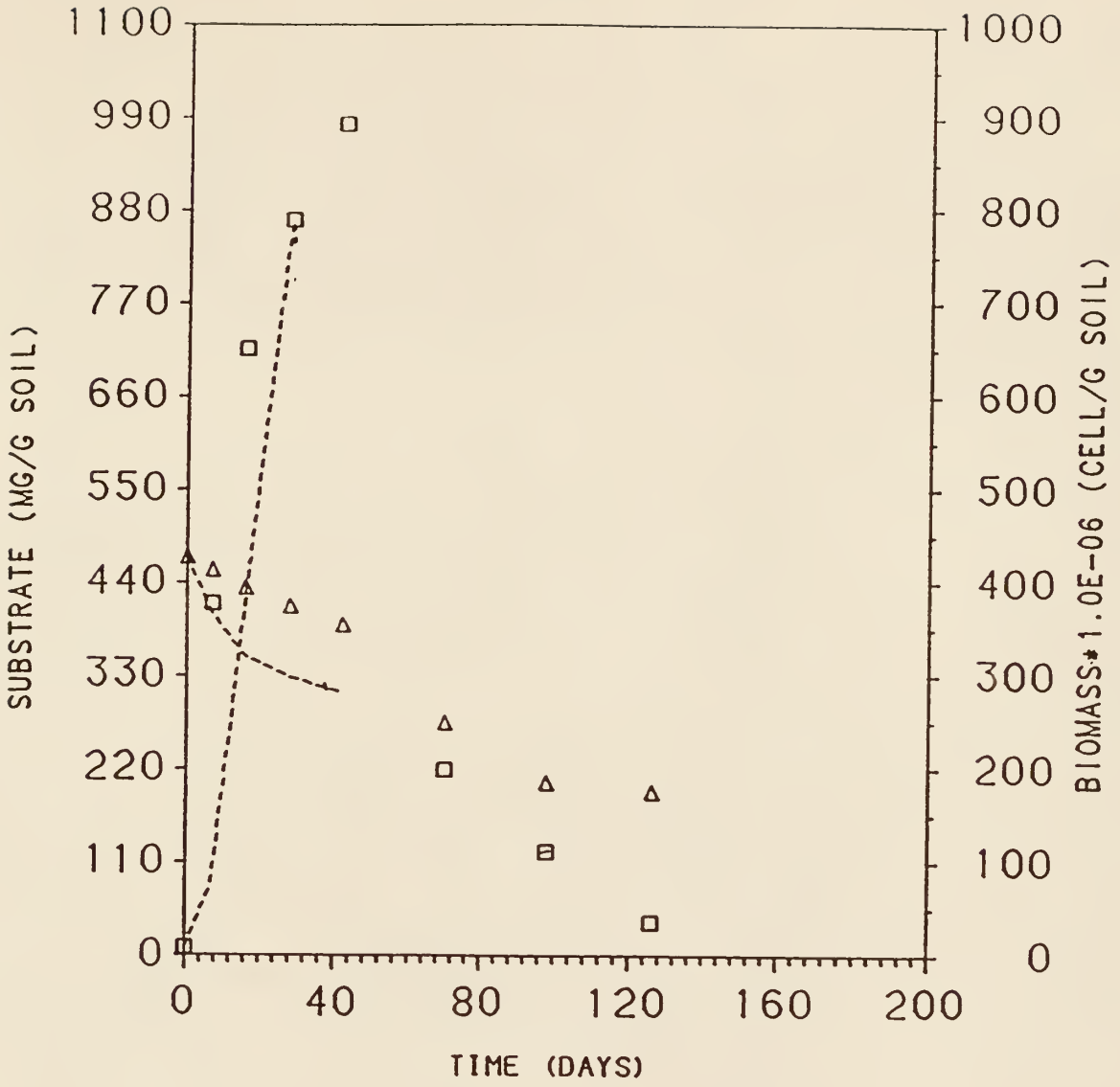

Figure III-21. Substrate as measured by COD, and biomass concentrations for acid sluge saturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.086 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0044 days^{-1}. Data from Table III-VII. Parameter estimates from Tables III-XII and III-XVI, for experiment AS.

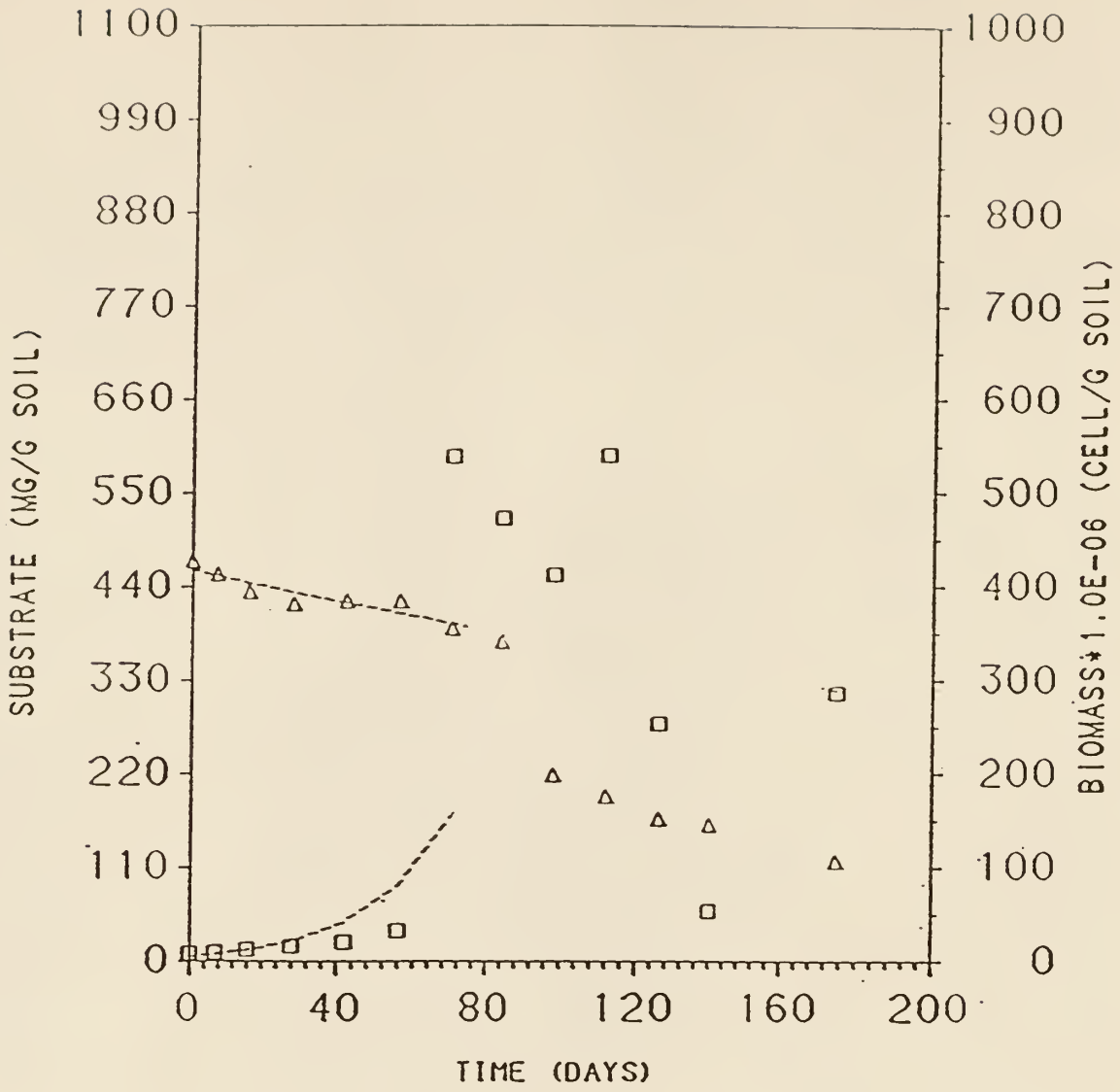

Figure III-22. Substrate as measured by COD, and biomass concentrations for acid sluge unsaturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.047 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0021 days^{-1}. Data from Table III-VIII. Parameter estimates from Tables III-XII and III-XVI, for experiment AU.

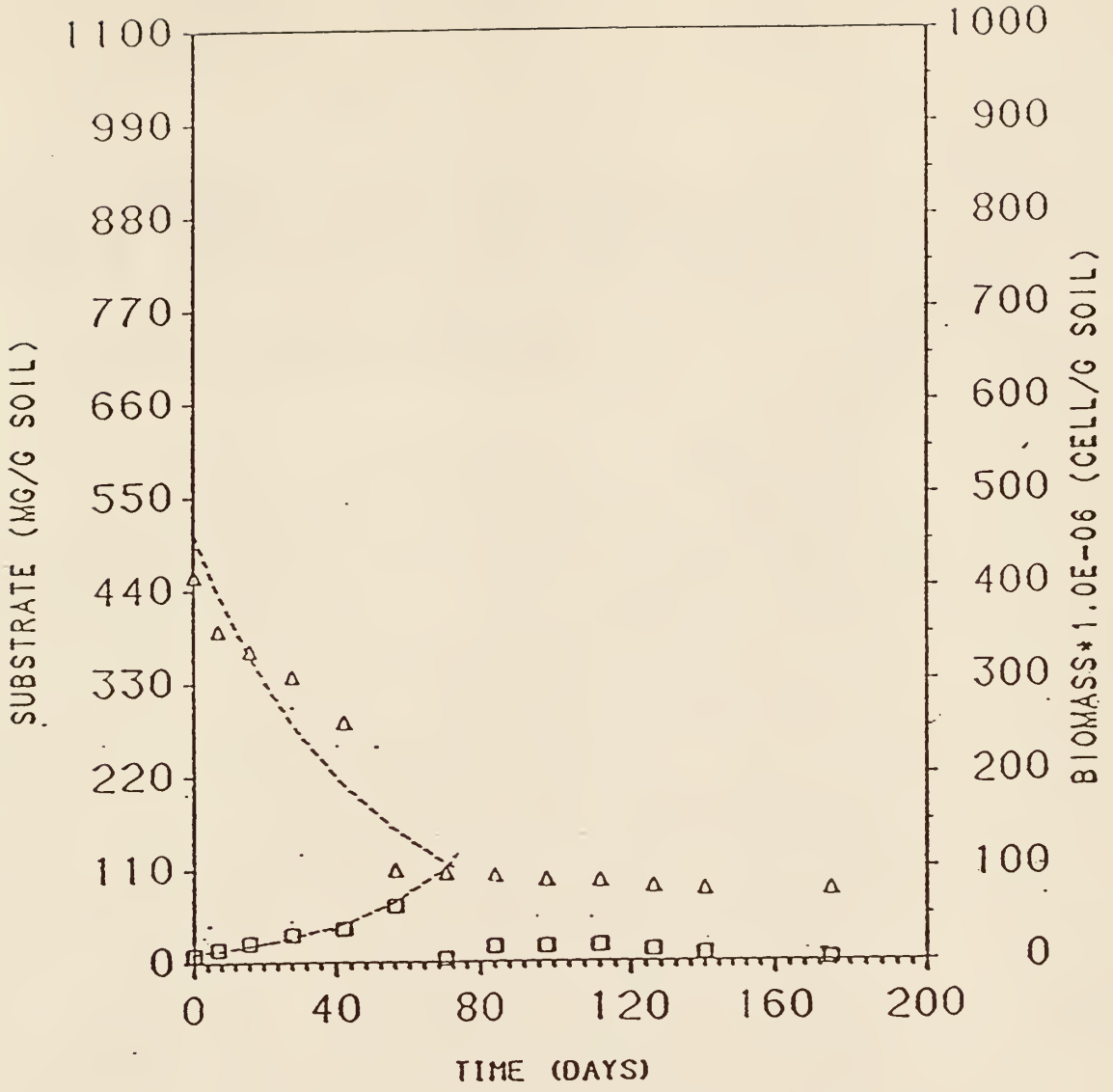

Figure III-23. Substrate as measured by COD, and biomass concentrations for ten feet unsaturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.032 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.021 days^{-1}. Data from Table III-IX. Parameter estimates from Tables III-XII and III-XVI, for experiment 10U.

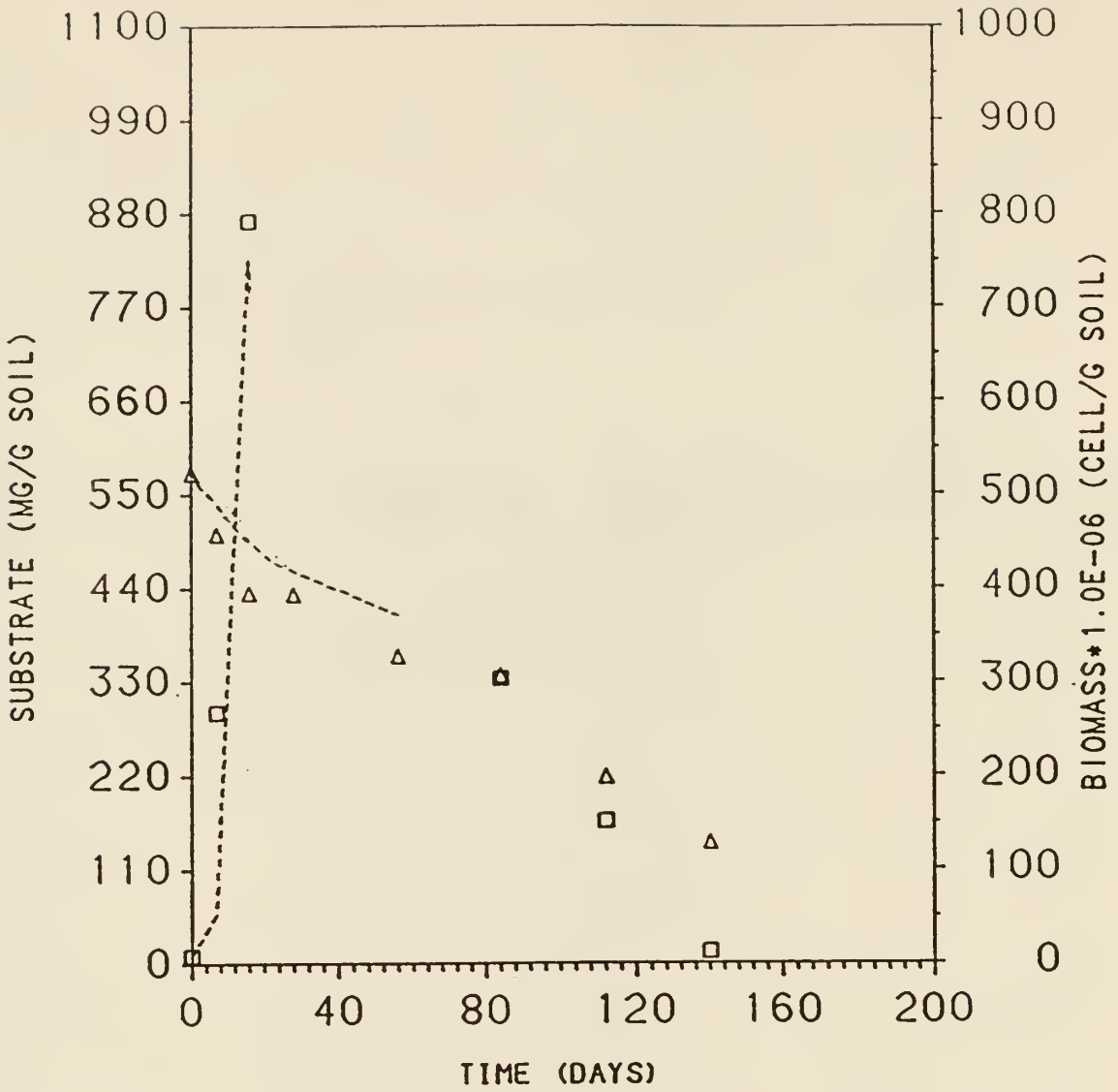

Figure III-24. Substrate as measured by COD, and biomass concentrations for twenty feet saturated plus surfactant; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.061 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0067 days^{-1}. Data from Table III-X. Parameter estimates from Tables III-XII and III-XVI, for experiment 20T.

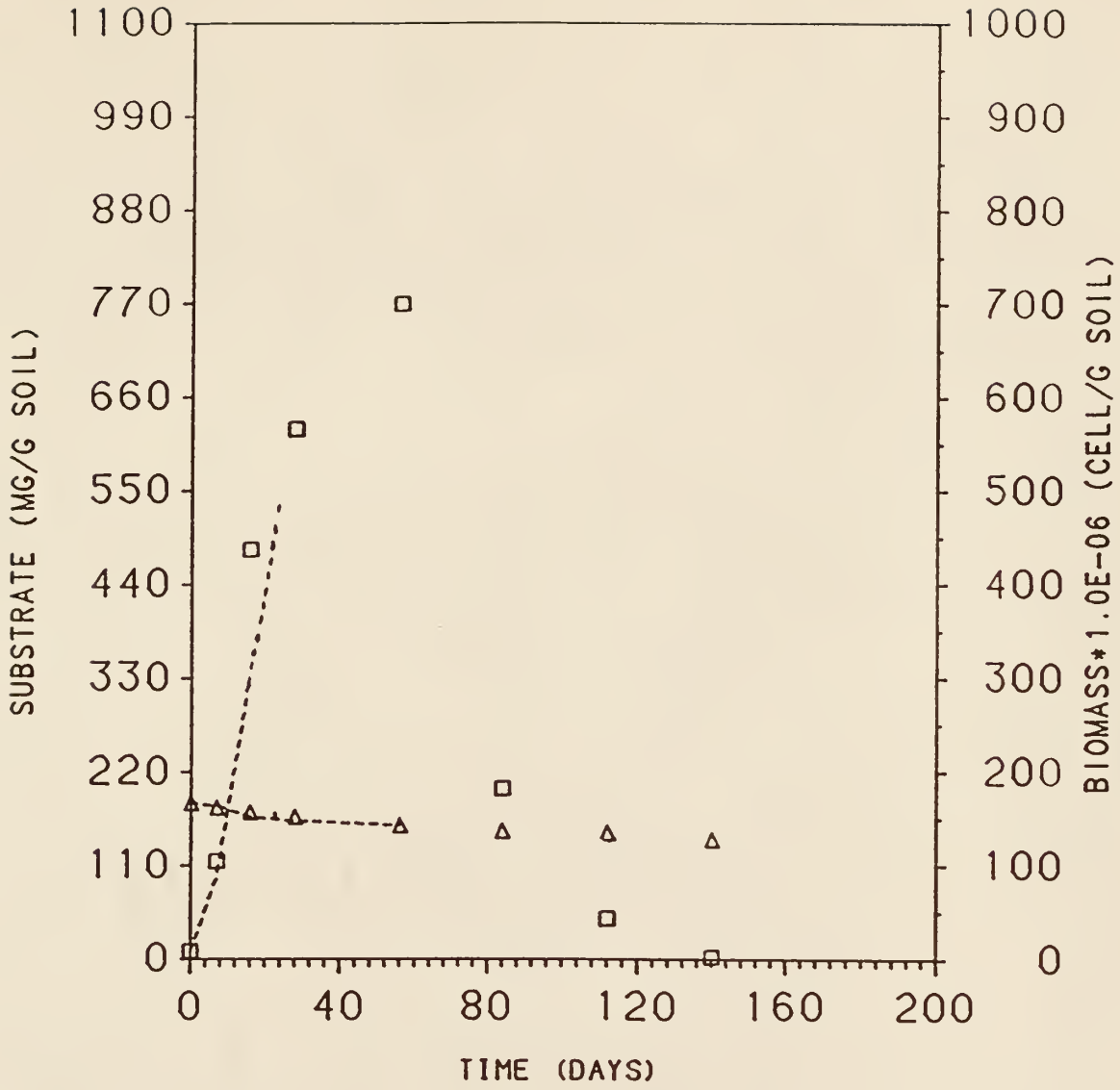

Figure III-25. Substrate as measured by COD, and biomass concentrations for twenty feet saturated; biomass concentration, □; substrate concentration, Δ; biomass predicted by an exponential model with μ = 0.064 days^{-1}; substrate concentration predicted by first order kinetic model with k = 0.0027 days^{-1}. Data from Table III-XI. Parameter estimates from Tables III-XII and IIIXVI, for experiment 20S.

Table III-I. Compounds and their initial concentration found in the soil in the acid sludge at the Arkansas City dumpsite.

Compound	Initial Concentration (μg/kg)
Ecosane	29,338
Fluoranthene	1,668
Hexatriacontane	141,314
Indeno(1,2,3-cd)-pyrene	834
Octacosane	136,765
Phenenthrene	17,156
4-$CH_3C_{14}H_9$	41,498
2,5,-$CH_3C_{14}H_9$	109,736
Pyrene	5,361
1-pentatriacontanol	30,532
Anthracene	20,016
Benz(a)anthracene	41,283
Benz(b)fluoranthene	15,250
Benzo(a)pyrene	46,465

Table III-I. Continued.

Compound	Initial Concentration (μg/kg)
Chrysene	165,191
Chrysene-5-methyl	122,145
Diben(a,h)-anthracene	1,251
Docosane	158,079
Dotriacontane	27,665
Tritetracontane	266,750
Heneicosane	122,684

Spruill, 1987.

Table III-II. HPLC Programs.

	Time (min)	Code	Value
Program 1:*	0.0	FLOW	0.1
	0.0	%	0.0
	0.0	RSVR	AB
	0.0	EVENT	0
	5.0	FLOW	1.0
	7.0	FLOW	2.0
	7.0	PROG	2
Program 2:			
	0.0	FLOW	2.0
	0.0	%	0.0
	0.0	RSVR	AB
	0.0	EVENT	0
	10.0	PRGM	2

*Program 1 is used only to bring the column to the initial conditions when starting up the HPLC.

Table III-III. Varian model 5000 chromatograph settings and conditions for HPLC analysis.

Parameter	Value
column	Varian MCH-10
solvent	HPLC acetonitrile
Pmax	150 atm
Pmin	0 atm
temperature	30 C
reservoir	AB
external events	0
analog out	%

Table III-IV. ISCO ISIS auto-injector settings for HPLC analysis.

Parameter	Value
Repeat Size	1/4
Transfer Pump*	auto
Wash	auto
Loop Loading Time	11 sec
Injections per sample	1
Analysis Time	10 min
Remote Inject	off

*The transfer pump itself is set to FWD.

Table III-V. Varian UV-Vis Detector and model 9176 Strip Chart Settings for HPLC Analysis.

Parameter	Value
UV-Vis Detector:	
Wavelength	254 nm
Time Constant	normal
Bandwidth Control	8
Absorbance Range	0.5
Sample Cell Position	front
Chart Recorder:	
Chart Speed	1 cm/min
Span	x 1
Span mV/FS	1

Table III-VI. Experimental data for biodegradation of acid sludge plus surfactant under saturated conditions. Experiment AT.

Time (days)	Temp. (C)	pH	COD (mg/ g soil)	Biomass 10^6cell/ g soil)	Substrate* (g/ g soil)
0	21	6.5	1051	84	0.148
7	22	6.5	888	197	0.143
16	22	6.4	842	504	0.135
28	22	6.5	808	724	0.133
42	21	6.6	753	870	0.126
52	22	6.6	613	990	0.109
70	21	6.5	594	14	0.096
84	21	6.6	575	79	0.069
98	22	6.4	540	38	0.069
112	22	6.5	487	19	0.069
126	21	6.4	495	17	0.068
140	21	6.4	504	12	0.069
175	20	6.5	487	1	0.068

*Measured using HPLC.

Table III-VII. Experimental data for biodegradation of acid sludge under saturated conditions. Experiment AS.

Time (days)	Temp. (C)	pH	COD (mg/ g soil)	Biomass 10^6cell/ g soil)	Substrate* (g/ g soil)
0	21	6.5	471	8	0.131
7	22	6.5	456	378	0.129
16	21	6.6	434	652	0.127
28	22	6.5	412	790	0.125
42	21	6.6	390	894	0.124
56	22	6.6	316	860	0.117
70	21	6.5	276	200	0.103
84	21	6.6	232	560	0.096
98	22	6.4	205	112	0.096
112	22	6.4	199	33	0.096
126	21	6.5	193	36	0.096
140	21	6.4	186	4	0.095
175	20	6.5	193	4	0.096

*Measured using HPLC.

Table III-VIII. Experimental data for biodegradation of acid sludge under unsaturated conditions. Experiment AU.

Time (days)	Temp. (C)	pH	COD (mg/ g soil)	Biomass 10^6cell/ g soil)	Substrate (g/ g soil)
0	21	6.5	471	8	0.131
7	22	6.4	456	10	0.129
16	22	6.5	434	13	0.128
28	22	6.5	420	17	0.126
42	21	6.5	423	21	0.125
56	22	6.6	423	33	0.122
70	21	6.6	391	540	0.121
84	21	6.5	376	474	0.122
98	22	6.5	219	413	0.098
112	22	6.4	193	541	0.081
126	21	6.4	167	254	0.077
140	21	6.4	160	54	0.076
175	20	6.5	118	287	0.072

*Measured using HPLC.

Table III-IX. Experimental data for biodegradation of soil sample from ten feet below the surface under unsaturated conditions. Experiment 10U.

Time (days)	Temp. (C)	pH	COD (mg/ g soil)	Biomass 10^6cell/ g soil)	Substrate* (g/ g soil)
0	21	6.9	458	8	0.114
7	22	7.0	392	15	0.109
16	22	6.8	368	21	0.103
28	22	6.9	338	30	0.099
42	21	7.0	283	37	0.093
56	22	6.9	109	61	0.070
70	21	6.7	105	4	0.059
84	21	6.7	103	17	0.056
98	22	6.6	97	17	0.039
112	22	6.8	96	18	0.039
126	21	6.9	89	13	0.038
140	21	7.0	86	10	0.038
175	20	7.0	85	3	0.038

*Measured using HPLC.

Table III-X. Experimental data for biodegradation of soil sample from twenty feet below the surface plus surfactant under saturated conditions. Experiment 20T.

Time (days)	Temp. (C)	pH	COD (mg/ g soil)	Biomass 10^6cell/ g soil)	Substrate* (g/ g soil)
0	21	6.9	575	8	0.121
7	22	7.0	503	268	0.099
16	22	6.8	435	792	0.079
28	22	6.8	433	1388	0.071
42	21	6.9	384	1875	0.065
56	22	7.0	361	2011	0.055
70	21	6.8	346	2823	0.052
84	21	6.9	338	304	0.043
98	22	6.7	275	234	0.030
112	22	6.8	219	152	0.025
126	21	6.9	180	47	0.025
140	21	6.7	142	13	0.025
175	20	7.0	130	3	0.024

*Measured using HPLC.

Table III-XI. Experimental data for biodegradation of soil sample from twenty feet below the surface under saturated conditions. Experiment 20S.

Time (days)	Temp. (C)	pH	COD (mg/ g soil)	Biomass 10^6cell/ g soil)	Substrate* (g/ g soil)
0	21	6.5	184	8	0.102
7	22	6.5	178	105	0.087
16	22	6.6	172	438	0.052
28	22	6.7	168	566	0.051
42	21	6.6	162	647	0.049
56	22	6.6	158	700	0.044
70	21	6.5	155	62	0.038
84	21	6.5	151	183	0.033
98	22	6.4	149	226	0.018
112	22	6.6	149	44	0.016
126	21	6.5	147	16	0.016
140	21	6.7	141	3	0.016
175	20	6.7	143	3	0.015

*Measured using HPLC.

Table III-XII. Specific growth rate estimates based on biomass data.

Experiment	Maximum Specific Growth Rate ($days^{-1}$)	Correlation Coefficient for μ_m coeff.	Time Span for Estimating μ_m (days)
AT	0.04	0.89	0 to 56
AS	0.086	0.73	0 to 56
AU	0.047	0.87	0 to 70
10U	0.032	0.97	0 to 56
20T	0.061	0.79	0 to 70
20S	0.0637	0.78	0 to 56

Where AT = acid sludge plus surfactant, AS = acid sludge saturated, and AU = acid sludge unsaturated; 10U = material 10 feet below the surface, unsaturated, 20S = material 20 feet below the surface saturated, and 20T = material 20 feet below the surface plus surfactant.

Table III-XIII. Specific Growth Rate Estimates for the Monod Model. Based on HPLC data.

Experiment	Maximum Specific Growth Rate ($days^{-1}$)	Saturation Constant (g/ g soil)	Correlation Coefficient for μ_m coeff.	Time Span for Estimating μ_m (days)
AT	0.0022	0.149	0.94	0 to 56
AS	0.0009	0.130	0.98	0 to 56
AU	0.0016	0.135	0.69	0 to 70
10U	0.0042	0.120	0.96	0 to 56
20T	0.0081	0.093	0.83	0 to 70
20S	0.0064	0.088	0.96	0 to 56

Where AT = acid sludge plus surfactant, AS = acid sludge saturated, and AU = acid sludge unsaturated; 10U = material 10 feet below the surface, unsaturated, 20S = material 20 feet below the surface saturated, and 20T = material 20 feet below the surface plus surfactant.

Table III-XIV. Specific Growth Rate Estimates for the Monod Model. Based on COD data.

Experiment	Maximum Specific Growth Rate ($days^{-1}$)	Saturation Constant (g/ g soil)	Correlation Coefficient for μ_m coeff.	Time Span for Estimating μ_m (days)
AT	0.0039	0.970	0.93	0 to 56
AS	0.0014	0.465	0.98	0 to 56
AU	0.0053	0.544	0.94	0 to 70
10U	0.018	0.587	0.93	0 to 56
20T	0.0066	0.536	0.92	0 to 70
20S	0.00087	0.180	0.92	0 to 56

Where AT = acid sludge plus surfactant, AS = acid sludge saturated, and AU = acid sludge unsaturated; 10U = material 10 feet below the surface, unsaturated, 20S = material 20 feet below the surface saturated, and 20T = material 20 feet below the surface plus surfactant.

Table III-XV. Kinetic parameter values derived from first order kinetic model. Data based on HPLC.

Experiment	Biomass yield (No. cell/ g substrate)	Rate Constant ($days^{-1}$)	Rate of Transformation (mg S/ g soil-day)
AT	2.4 E+10	0.0048	0.712
AS	1.2 E+11	0.0013	0.171
AU	5.0 E+10	0.0012	0.158
10U	1.2 E+09	0.0076	0.872
20T	4.1 E+10	0.011	1.33
20S	1.2 E+10	0.014	1.43

Where AT = acid sludge plus surfactant, AS = acid sludge saturated, and AU = acid sludge unsaturated; 10U = material 10 feet below the surface, unsaturated, 20S = material 20 feet below the surface saturated, and 20T = material 20 feet below the surface plus surfactant.

Table III-XVI. Kinetic parameter values derived from first order kinetic model. Data based on COD.

Experiment	Biomass yield (No.cell/ g COD)	Rate Constant ($days^{-1}$)	Rate of Transformation (mg S/ g soil-day)
AT	2.1 E+10	0.0080	8.41
AS	5.5 E+10	0.0044	2.07
AU	6.7 E+10	0.0021	0.989
10U	1.5 E+09	0.021	9.61
20T	1.2 E+11	0.0067	3.86
20S	2.6 E+11	0.0027	0.497

Where AT = acid sludge plus surfactant, AS = acid sludge saturated, and AU = acid sludge unsaturated; 10U = material 10 feet below the surface, unsaturated, 20S = material 20 feet below the surface saturated, and 20T = material 20 feet below the surface plus surfactant.

CHAPTER IV

EVALUATION OF IN SITUBIODEGRADATION AT AN ABANDONED REFINERY

INTRODUCTION

The laboratory studies of the material in the Arkansas City dumpsite have shown that the materials found in the site are biodegradable and that the addition of surfactant improves the transformation rate. The next step is to evaluate the feasibility of in situ biodegradation as a possible remedial action to cleanup.

This chapter studies the applications and limitations of this technique, its operation and maintenance, costs of some past and hypothetical cases, and presents a design of the system. Also, a comparison between this treatment and other treatments already used is presented. Based on this information it is recommended that the in situ biodegradation be considered for implementation at the site under investigation.

In situ biological treatment of soils and groundwater contaminated with organic compounds is based on stimulating

the indigenous subsurface microbial population to degrade the organic contaminants. Conditions for contaminant biodegradation are optimized by providing the nutrients and oxygen which may be limiting factors for the growth of aerobic microbes in the subsurface.

In situ treatment offers advantages over conventional methods such as pumping groundwater to the surface for treatment. Because the active treatment zone is in the subsurface, in situ biological treatment has the potential to both remove contaminants adsorbed in the soil matrix and treat the contaminated groundwater. Pump-and-treat methods treat only the groundwater, allowing clean groundwater to become contaminated because desorption of the pollutants occurs when the groundwater contacts the untreated soil. In situ biological treatment is more desirable environmentally than excavation, removal and disposal of contaminated soils which only transfer contaminants to a more secure disposal area without treatment. It offers treatment of organic-contaminated soils and groundwater and can be less expensive than conventional treatment or disposal methods.

In the process of designing the microbial waste treatment system, we must determine the oxygen, emulsifier (if the wastes are insoluble), and fertilizer requirements for optimum waste treatment rates. Microorganisms require the maintenance of sufficient concentrations of nitrogen,

phosphorus and trace elements, and a pH range that will support their growth.

The attention that in situ biodegradation has received may be caused by the fact that it does not demand intensive long-term controls over many years as is necessary for depositing or containing hazardous materials. An important requirement for the biological degradation is the establishment and control of an artificial large scale subsurface flow system. A partial benefit of these hydraulic measures is the effect of flushing the contaminated soil. The injected water, after passage through the contaminated aquifer, is totally pumped out. The flushing products are separated by strip-aeration and sand-filtration to be ready for reinjection.

Geldner (1986) found that in situ biodegradation had been accepted by the responsible environmental authorities as successful in the sense that the contamination is reduced down to a level that does not exert any harm to human health or violates environmental interests. He reported that all aromatic hydrocarbons were totally remediated so that the predominant goal to abolish potential danger of carcinogenic substances in the ground was achieved in the site studied.

APPLICATIONS AND LIMITATIONS

Methods for in situ chemical treatment of soil will probably be most effective for certain cleanups situations such as (EPA, 1987):

1. The contamination is spread over a relative large volume of subsurface soil, e.g. 100 to 100,000 m^3 at a depth of 1 to 10 meters.

2. The contamination is not highly concentrated, e.g. not over 10,000 ppm, or the highly contaminated portion of the site has been removed or sealed off.

3. The contaminants can be dissolved or suspended in water, degraded to non toxic products or rendered immobile, using chemicals that can be carried in water to the zones of contamination.

The use of an aqueous nonionic surfactant pair for cleaning soil spiked with PCB's , petroleum hydrocarbons and chlorophenols has been used. Ellis et al (1985) developed this technique through bench scale shaker table tests. The extent of contaminant removal from the soil was 92% for PCB's using 0.75 mg/l of each Adsee 799 (Witcfo Chemical) and Hyonic NP-90 (Diamond Shanrock) in water. For the petroleum hydrocarbons, the removal with a 2% aqueous solution of each surfactant was 93%. These removals are

orders of magnitude greater than obtained with just water washing and represent a significant improvement over existing in situ cleanup technologies.

Considerable research conducted over the past several decades has confirmed that microorganisms are capable of breaking down many of the organic compounds considered to be environmental and health hazards at spill sites and uncontrolled hazardous waste sites. Laboratory, pilot, and field studies have demonstrated that it is feasible to use this capability of microorganisms in situ to reclaim contaminated soils and groundwater.

Aerobic respiration, in which oxygen is required as a terminal electron acceptor, is the method that has been most developed and is most feasible for in situ treatment. This method involves optimizing environmental conditions by providing an oxygen source and nutrients which are delivered to the subsurface through an injection well or infiltration system to enhance microbial activity. Indigenous microorganisms can generally be relied upon to degrade a wide range of compounds given proper nutrients and sufficient oxygen. Specially adapted or genetically manipulated microorganisms are also available and may be added to the treatment zone. It can be generalized that for the degradation of petroleum hydrocarbons, aromatics, halogenated aromatics, polynuclear aromatic hydrocarbons,

phenols, halophenols, biphenyls, organophosphates, and most pesticides and herbicides, aerobic techniques are most suitable.

Anaerobic microorganisms are also capable of degrading certain organic contaminants. The potential for anaerobic degradation has been studied in numerous laboratories; several reviews have appeared (Evans, 1977; Kobayashi and Rittmann, 1982; Ghisalba, 1983; Rochkind-Dubinski et al., 1987; and Erickson and Fan, 1988). Sleat and Robinson (1984) have reviewed anaerobic biodegradation of aromatic compounds giving separate attention to photometabolism, nitrate-dependent respiration, sulfate-dependent metabolism, and methanogenic fermentation. The use of anaerobic degradation as an in situ degradation approach is reported as theoretically feasible by the EPA (1985).

Comparative studies were conducted between aerated and non-aerated soils by Yong and Mourato (1986). While aeration was observed to significantly enhance CO_2 evolution and hydrocarbon degradation, non-aerated soils had also displayed some biodegradation. The rates of CO_2 evolution and hydrocarbon degradation were shown to be significantly slower in the non-aerated soils, whereas the addition of external sources of nitrogen and phosphorous was more important for the degradation to occur. Jet fuel

degradation was only initiated after a lag time in the non-exposed soils. This phenomenon appeared to be related to the presence of jet fuel adapted microbial species within the soils which have been contaminated for a period of 5 to 10 years (Yong and Mourato, 1986).

Microorganisms require specific inorganic nutrients and a carbon and energy source to survive. Many organic contaminants provide the carbon and energy and thus serve as primary substrates. If the organic compound which is the target of the biodegradation is only degraded cometabolically, a primary substrate must be available. Optimum microbial activity for degradation purposes occurs within a pH range of 6.0 to 8.0 with slightly alkaline conditions being more favorable.

It is feasible to manipulate some factors to improve the degradation of the contaminants. Nutrient, oxygen and surface active agents can be added to the subsurface. The pH can be adjusted with the addition of dilute acids or bases. Water could be pumped into an arid zone. Temperature can be increased at the subsurface with heated water.

Wetzel et al. (1986) tested in situ biological degradation of organic contaminants at a waste disposal site at Kelly Air Force Base, Texas. They selected hydrogen peroxide as the source of oxygen since it can provide

approximately five times more oxygen to the subsurface than aeration techniques. Although hydrogen peroxide can be toxic to bacteria at high concentration, studies indicate that it can be added to soil or groundwater systems at concentrations up to 100 mg/l without being toxic to microbial populations. Higher concentrations can be added if the proper acclimation period is provided for the bacteria. Aerobic and anaerobic populations were present that were capable of degrading the organic contaminants.

At Kelly Air Force Base site, tetrachloroethylene (PCE) and trichloroethylene (TCE) concentrations decreased from 4.0 ppm to 0.93 mg/l,while trans-1-2 DCE increased from 0.03 to 1.4 mg/l in the period from June 1985 to February 1986. They concluded that the cost of performing in situ treatment is less than that for removal and redisposal of soils. It is estimated that in situ treatment can be performed for $50 to $ 100/ton of contaminated soil for a typical site (Wetzel et al., 1986).

Flushing or mobilization of wastes can serve two purposes: to promote the recovery of wastes from the subsurface for treatment on the surface, and to solubilize adsorbed compounds in order to to enhance the rate of other in situ treatment techniques. The use of surfactants will be required for significant solubilization of insoluble (hydrophobic) compounds.

Laboratory tests suggest that surfactants may enhance the recovery of subsurface gasoline from leaks by groundwater pumping, and promote the mobilization of crude oil and PCB's from soils. However, certain environmental factors may reduce the in situ effectiveness of surfactants. These include precipitation of the surfactant by groundwater with high TDS or alkaline earth cation concentrations; reduction of surfactant effectiveness due to unfavorable pH or temperature; and adsorption of the surfactant by soil particles.

There are some factors that cannot be corrected, such as the presence of predators, competition between microbial populations or the salinity of groundwater. Indigenous microorganisms will be able to survive subsurface conditions that specialized microorganisms with higher degradation capabilities may not survive. However, the use of specialized microorganisms can be expected to have the greatest application at spill sites where the exposure time has not been long enough for a substantial adapted indigenous population to evolve.

Even if active microbial populations are present, the wastes are biodegradable, and the in situ environment can be optimized, biodegradation will not be feasible if the hydrogeology of the site is not suitable. the hydraulic conductivity must be great enough and the residence time

short enough so that added substances, oxygen, and nutrients are not used before reaching the distant portions of the treatment zone. Sandy and other highly permeable sites will be far easier to treat than sites containing clay soils. There is the possibility that added substances may react with the soil components. Oxidizing the subsurface could result in the precipitation of iron and manganese oxides and hydroxides. Addition of phosphates could result in the precipitation of calcium and iron phosphates.

DESIGN

The first site remediation to treat hydrocarbon contamination in situ was conducted by Raymond et al. (1976). The first treatment approaches involved stimulation of the indigenous microorganisms through the delivery of nutrients and oxygen to the subsurface. Recently specialized microorganisms are being used at other remediation sites. Hydrogen peroxide or possibly ozone appear to be feasible options to air, and the technique is being applied to sites with hazardous waste containing a complex range of organic compounds.

Oxygen can be provided to the subsurface through the use of air, pure oxygen, hydrogen peroxide, or ozone. Air

can be added to extracted groundwater before reinjection or it can be injected directly into the aquifer. There are different methods to accomplish this, some are in line aeration, aeration wells, sparging air into wells using diffusers, blowers, and using colloidal gas aphrons for microdispersion of air into water.

Three oxidants, ozone, hydrogen peroxide, and hypochlorites have been investigated for their possible application to in situ treatments. Significant problems may preclude their effective implementation as in situ treatment agents for waste deposits. Hypochlorite reacts with organic compounds as both a chlorinating agent and an oxidizing agent, its addition may lead to production of undesirable chlorinated by products rather than oxidative degradation products. Ozone is an effective oxidizing agent for many organic compounds, but its relatively rapid decomposition rates in aqueous systems preclude its effective application to subsurface waste deposits. Hydrogen peroxide is a weaker oxidizing agent than ozone, but its stability in water is considerably greater. Since decomposition of hydrogen peroxide to oxygen may be catalyzed by iron or certain other metals, effective delivery throughout an entire waste deposit may be difficult (EPA, 1987).

Many of the nutrients required for microbial metabolism may already be present in the environment and do not need to

be supplemented. The optimum nutrient mixture can be determined by laboratory growth studies and from a chemical evaluation of the site. If the contaminants are only degraded by cometabolism and a primary source of carbon is needed, glucose could be added. It can also be included in the nutrient solution if the concentration of contaminants is too low to maintin an active microflora.

The injection and recovery systems should be designed to provide adequate contact between treatment agents and contaminated soil or groundwater, to provide hydrologic control of treatment agents and contaminants to prevent their migration beyond the treatment zone, and to provide for complete recovery of spent treatment solutions and/or contaminants where necessary.

The proposed process by Kosson et al. (1987) consists of in situ alkaline sludge extraction coupled with on site, sequential aerobic/anaerobic, soil based microbial destruction of recovered organic contaminants. The results obtained in the pilot plant duiring the first year indicated rapid organic species extraction from sludges and greater than 95% destruction of recovered extract TOC. Solid based sequential aerobic/anaerobic microbial treatment removed greater than 99% of TOC present in the recovered extract for the second year.

Figure IV-1 presents a simplified flow diagram of this process. The first process step was extraction of sludges present in a representative section of the lagoon. Sodium hydroxide solution was mixed and injected into the sludges applied to the surface of the extraction bed. Extract was recovered from the extraction bed using two wells and collected in a process tank. This extract was diluted if necessary, the pH was adjusted, and some nutrients were added in a baffled mixing tank. The third step was treatment of the modified extract in an aerobic/anaerobic bioreactor. Effluent from the treatment bed was recovered through a screened well and pumped into a storage tank prior to recycle or discharge.

OPERATION AND MAINTENANCE

Monitoring a number of parameters is necessary to determine process performance. Monitoring of groundwater can be performed at the injection, extraction, and monitoring wells. The monitoring wells should be placed on-site to monitor the process performance and off-site to monitor for the migration of contaminants. Some of the suggested parameters to follow are the microbial concentration, total organic carbon, priority pollutants,

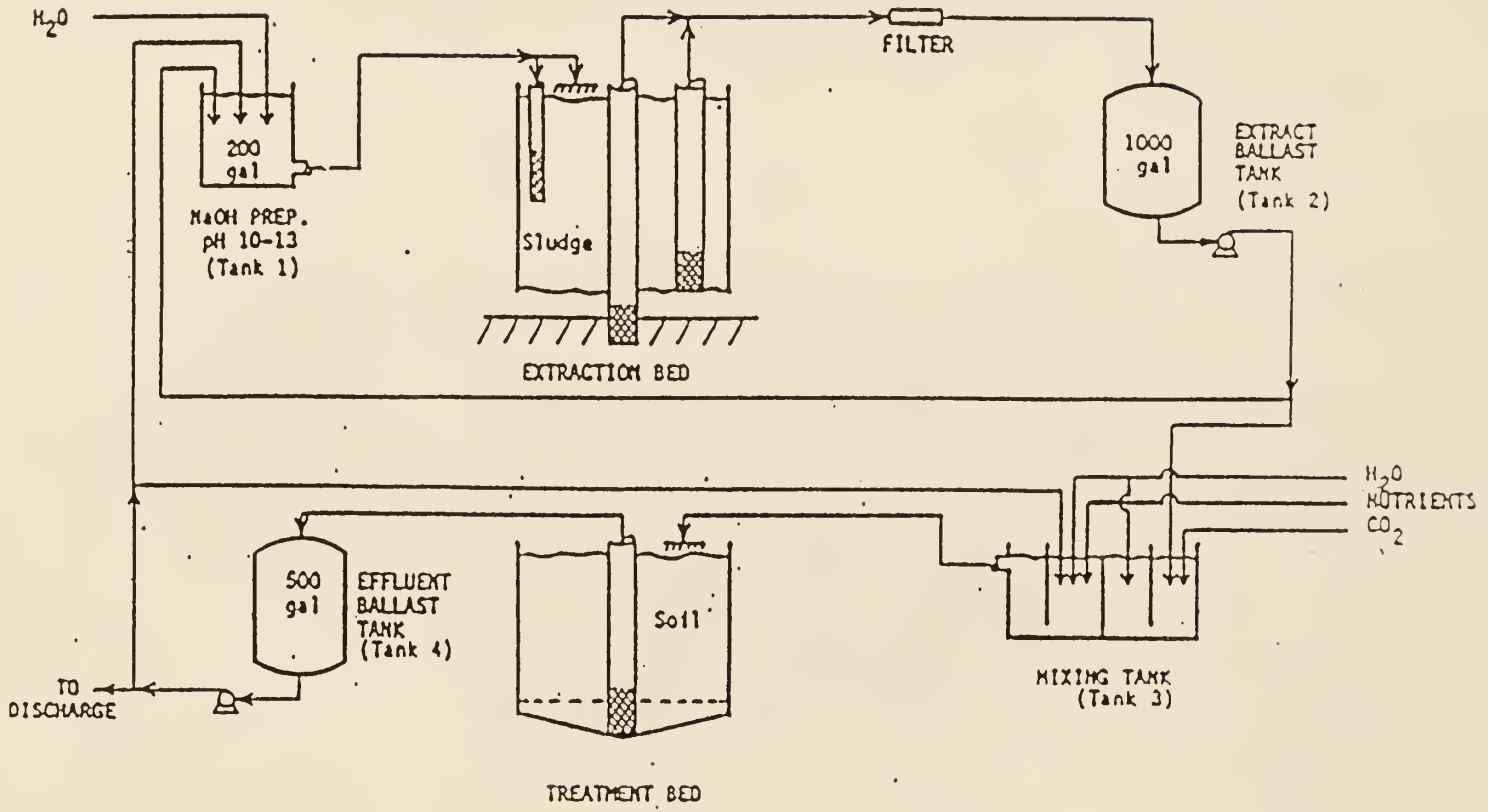

Figure IV-1. Simplified process flow diagram. Kosson et al., 1987

pH, dissolved oxygen, and alkalinity.

Maintenance of the microbial population is very important, especially for selective mutant organisms which tend to be more sensitive than naturally occurring species. A continuous incubation facility operating at higher temperatures and under more controlled conditions could be used to maintain the population, and the subsurface could be reinoculated continuously with microorganisms.

Nutrient formulations should be devised with the help of experienced geochemists for minimizing problems with precipitation and dispersion of clays. The pH should be maintained at the optimum level, and concentrations of both nutrients and organic compounds should be kept as uniform as possible to protect against shock loading.

COST

Cost for biological in situ treatment depends on various factors. Some of them are the extent of contamination, the kinds and concentrations of contaminants, and the site geology and geohydrology. Well construction, pumping, chemicals, nutrients, and hydrogen peroxide, if needed, have to be taken into consideration too. Reuse of

surfactants is important for this process to be cost effective.

Here is some cost data for an hypothetical site involving the use of hydrogen peroxide as an oxygen source for the enhancement of biodegradation (FMC, 1985). The cleanup of 300 gallons of gasoline from a sand gravel aquifer over a period of 6 to 9 months is between $70,000 and $120,000. Cleanup for 3,000 gallons of diesel fuel from a fractured bedrock formation is estimated to require 9 to 12 months and $160,000 to $250,000. The cost estimate for degrading 10,000 gallons of jet fuel from a fine gravel formation is estimated to cost $400,000 to $600,000 and take 14 to 18 months (EPA, 1985).

At Kelly Air Force Base, Texas it was found that the major construction costs were incurred through well installation and labor. Construction materials will vary in cost depending on the site conditions, but should not constitute a large portion of capital costs. During operation of a system, the major costs incurred are due to sampling and analysis to monitor system performance. The cost of treatment chemicals will be minor in comparison. The estimated costs for treating the Kelly AFB site were $35/ton of contaminated soil; analytical costs accounted for $9/ton or 25% of the total cost (Wetzel et al., 1986).

At the Arkansas City dumpsite, the cost for in situ biodegradation was calculated as $1360 per ton of acid sludge for equipment, labor, materials, and neutralization (Ott et al., 1988). These figures were calculated using hydrogen peroxide as the oxygen source. It was found that the cost of biodegradation is proportional to the hydrocarbon content, since the H_2O_2 material cost makes up nearly 80% of the cost of in situ biodegradation.

EVALUATION

Relative to conventional pump and treat methods, biodegradation may be more effective since it is capable of degrading organic compounds adsorbed in the soil. Adsorbed organic compounds are not removed using conventional pump and treat methods. Pumping systems are prone to mechanical and electrical failure, but repairs can be done relatively quickly. Subsurface drains are less prone to failure since there are no electrical components. Where mechanical failures do occur, repairs can be costly and time consuming.

Excavation and removal of the contaminants will take less time than in situ biodegradation. Depending on the size of the site, it could also take longer than a

conventional pump and treat approach. However, in situ biodegradation will treat contaminated subsurface soils removing the source of contamination of groundwater. The important factors affecting the time required for biodegradation are oxygen availability and aquifer permeability. As discussed before, both of these can be improved by the use of hydrogen peroxide and surfactants.

There are fewer hazards associated with in situ biodegradation than with excavation and removal. Since the treatment occurs only under the surface, there is little if any contact with the contaminants. The nutrient solution does not represent any hazard even if it contains hydrogen peroxide, which will rapidly decompose in the subsurface to oxygen and water. Hydrogen peroxide will be hazardous before it is diluted in the nutrient solution; therefore, safety training in the use of hydrogen peroxide should be provided by qualified personnel.

CONCLUSIONS

Laboratory experiments showed that the material in the Arkansas City dumpsite was biodegradable over a period of five to six months. The addition of surfactant showed an improvement of the transformation rates for all materials,

more for the acid sludge than the material below the water table.

Arkansas City dumpsite meets two of the three main criteria to be a good candidate for in situ biodegradation. It is spread over a large volume, and the contaminant concentration is not too high, except in the northern acid sludge area. The third criteria, contaminants can be dissolved or suspended in water, can be met with the addition of surfactant.

The bench scale experiments did not show any decrease in the properties of the surfactant used, Tween 20. There was no precipitation of surfactant, and there was no adsorption into the soil since it was visible during the course of the experiments. Also, the addition of surfactant appears to enhance the microbial growth rate instead of being toxic to the flora.

The cost for the in situ biodegradation was found to be too high when compared with other technologies. There are factors that increased the cost for the whole site but that are only present in some areas. The acid sludge pits need neutralization and hydrogen peroxide as source of oxygen in proportion to the amount of PNA's. The rest of the site is already neutralized and has considerably lower concentrations of PNA's.

Based on all these facts the Arkansas City dumpsite is recommended for in situ biological treatment as remedial action for cleanup. A small scale soil and groundwater treatment system with neutralization and the addition of surfactant and nutrient solution should be started as soon as possible. This will give an estimate of the limitations that in situ treatment has in this site. In situ biodegradation is not recommended for the acid sludge pits since it is not very economical. Less expensive alternatives to in situ biodegradation with hydrogen peroxide as the source id oxygen need to be considered for the acid sludge pits.

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CHAPTER V

RECOMMENDATIONS FOR FUTURE WORK

In this chapter some of the problems encountered during the course of this work and suggestions for improving the experimental procedure are presented. There were problems with the resolution of the substrate and product concentrations and with contamination in the biomass concentration measurements. The improvement for the HPLC procedure is very important in the understanding of the biodegradation of polynuclear aromatic hydrocarbons.

HIGH PRESSURE LIQUID CHROMATOGRAPHY

The HPLC procedure recommended for the substrate concentration measurement for PNA's is explained in detail in Chapter III. HPLC analysis of a mixture of PNAs adsorbed in soil was used as one of the methods to determine the substrate concentration in the soil. The soil used for this experiment was that found in Arkansas City dumpsite.

Separation was accomplished by reverse-phase HPLC utilizing a Varian MCH-10 column. The column is packed with octadecylsilane ($C_{18}H_{37}$ covalently bond to silica). In reverse-phase chromatography the separation is governed by the hydrophobic character of the solute compounds; the more hydrophobic the compound, the longer it is retained in the column. Isocratic elution for one minute using 40% acetonitrile/ 60% water, then linear gradient elution to 100% acetonitrile over 15 minutes at a flow rate of 2.0 ml/minute was the method tried.

Due to technical difficulties, this gradient or any other gradient containing water, could not be used and 100% acetonitrile was used. The problem was that the samples precipitated if there was any water in the column. This caused an increase in the column pressure and the system stopped after reaching the maximum pressure. Thus a gradient analysis was impossible to perform and a 100% acetonitrile had to be used as carrier. The system worked fine after acetonitrile was run through the column for fifteen minutes. The samples showed similar resolutions, which were not selective enough to identify any PNA compound.

Another factor that may have contributed to this problem is the fact that the column used had been used

before by two other investigators, so there is the possibility that the column was not as efficient as a new one.

It is recommended that the present column be changed for a new one. Column CH-10 is designed specially for polynuclear aromatic hydrocarbons. The column used, MCH-10, can be used for the type of analysis done in this study, but is not designed for high coverage as CH-10 is. Another possibility would be to use a different carrier, or mixture of carriers that does not contain water. Since methanol has been used in other studies (Guerin and Jones, 1988a, 1988b), it may be a good choice for experimentation.

BIOMASS MEASUREMENTS

The determination of the number of bacteria in a highly heterogeneous biological system such as soil is difficult since conventional microbiological techniques only estimate a portion of the total number of bacteria. No one medium is adequate nutritionally for all the species present since the growth requirements for many strains are known, and the observed count represents only a fraction of the total. A second limitation arises from the fact that bacteria usually occur in the soil as colonies, and these may not

desintegrate when the soil dilutions are shaken so that the estimates tend to be low.

Guerin and Jones (1988a, 1988b) used the Lowry Method to determine the biomass concentration. This method uses protein to estimate biomass using bovine serum albumin as a standard. They found that the plate count and the epifluorescence microscopic count methods were unsuitable.

During the course of this work the only problem encounter with the Standard Plate Count Method was contamination of the sample. This was solutionated by working under sterile conditions. To avoid the under estimate of cells due to colonies, the samples were shaken and then they were allowed to settle before shaking them again. After the second shake the sample was plated.

The other aspect of biomass concentration that needs to be studied in some detail is the microbial flora. The microorganisms used in these experiments were those found in the soil surrounding the site. From looking under the microscope, the dominant species were bacteria.

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APPENDIX

Procedure to Estimate the Substrate Concentration

The following procedure was used in the determination of the standard curves to convert integrater units to grams of substrate for the HPLC analysis. A known amount of the polynuclear aromatic hydrocarbon, naphthalene or phenanthrene, was diluted in 50 ml of methylene chloride and let sit covered for thirty minutes. The methylene chloride was evaporated to a volume less than 5 ml. The volume was brought to 10 ml by adding methylene chloride quantitatively. It was diluted 1:20 with acetonitrile and injected into the HPLC at the same conditions that were used during the rest of the experiments. The area under the peak was recorded in integrater units.

From this information the grams of substrate can be related to the integrater units. Tables A-I and A-II present the results obtained from these analyses. The regression wquation wmployed to estimate the grams of PNA's per grams of soil was obtained by averaging the two results; that is:

$$g\ PNA's/10\ g\ soil = 0.00289(I.U.) - 0.00835$$

Table A-I. Data for the Substrate Standard Curve for UV Detection at 254 nm.

Naphthalene (g)	Area (I.U.)
0.0	0.0
0.1	34
0.2	71
1.0	356
2.0	492
3.0	626

Integrater units = I.U.

Linear regression of these data gives :

Substrate (g) = 0.00449(I.U.) - 0.1317

correlation coefficient = .95

Table A-II. Data for the Substrate Standard Curve for UV Detection at 254nm.

Phenanthrene (g)	Area I.U.
0.0	0.0
0.1	57.7
0.2	64.3
0.5	72.1
1.0	844
2.0	1379

Integrater units = I.U.

Linear regression of these data gives :

Substrate (g) = .00129(I.U.) + .115

correlation coefficient = .94

BIODEGRADATION OF ORGANIC COMPOUNDS FROM AN ABANDONED REFINERY SITE

by

Lourdes M. Taladriz

B.S., Iowa State University, 1986

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the
requirements for the degree

MASTER OF SCIENCE

College of Engineering
Department of Chemical Engineering

KANSAS STATE UNIVERSITY
Manhattan, Kansas
1988

ABSTRACT

It has been suggested that microorganisms exist which are capable of oxidizing all naturally produced organic compounds under suitable conditions. The problem is that many environments, such as anaerobic or acidic ones, do not represent suitable conditions, and in this type of situation many organic molecules may remain more or less unchanged over very long periods of time. This is exactly what has happened in Arkansas City, Kansas.

The Milliken Oil Refinery operated in the vicinity of Arkansas City during the early part of the 20th century. Following a fire in the mid 1920's the property was used for disposal of various waste materials from unknown sources. The presence of carcinogenic polynuclear aromatic compounds (PNA's) in soil and ground water samples has been detected. We are interested in the aliphatic hydrocarbons and the polynuclear aromatic compounds since many are known to be carcinogenic.

Samples were classified into three different types according to location; acid sludge, soil found 10 feet below the surface and soil found 20 feet below the surface, which was also below the water table. A mixed culture was harvested from the surrounding soil of the acid sludge pond

and used as inocula. The treatments applied to the soil were saturated, unsaturated, and saturated with the addition of surfactant. The surfactant used was Tween 20 due to its properties and availability.

The material in the site can be biodegraded by a mixed soil culture, if it is neutralized and fed with a nutrient solution. The results of this experiment show that it can be reduced from 70% to 90% in a period of 3 months. The addition of surfactant enhances degradation. The unsaturated treatment did not show an improvement over the saturated conditions found at the site.

The cost for the in situ biodegradation was found to be high when compared with other technologies. The acid sludge pits need neutralization and a source of oxygen in proportion to the amount of PNA's. The rest of the site is already neutralized and has considerably lower concentrations of PNA's.

In situ biological treatment is recommended as a potential alternative for the remediation of the Arkansas City dumpsite. A small scale soil and groundwater treatment system with the addition of surfactant and nutrient solution should be started as soon as possible. This will give an estimate of the limitations that in situ treatment has in this site.