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Degradation and Biotransformation of Isophorone, Xylenols, Cresols, and Polyaromatic Hydrocarbons in Acclimated Activated Sludge: Use of Enrichment Reactors to Enhance this Process

A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Civil Engineering

by

Lynne Jane Cardinal

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Cardinal, L.J. and Stenstrom, M.K. (1988) "Treatment of Hazardous Substances in Conventional Biological Treatment Plants, Use of Enrichment Reactor to Enhance This Process", Poster presented at the Second Annual Research Symposium, University of California, Los Angeles, CA.

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Cardinal, Lynne (1989) "Degradation and Biotransformation of Xylenols, Cresols and Polyaromatic Hydrocarbons in Acclimated Activated Sludge: Use of Enrichment Reactors to Enhance this Process." Ph.D Thesis, School of Civil Engineering, University of California, Los Angeles.

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ABSTRACT OF DISSERTATION

Degradation and Biotransformation of Isophorone, Xylenols, Cresols and Polyaromatic Hydrocarbons in Acclimated Activated Sludge: Use of Enrichment Reactors to Enhance this Process

by

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Doctor of Philosophy in Civil Engineering
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Professor Michael K. Stenstrom, Chair

The activated sludge process, which is widely used to treat organic wastes in wastewater treatment plants, is being increasingly considered for treating organic toxic wastes. This study focused on the development of activated sludge reactors which are enriched in specific microbial populations capable of degrading particular compounds or classes of compounds. These reactors can be used to provide conditioned inocula for conventional treatment plants thereby enhancing toxic waste removal form wastewater.

Three separate continuous flow reactors acclimated to isophorone, 2,4 xylenol, or naphthalene reduced the concentrations of these compounds from 99.6 mg/l 2,4 xylenol, 18.7 mg/l naphthalene, and 100 mg/l isophorone in the influent to less than 8.97 ug/l 2,4 xylenol, 0.0714 ug/l naphthalene, and 1.72 ug/l

isophorone in the effluent. No stable intermediates were detected in the effluent. Activated sludge acclimated to 2,4 xylenol biodegraded isomers of xylenol, cresol and trimethylphenol. Activated sludge acclimated to naphthalene biodegraded phenanthrene.

Enrichment reactors, operated as sequencing batch reactors, were started using activated sludge acclimated to naphthalene. These reactors were maintained on either a salicylate feed solution or a 2-aminobenzoic acid plus succinate feed solution and 160 ug/l naphthalene. Salicylate and 2-aminobenzoic acid gratuitously induce the production of the enzymes used in the beginning oxidation reactions involved in the degradation of naphthalene. The enrichment reactor maintained on salicylate showed good removal of naphthalene for more than 9 months. The reactor maintianed on 2 aminobenzoic acid did not show significant removal of naphthalene.

A continuous flow reactor and a sequencing batch reactor were then operated together of form an enrichment reactor system. The continuous flow reactor which received an influent of glucose/nutrient broth feed and 5.7 ug/l naphthalene demonstrated enhanced ability to degrade naphthalene when given periodic doses of activated sludge from the sequencing batch enrichment reactor maintained on salicylate. No significant removal of naphthalene occurred when the continuously fed reactor did not receive an influent of 5.7 ug/l naphthalene.

The effects of K (the decay rate) on the mechanism of volatilization of naphthalene, 2,4 xylenol , and isophorone were modeled. Steady state conditions and a first- order rate of decay were assumed.

INTRODUCTION

Since the passage of the ammendment to the clean water act in 1972, most municipalities in the United States have constructed some type of secondary wastewater treatment facilities. The vast majority off these use the activated sludge process to remove organic soluble and colloidal pollutants. In this process biodegradable material is oxidized to carbon dioxide and water or converted to biomass. Non-biodegradable pollutants may pass through the process, but are more often removed from the liquid phase, either by adsorption onto biological flocs which are subsequently removed in secondary clarifiers, or volatilization to the atmosphere during aeration.

These secondary wastewater treatment facilities in publicly owned treatment works (POTW's) were designed to treat municipal wastewaters which are comprised primarily of biodegradable materials from domestic operations, such as human wastes, kitchen wastes and washing by-products. Consequently, the process has been optimized for treating these easily biodegraded materials.

Commercial and industrial discharges often have their own treatment systems. Frequently these treatment systems discharge their effluents into POTW's, and for this reason they are actually functioning as pretreatment systems.

Large municipalities such as Los Angeles have many industries discharging into their treatment system. Consequently, their wastewaters are composed of both municipal and industrial wastes. The industrial contribution results from imperfect pretreatment systems, fugitive emissions, illegal

discharges or urban runoff. Table 1 shows potential sources for the compounds being investigated in this project.

In the current environment of increasing regulation of hazardous wastes, POTW's are being evaluated for a larger treatment roll. The state-of-the-art approach for these wastes is to remove them from wastewaters, to concentrate them to reduce volume, and finally to destroy them in an approved hazardous waste facility. Undoubtedly this is the desirable approach for compounds which are extremely hazardous or highly non-degradable. For many types of hazardous wastes, particularly high volume, dilute wastewaters, biological treatment in existing facilities, perhaps in selected POTW's, may be a more cost effective alternative.

Therefore it is proposed that municipal systems be re-evaluated as a treatment technology for commercially significant, "semi-hazardous" waste. Waste which can be biodegraded or removed by adsorption in the activated sludge process and those which cannot be conveniently isolated from municipal waste or urban runoff, are the best candidates for treatment in POTW's. Removal of these toxic compounds by biodegradation in the activated sludge process and the use of enrichment reactors to enhance this process is the focus of this study.

The initial approach of this study was to review the types of compounds that may enter POTW's. The degradation pathways of selected compounds was then delineated. Further investigation of the biochemical and genetic aspects of the removal of the compounds by biodegradation was subsequently used to select the appropriate substrates used in the development of enrichment reactors.

Table 1. Sources of Toxic Compounds.

Compound	Anthropogenic Sources	Natural Sources
Cresols	Auto ExhaustCoal	
	Roadway Runoff	Petroleum
	Asphalt Runoff	Wood Constituents
	Petroleum Distillates	Natural Runoff
	Oil, Lubricants	
Xylenols	Roadway Runoff	Coal
	Asphalt Runoff	
	Fuels, Solvents	
	Plastics	
	Pesticides	
	Catalytic Cracking	
Isophorone	Solvent for Resins	
	Lacquers and Finishes	
	Pesticides	
	Organic Chemical Mfg.	
PAH's *	Air Pollution	Plant and Animal
	Catalytic Cracking	Pigment
	Processing for Fossil Fuel	Crude Oil
	Combustion of Fossil Fuel	Grass and Forest
	Roadway Runoff	fires

* Polycyclicaromatic Hydrocarbons

Models in biodegradation and volatilization were also reviewed. Using these models volatilization of specific compounds was evaluated at different rates of biodegradation.

Bench scale continuous flow and sequencing batch reactors were used both as independent units and together. As single units they were used to study the degradation of these compounds while being maintained on an influent containing various substrates. Sequencing batch reactors were then used in conjunction with the continuous flow reactors to form an enrichment reactor system. This system demonstrated the concept of the use of enrichment sequencing batch reactor to enhance the removal naphthalene compound from a continuous flow reactor. The maintenance schedules, operating parameters and necessary feed solutions to maintain viable activated sludge for the experiments are presented.

A variety of analytical methods were used to separate compounds from the activated sludge and measure the presence and/or the concentration of a particular compound. Liquid/liquid and solid/liquid extraction methods were used to recover the toxic compounds of interest. Gas chromatography and GC/MS were the predominate methods used to detect and/or quantify the compounds; ultraviolet spectrophotometery was used to a lesser extent.

In the first part of the study activated sludge in continuous feed reactors, which was acclimated to either isophorone, 2,4-xylenol or naphthalene, was tested for its ability to degrade the target compounds and structural analogues of the target compounds. The relationship between the structure of the analogous compounds and their subsequent degradability is discussed.

The second part of the study focused on the development of a microbial population capable of degrading naphthalene. Activated sludge capable of degrading naphthalene was placed in an enrichment sequencing batch reactor and maintained on a substrate other than naphthalene. This enrichment reactor was then used in the enrichment reactor system where the continuously fed reactor was periodically inoculated with activated sludge from the enrichment sequencing batch reactor. The continuous feed reactor which was inoculated with enrichment reactor cells was then tested for an enhanced ability to degrade naphthalene and compared to controls.

DEGRADATION PATHWAYS

Aerobic microorganisms make use of many of the metabolic pathways which are known. They have the ability to catalyze the oxidation of numerous biochemically inert compounds using molecular oxygen. Once oxidized these compounds can then participate in the reaction sequences that enter the central pathways of metabolism.

Degradation of Xylenols and Cresols

For aromatic compounds to undergo ring-fission, the benzene nucleus must be oxidized to contain at least two hydroxyl groups. The hydroxylation of these compounds usually results in the formation of a catechol, protocatechutate or gentisate.

The degradation of the aromatic compound 2,4-xylenol, which is an industrial pollutant commonly found in wastewater, has been extensively studied. Chapman (1968) found that the degradation of 2,4-xylenol was initiated by the oxidation of the methyl substituent para to the hydroxyl group. P-cresol and 3,4-xylenol (Dagley 1956) were attacked in a similar manner. The further degradation of 4-hydroxy-3-methylbenzoic acid, from 2,4-xylenol, involves oxidation of the methyl substituent to a carboxyl group forming 4-hydroxy-isophthalic acid followed by an oxidative decarboxylation of the newly formed carboxyl to give protocatechuic acid. Protocatechuic acid is then metabolized to b-ketoadipic acid by protocatechuic acid 3-4-dioxygenase.

Figure 1 shows the overall pathway. Thus, in 2,4-xylenol both methyl groups undergo oxidation in succession, with the original ortho methyl group being replaced by hydroxyl to produce protocatechuic acid.

Microorganisms which oxidize other isomers of the xylenols and cresols utilize the pathways involving catechol or gentisate (Figure 2). Xylenols oxidized by these pathways retain one or both of their methyl groups intact until after ring cleavage.

M-cresol, 2,5-xylenol and 3,5-xylenol are oxidized to gentisic acid, 4-methylgentisic acid and 3-methylgentisic acid respectively, (Hooper 1970). The Pseudomonas species here initiate the degradation of cresols and xylenols by oxidizing a methyl group placed meta to the hydroxyl group, and add a second hydroxyl group para to the first.

The compounds 2,3-xylenol, 3,4-xylenol and o-,m-,p-cresol, are oxidized by direct ring hydroxylation producing catechols which undergo meta ring cleavage. In this case degradation, also by a species of Pseudomonas, is accomplished by direct ring hydroxylation of the cresols and xylenols to form catechols.

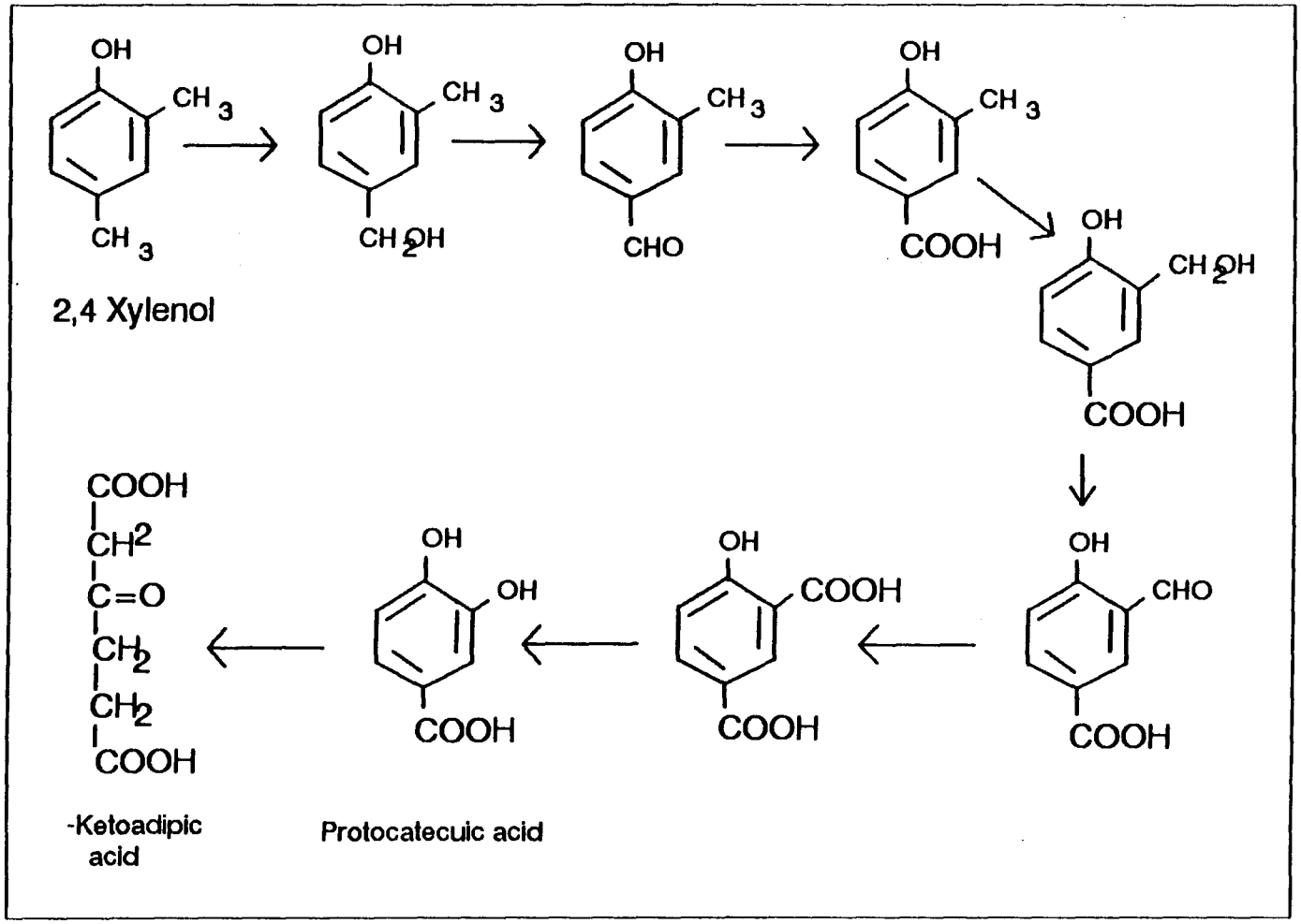

Fig. 1 Pathway for the degradation of 2,4 xylenol by bacteria (Gibson,1984).

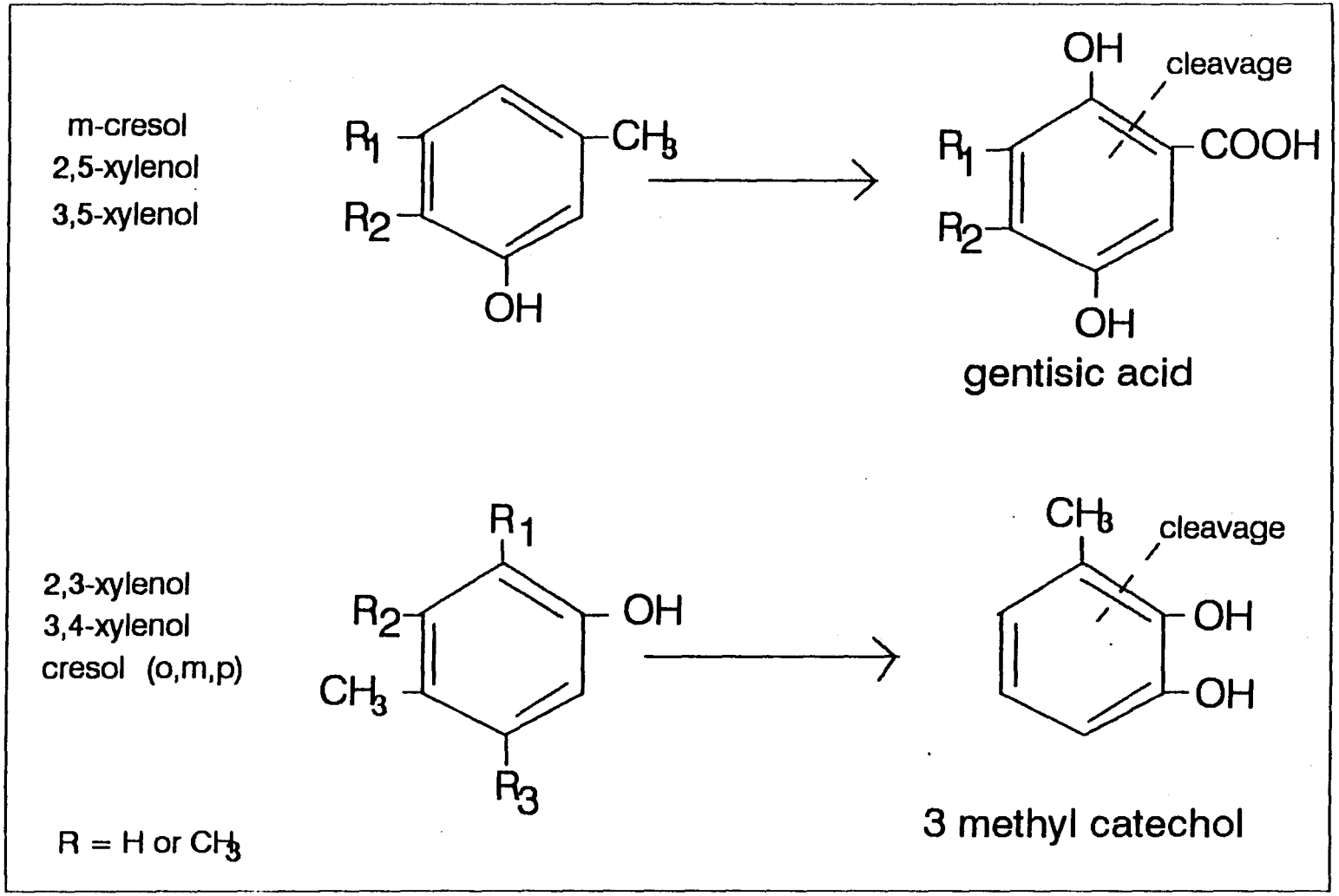

Fig. 2. Degradation pathways of isomers of xylenol and cresol

Degradation of Alicyclic Hydrocarbons

The pathways for the oxidation of the alicyclic hydrocarbons, such as isophorone, have not been as well researched. Alicyclic hydrocarbons are resistant to microbial attack and microorganisms capable of utilizing them as sole carbon sources are not easily isolated (Trudgill 1984). The difficulty in isolating such microorganisms may arise because degradation occurs primarily under conditions of co-oxidative metabolism and in commensal situations. Trudgill et al. (1984) achieved their best results when testing for the degradation of alicyclic compounds using mixed cultures.

Degradation of Polycyclic Aromatic Hydrocarbons

The initial steps for the oxidation of polycyclic aromatic hydrocarbons is similar to that of the monocyclic aromatic hydrocarbons xylenol and cresol (Cerniglia 1984). The aromatic hydrocarbons are initially oxidized to form dihydroxylated derivatives by incorporation of both atoms of molecular oxygen into the aromatic nucleus by a dioxygenase. Naphthalene (Figure 3), in the presence of oxygen and NADH, is oxidized by incorporating both atoms of molecular oxygen into the aromatic nucleus to form the dihydrodiol 1,2-dihydroxynaphthalene.

Further oxidation of the dihydrodiols leads to the formation of catechols that are substrates for other dioxygenases that bring about enzymatic cleavage of the aromatic ring. Cleavage of the catechol can proceed via the ortho

pathway, which involves cleavage of the bond between carbon atoms of the two hydroxyl groups to yield cis,cis-muconic acid, or via the meta pathway, which involves cleavage of the bond between a carbon atom with a hydroxyl group and the adjacent carbon atom without a hydroxyl group.

The bacteria commonly associated with this type of naphthalene degradation are Fluorescent Pseudomonas putida, other Pseudomonas sp (Stanier 1966) (Jeffery 1975) and Aeromones sp (Cerniglia 1984). Prokaryotic and eukaryotic photosynthetic algae can also hydroxylate aromatic compounds such as naphthalene (Cerniglia 1984).

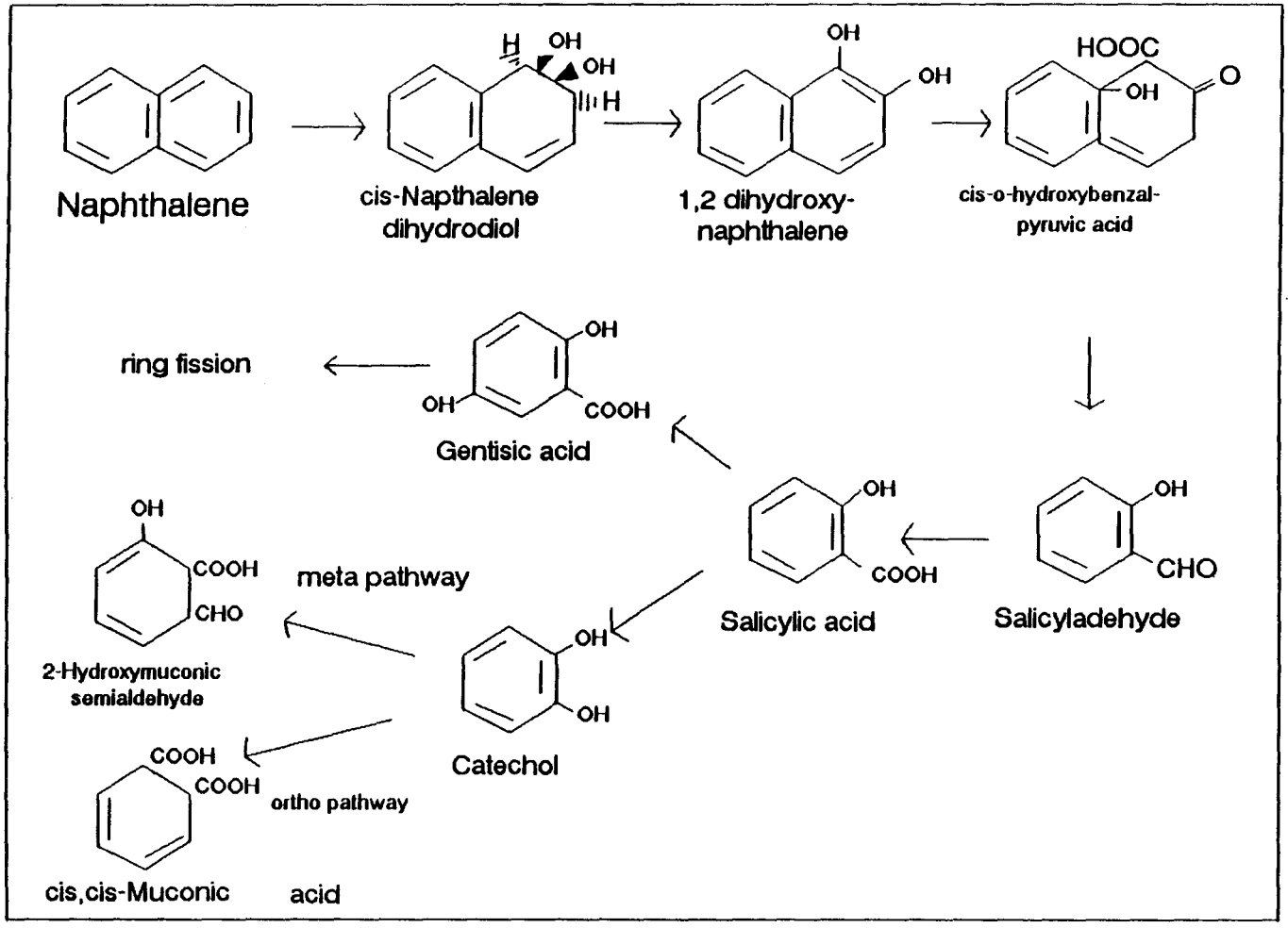

Fig. 3. Pathway for the degradation of naphthalene by bacteria.
(Gibson 1984, Cerniglia 1984, Zuniga 1981)

ENRICHMENT SUBSTRATES

Degradation via Enzymes of Broad Specificity

Many of the early reactions involved in biodegradation of the xylenols are mediated by enzymes of broad specificity which are inducible by structurally related compounds (structural analogues). Studies by Chapman (1971) show that a strain of Pseudomonas putida capable of degrading 2,4-xylenol readily oxidizes 3,4-xylenol and p-cresol and slowly oxidizes the meta methyl substituents of phenols such as m-cresol and 3,5-xylenol. In this case, exposure of bacteria to a single compound induce the production of enzymes that can partially or completely metabolize a whole class of compounds.

Much work has been done to determine the ability of naphthalene degrading pseudomonads to metabolize other polyaromatic hydrocarbons. Pseudomonas putida grown on naphthalene will oxidize phenanthrene as rapidly as naphthalene, while benzene, substituted benzenes and naphthalenes, and anthracene show 30-80% relative activity (Jeffery 1975, Ribbons 1982). In Jeffery's study, only naphthalene supports significant growth with the principle product of phenanthrene oxidation being cis 3,4-dihydrophenanthrene.

When naphthalene and phenanthrene are both used as a primary energy source for growth, both compounds undergo complete catabolism with protocatechuate as an intermediate. Kiyohara et al.(1978) found that this is the case with Aeromonas sp. and fluorescent pseudomonads and believed that the pathways of degradation of phenanthrene and naphthalene are separate. Results by Barnsly (1983) provide further evidence by showing that an

organism which can grow on both naphthalene and phenanthrene induces a separate enzyme for the initial oxidation of each hydrocarbon.

Induction of the Initial Enzymes of Naphthalene Oxidation

Due to the diversity of the microbial population present in activated sludge the potential for the induction of many degradation pathways is present in the system. In the case of naphthalene an understanding of the location of the chromosomes that code for the necessary enzymes, and which intermediates are responsible for the induction of these enzymes, leads to more efficient and feasible methods of enrichment.

Pseudomonas putida and related species may contain the degradative plasmids NAH and SAL that are responsible for the degradation of naphthalene and salicylate respectively. The NAH plasmid is involved in the degradation of naphthalene by a series of reactions through salicylate which is then metabolized further through catechol via the ortho or meta pathways (Fig. 3).

The actual enzymes that are produced by these plasmids or whether they are actually the same plasmid is a matter of debate. Dunn (1973) felt that it was possible that at least the initial enzyme in the pathway, the 2,3-oxygenase, is present on a plasmid or the induction mechanism for this oxygenase is on this plasmid. Williams (1974) concluded that the majority, if not all of the enzymes of the meta pathway are coded for on a transmissible plasmid and both Dunn and Williams found that the loss of this plasmid caused total disappearance of all the meta enzymes. Zungia (1981) found that the presence of a plasmid was

directly related to the ability of Pseudomonas putida to use naphthalene and salicylic acid as sole carbon source. Salicylic acid degradation via meta cleavage was found to be entirely plasma encoded, whereas naphthalene degradation, which proceeds through salicylate, requires some chromosomal genes.

The characterization of these plasmids is important because of the role they play in the evolution of metabolic diversity among the pseudonomas species. Plasmids are important in increasing the rate and degree of removal via degradation of these compounds by the use of transduction or conjugation. By transferring plasmids between pseudomonas of the same and different species the number of organisms capable of utilizing naphthalene increases at a faster rate than in the case of cell multiplication. As a result of this the removal of these compounds, which is first order with respect to the microbial population, will occur at a much faster rate. This is similar to the nutritional versatility by plasmid transfer found in the genera of bacteria that develop drug resistance.

Induction of the enzymes for the degradation of naphthalene is brought about by naphthalene, intermediates formed during the catabolic process, as well as compounds not directly involved in the metabolic pathway. Results of Shamsuzzman (1974) show that the early enzymes of naphthalene metabolism, naphthalene oxygenase, 1,2-dihydroxynaphthalene oxygenase and salicylaldehyde dehydrogenase, are induced when the organism is grown on naphthalene or salicylate, but not catechol. In later studies, (Williams, Shamsuzzman and Barnsley 1976), using Pseudomonas putida, show induction of the early enzymes of naphthalene oxidation in response to salicylate or gratuitously in response to 2-amino benzoate. Thus with growth on

naphthalene, salicylate or succinate plus 2-aminobenzoate, naphthalene oxygenase, 1,2-dihydroxynaphthalene oxygenase, salicylaldehyde dehydrogenase and salicylate hydroxylase are all induced.

Substrate for Maintenance and Growth of Activated Sludge Enriched in a NAP+ Microbial Population

Maintaining activated sludge, which has been enriched for microorganisms that mineralize a toxic compound, using the toxic compound itself can be difficult because of economic or safety reasons. These problems may be circumvented by using a substrate which is structurally very similar to the target compound, an intermediate of the degradation pathway for that compound, and/or a substrate that induces the enzymes necessary for the degradation of the target compound.

The substrate must serve both as an energy source and maintain the genetic integrity of activated sludge enriched in a microbial population capable of degrading the target compound. In this project the choice of substrates for the maintenance of the activated sludge acclimated to naphthalene are salicylate and 2-aminobenzoate plus succinate. The compounds salicylate and 2-aminobenzoate gratuitously induce the early enzymes of naphthalene degradation, as previously discussed, and salicylate and succinate serve as energy sources for the activated sludge.

Kiyohara (1978) noted that pseudomonads with the phenotype to assimilate naphthalene and/or phenanthrene lost both characteristics during prolonged storage on nutrient agar slants. This same phenomena may occur in acclimated activated sludge which is maintained over an extended period of time on a substrate other than the target compound.

KINETICS

Many researchers have found that the effect of a limiting substrate or nutrient, on microbial growth, can be defined using the Monod expression (Metcalf 1979), (Benefield 1980):

$$U = u_m(S/(K_s+S)) \qquad (1)$$

where U_m = maximum specific growth rate, $time^{-1}$

S = concentration of growth limiting substrate in solution, mass/unit volume

K_s = half-velocity constant, substrate concentration mass/unit volume

U = specific growth rate $time^{-1}$

The overall bacterial growth rate can be written as:

$$r_g = u_m XS/(K_s+S) = -Yr_{su} \qquad (2)$$

where r_g = rate of bacterial growth, mass/unit volume

X = mass of microorganisms

Y = maximum yield measured during exponential growth. The ratio of the mass of cells formed to the mass of substrate consumed, mass/mass

By rearranging equation 2 the rate of substrate utilization (r_{su}) can be written in the terms of the growth rate resulting in the relationship:

$$r_{su} = -u_m XS/(Y(K_s+S)) \quad (3)$$

where r_{su} = substrate utilization rate, mass/unit volume

Using a more conventional notation where the term u_m/Y is replaced by k, which is the maximum rate of substrate utilization per unit mass of microorganisms, equation 3 becomes:

$$r_{su} = kXS/(K_s+S) \quad (4)$$

For the limiting case, when S is much greater than K_s, K_s can be neglected.This represents a zero-order reaction with respect to substrate. Equation 4 reduces to;

$$r_{su} = ds/dt = kX \quad (5)$$

For the limiting case, when S is much less than K_s, S can be neglected. This represents a first order reaction rate. Equation 4 then reduces to;

$$r_{su} = ds/dt = KxS \quad (6)$$

where $K = k/K_s$ = Specific substrate utilization rate constant.

The rate of substrate utilization can also be written as:

$$r_{su} = -(S_o-S)/(V/Q) \quad (7)$$

where V/Q = hydraulic retention time (HRT)

Q = flow rate

and using equations 1,3,7 the specific utilization rate can be defined as:

$$U = -(r_{su}/X_a) = (So-S)/(HRT)(X_a) \quad (8)$$

where X_a = average mass of microorganism

The equation commonly used to determine rate constants is derived by using a linear form of the two substrate utilization equations, equation 7 and 4, and is as follows:

$$X_a{*}HRT/(S_o-S) = (K_s/k)(1/S) + 1/k \quad (9)$$

where all terms are as previously defined (Metcalf and Eddy 1979).

With the use of a CFSTR it is possible to accurately determine the reaction constants involved in these relationships. This is done by operating the CFSTR at a number of mean cell residence times, while measuring the residual substrate concentration at steady state. This process is very time consuming in that at each residence time steady state must be achieved before a measurement can be made.

When batch reactors are used to evaluate reactions constants a number of factors must be taken into consideration because batch reactors do not operate under steady state conditions (Braha 1985,Philbrook 1986).

i) When determining the kinetics of removal of a single component from a multicomponent wastewater the unknown character of the metabolic control mechanisms that may be operative may interfere with the degradation the specific compound. An example of this is when bacterial cells undergo rapid growth conditions, the resultant high levels of ATP may trigger these metabolic controls. This may result in sequential substrate removal under batch conditions where concurrent removal occurs in a continuous flow wastewater treatment system.

ii) The batch reactor is always in a state of transition. The microbial mass increases in proportion to the amount of substrate utilized. This causes a condition in which the substrate removal rate accelerates with increase in the microbial population. An average value of the initial and final MLVSS has been used.

iii) The constantly changing substrate concentration experienced by the cells will prevent them from ever fully adapting to their growth environment.

The use of batch reactors persists, in light of these problems because they are less expensive, more expedient and there is no transition time between operating periods. Evaluation of reaction constants has been accomplished using single batch test while taking the above consideration into account (Hafner 1985).

At low chemical concentrations little or no biodegradation may occur and a threshold exists below which no significant mineralization occurs. One explanation is that energy is obtained too slowly from oxidation of the low substrate concentrations to meet the energy demands of the small population utilizing the compound. As a result the bacterial population is unable to proliferate and reach cell densities sufficient to cause significant chemical loss (Boethling 1979). Tests involving relatively high chemical concentrations would not predict this behavior.

SEQUENCING BATCH REACTORS

The enrichment reactors used in this study were set up as sequencing batch reactors, also known as semi-batch biological reactors and fill-and-draw batch reactors. Typical batch reactors, which have been extensively studied by Dennis and Irvine (1979), may be composed of one or more tanks. Each tank has five basic operating modes or periods; these are the fill mode, draw mode, react mode, settle mode, and idle mode.

1. The fill mode is when the raw waste is pumped into the reactor.
2. The draw mode is when treated effluent is removed from the reactor and occurs each cycle for a given tank.
3. The react mode is the contact time, between the activated sludge and influent, in which the desired reactions may go to completion. This occurs after the fill mode.
4. The settle mode is when the organisms are separated from the treated effluent.
5. The idle mode is when little or no reactions occur and takes place after discharge and before filling.

Periods 3,4 and 5 can be eliminated depending on the objective of the treatment. Figure 4 is a schematic of a typical batch reactor. A continuous flow of wastewater can be treated using multiple sequencing batch reactors operated in parallel. In this case the reactors fill in sequence with the stipulation that one reactor must have completed draw prior to another completing fill.

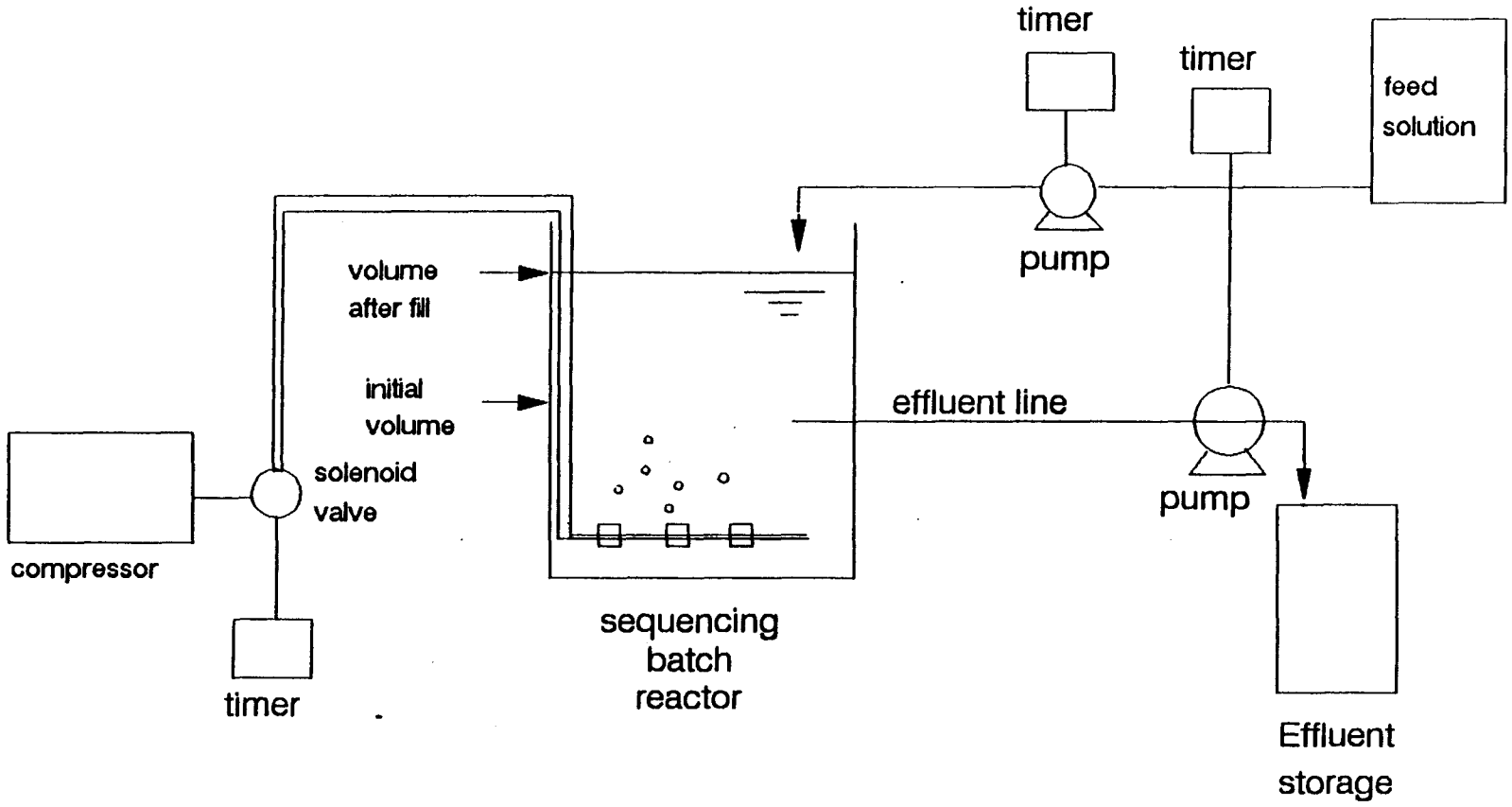

Fig. 4. Set up for sequencing batch reactor operation (Orhon et al. 1986, Irvine et al. 1979)

Organisms remain in each reactor until wasting is necessary. The time period between the wasting of the activated sludge may range from bimonthly in a low-yield single-tank system to once each cycle in a high-yield multiple tank system. Solids wasting is done after the settle mode or during the react mode.

There is not a steady relationship between sludge wasting and MLVSS. Hoepker (1979) found that there was variation of the MLVSS during a single cycle because of endogenous respiration. Thus the MLVSS values reported at any time can only be used in a qualitative sense when compared with other systems.

Due to the non steady state condition of these reactors any relationship which is developed between growth rate and mean cell residence time from sequencing batch reactor data is invalid for continuous flow stirred tank reactors at steady state.

By controlling the aeration, each tank can operate as both a biological reactor and a clarifier. Aeration control is also used to enhance the settling characteristics of the activated sludge. Irvine (1979) has shown that the growth of filamentous organisms in the system is readily controlled by anoxic conditions during the fill period. The anaerobic fill period suppresses filamentous growth because of the anaerobiosis and the higher organic concentration when aeration is started.

By varying the length of the fill and react period the system can be made to model closely a CFSTR or a plug flow reactor (Dennis et al. 1979). The mass balance of substrate in a sequencing batch reactor is:

$$d(VC_s)/dt = qC_s(0) + Vr_{su} \qquad (10)$$

Where $C_s(0)$, the initial concentration, is known

V = volume of substrate, l

C_s = substrate concentration, mg/l

q = influent flow rate l/d,

r_{su} = rate of utilization of substrate, mg/l.d

Differentiating equation 10, rearranging and letting dV/dt = q gives

$$dC_s/dt = (q/V)(C_s(0)-C_s)+r_{su} \quad (11)$$

This is identical in form to an unsteady-state mass balance on a continuously stirred tank reactor with recycle. The volume however, is a function of time. During the react period, q is equal to zero, and the volume is constant. The mass balance reduces to:

$$dC_s/dt = r_{su} \quad (12)$$

This is identical to a steady-state mass balance on a plug-flow reactor. Thus, as the length of the fill period increases and the length of the react period decreases, the treatment in the reactor appears to be similar to a CFSTR with varying liquid volume. As the length of the fill time decreases, the reactor more closely resembles a plug-flow reactor.

Sequencing batch reactors are very versatile and efficient. By modifying the various modes to accommodate a variety of conditions, a properly designed

VOLATILIZATION

The two film model used to estimate oxygen transfer can also be used to estimate volatilization losses of trace organics (Roberts 1984). The volumetric mass transfer coefficient and equilibrium dissolved oxygen concentration are estimated by fitting the concentration (DO) versus the time data to the two film model (Stenstrom et al.1981):

$$\frac{dC}{dt} = K_L a(C^* - C) \tag{13}$$

where

C = dissolved concentration in mg/l

C^* = saturation DO in mg/l at equilibrium

$K_L a$ = volumetric oxygen transfer coefficient per unit time (t^{-1})

t = time

The materials balance equations for a single volatile species C, which has a first order rate of degradation is:

$$\frac{dC_g}{dt} = \frac{Q_g(C_{go} - C_g)}{V_g} - K_L a_C\,(C^*_{g-} - C_l)\,\frac{V_l}{V_g} \tag{14}$$

$$\frac{dC_l}{dt} = Q_l\,\frac{(C_o - C_l)}{V_l} + K_L a_C\,(C^*_{g-} - C_l) - KC_l \tag{15}$$

The decay parameter, K, is shown as a first-order reaction rate coefficient. In reference to the concentrations of the organic compound in solution this can be either first-order or zero-order. In reference to the concentration of active biomass, often measured as volatile suspended solids concentration, or MLVSS, this is always first-order. With a fixed MLVSS and low concentrations of organics in the reactors the decay parameter can be modeled as first-order for the volatilization analysis.

Roberts et. al (1984) have suggested that the proportional relationship between transfer rate constants and volatile solutes can be expressed as:

$$K_L a_C = \Psi K_L a_{02} \qquad (17)$$

where Ψ = transfer rate constant proportionality coefficient.

This relationship is based on the assumptions that the transfer rate constants for volatile solutes are proportional to one another. Here Ψ depends on the ratio of the liquid phase diffusivity of the compound and dissolved oxygen diffusivity (D_i/D_{O2}) and is approximately constant over a wide range of temperatures and mixing intensity (Roberts 1984). In clean water tests they have tabulated ranges of between 0.5 and 0.7 for a variety of 2 or 3 chlorinated organic solutes. Considering this a worst case for larger molecules, which should have lower diffusivity coefficients, this is conservative and provides an upper bound for stripping estimates. The maximum transfer rate is also further reduced by contaminants in wastewater, and is generally correlated by an empirical alpha factor, ranging from 0.2 to 0.8 (Stenstrom 1981). compound at any one time or increasing the idle mode creating anoxic conditions which

reduce filamentious growth are all modifications which can easily be made to enhance organic removal in a SBR, whereas the CFSTR does not have this type of versitility. Batch tests performed by Dennis (1979) showed a reduction of a five day BOD from 400 mg/l to 3 mg/l in a 24hr aeration period. Hoepker (1979) ran a batch system that could handle an influent BOD_5 up to 640 mg/m^3. Since it is difficult to overload a sequencing batch reactor with soluble substrate it is usually the sludge settling characteristics of the activated sludge, rather than the influent substrate concentrations, that controls the system design (Dennis et al. 1979).

EXPERIMENTAL METHODS

Four identical "Eckenfelder-type" continuous flow activated sludge reactors (Ng 1987) and three activated sludge batch fed reactors (Irvine et al. 1979) were operated in parallel and used as a source of microorganisms for the experiments. Three of the continuous flow reactors, and one of the batch reactors were fed toxic compounds as well as glucose/nutrient broth feed. The other two batch reactors were fed an influent naphthalene concentration from 0.16 - 0.32 mg/l and substrates which were either intermediates of naphthalene degradation and/or induced enzymes necessary for naphthalene degradation. The fourth continuous fed reactor was fed glucose/nutrient broth feed and used as a control.

Protocol of Study

Step 1. Acclimation of activated sludge:

- obtain activated sludge from waste water plants that are routinely exposed to refinery wastes, such as Hyperion and Chevron.
- continually feed the reactors the toxic compound being studied; gradually increase the amounts of toxic feed until the desired level of degradation is achieved.
- periodically seed the reactors with cells from waste water plants that are exposed to refinery wastes until acclimation is achieved.
- stabilize the activated sludge which is acclimated to a specific compound. This is done by operating the reactors at a constant

influent concentration, of the toxic compound, for a time period equal to a number of mean cell retention times, i.e. 1 to 3 months or 2 to 6 mean cell retention times.

Step 2. Biodegradation - initial observations

- using activated sludge acclimated to these compounds measure the utilization rates of the toxic compounds in question: i.e. 2,4 xylenol, isophorone, naphthalene
- measure the utilization rates of compounds which are related structurally to the toxic compounds: xylenol isomers, cresols, phenanthrene, etc.

Step 3. Enrichment reactor setup

- start up enrichment reactors with cells that are acclimated to the target compound, in this case naphthalene. Operate these reactors as batch reactors.
- feed enrichment reactor a predetermined substrate, such as salicylate or 2 aminobenzoic acid plus succinate, which will maintain the cell population capable of degrading naphthalene. Also feed 1/100th (i.e. influent concentrations of 0.16 - 0.32 mg/l) of the amount of naphthalene fed to the continuous feed reactors in a 24 hour period.
- allow these reactors to stabilize for at least a 2-3 month period.

Step 4. Biodegradation observations

- measure rate of removal of naphthalene in the enrichment reactors, the continuous feed reactors, and the control. Compare the results.

Step 5. Use biomass from enrichment reactors to enhance naphthalene removal in continuous feed reactors.

- maintain three continuously fed reactors in parallel; two reactors receive an influent containing low levels of naphthalene as well as the glucose/nutrient broth feed, the third receives influent containing only glucose/nutrient broth. Periodically seed one of the reactors, which is receiving naphthalene, with cells from an enrichment reactors.
- allow the reactors to stabilize under these conditions for 1-2 months.
- measure the rate of naphthalene utilization in all three continuously fed reactors and compare.
- to determine the effect of the low levels of naphthalene influent on naphthalene utilization in the CFSTRs repeat the above experiment, with the only change being that none of the continuously fed reactors receive naphthalene for a period of 1-2 months.

Step 6. Measure the removal of phenanthrene by activated sludge from the batch reactor fed salicylate.

Reactor design and operation

Continuous Flow Activated Sludge Reactors

Reactors and associated equipment

The four continuous flow reactors were constructed of 0.5" plexiglass (Figure 5). Each reactor had a working volume of 12.2 liters in the aeration section and 1.5 liters in the solids-liquid separator section. The two sections were separated by a sliding baffle. Access for the addition of feed, caustic, toxic compounds, air, and pH probes was through holes in the cover of the reactors. The tubing for the ventilation system employed was also attached to a hole in the lid. A hole on the side of the aeration section was used to withdraw mixed liquor.

Air was added through diffuser stones located at the bottom of the mixed liquor aeration section and provided oxygen for the microbial activity as well as turbulence for the mixing process. The air flow rates ranged form 7.9 x 10^{-5} to 11.8 x 10^{-5} m^3/s and were monitored independently in each reactor by routing the air through a rotometer prior to its introduction into the mixed liquor. The dissolved oxygen concentration in the mixed liquor was always above a level that would be expected to limit the growth of the heterotrophs (i.e. greater than 3 mg/l).

The pH was maintained independently in each reactor within a range of 7.0 to 8.0 by the use of four pH control units manufactured by Horizon Ecology Co. (Model 59997-20). Each pH control system consisted of a combination pH

electrode (Orion model 34), in direct contact with the mixed liquor, and a pH control unit equipped with set point dials to control the action of the base pump. When the pH would fall below pH 7 the base pump was actuated, delivering a solution of Soda Ash (106 g/l Na_2CO_3) to the aeration section until the pH reached 7.0.

Feed Dilution System

In this system a concentrated feed was automatically diluted before being pumped into the reactors. The concentrated feed and the mixing reservoir was contained in a refrigerator at 5-10^{o}C. A schematic of the dilution system is shown in Figure 5.

The liquid level in the mixing reservoir was electronically sensed by two float switches which controlled both the concentrate feed pumps to the reservoir and an external solenoid valve for the flow of dilution tap water.

The diluted substrate was pumped directly from the mixing reservoir into the reactors using a separate pump system. For the first set of experiments a feed solution consisting mainly of glucose as the source of energy was used. For the second set of experiments a feed solution consisting of glucose and nutrient broth was used as a source of energy for the microbes. The feed was adjusted to reduce bulking during the enrichment reactor experiments. The composition of the feed solutions are shown in Table 2,3,4, and 5. In both

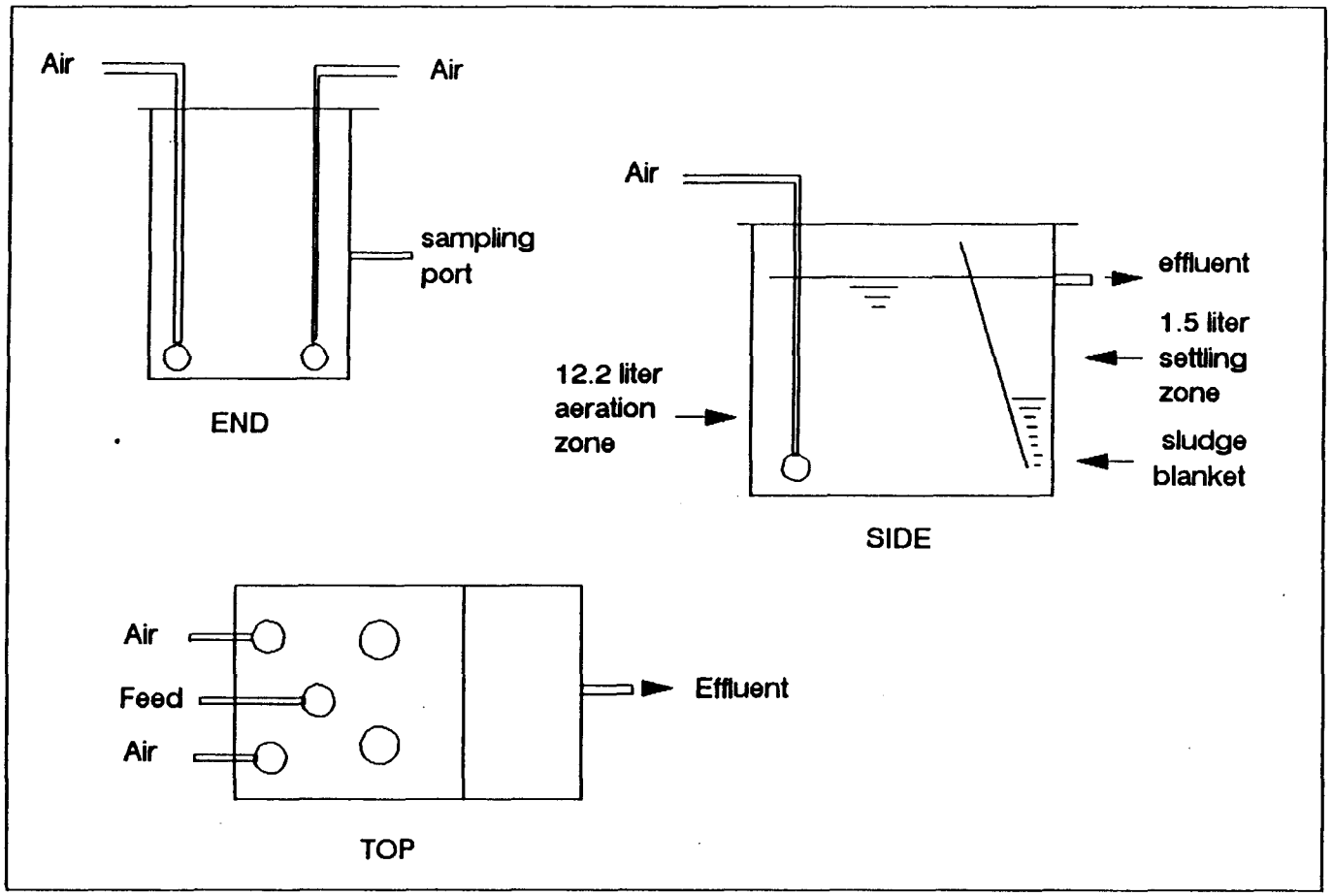

Fig. 5. Schematic of Reactor and Associated Apparatus (Ng et al. 1987)

cases the $CaCl_2$-$MgCl_2$ solution was separately pumped into the reservoir at each dilution cycle. This was done to prevent the formation of calcium phosphate precipitates in the concentrate.

The concentrated feed was diluted approximately 415 times and the $CaCl_2$-$MgCl_2$ solution was diluted approximately 5763 times during each cycle. The resulting influent for each feed is shown on Tables 6 and 7.

The semi-toxic compounds were pumped into the reactors from a separate system of pumps, feed lines and reservoirs. Concentrated solutions of Isophorone and 2,4 xylenol in water were pumped into the reactors at such a rate as to give a specific overall influent concentration. Due to the low solubility of naphthalene concentrated solutions of naphthalene in methanol were pumped into the reactors at timed intervals. Small quantities of very concentrated solutions of naphthalene were used to minimized the amount of methanol being feed to the reactors.

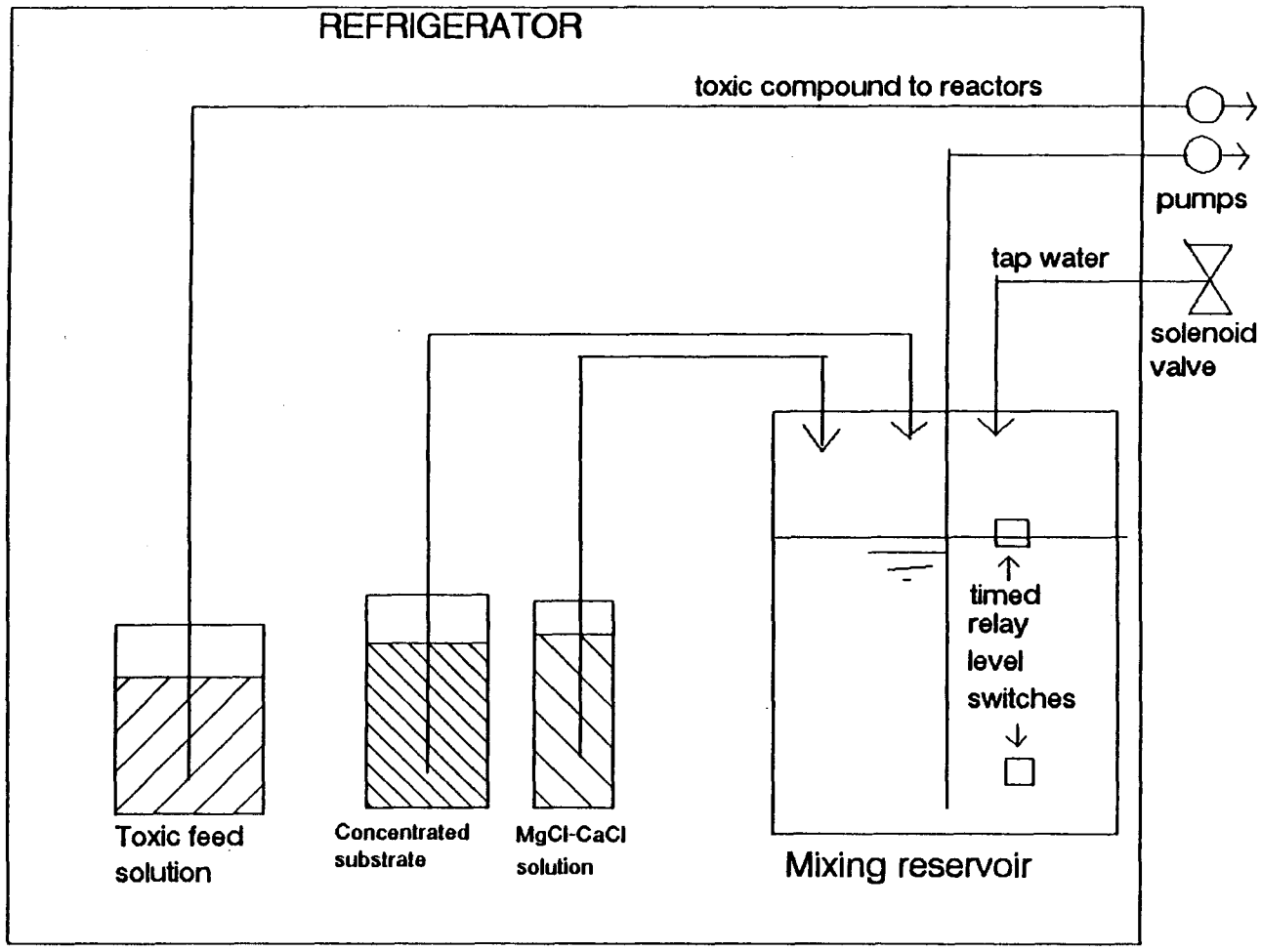

fig. 6 Schematic of feed dilution system.

Table 2. Calcium/Magnesium solution for feed

H_20	200ml
$CaCl_2 \cdot 2H_2O$	5.26g
$MgCl_2 \cdot 6H_2O$	8.20g

Table 3. Trace mineral solution used in concentrated feed

H_2O	500ml
$FeCl_3$	19.50g
$MnCl_2 \cdot 4H_2O$	4.75g
$ZnCl_2$	3.30g
$CuCl_2 \cdot 2H_2O$	2.05g
$CoCl_2 \cdot 6H_2O$	2.90g
$(NH_4)Mo_7O_{24} \cdot 4H_2O$	2.10g
Na_3 Citrate	176.50g
$Na_2B_4O_7 \cdot 10H_2O$	1.20g

Table 4. Concentrated glucose feed composition

H_2O	1000ml
Trace mineral soln	2ml
K_2HPO_4	25.00g
Yeast extract	10.00g
Glucose	103.50g
NH_4Cl	45.00g
$(NH_4)_2SO_4$	10.00g

Table 5. Concentrated glucose nutrient broth composition

H_2O	1000ml
Trace mineral soln	2ml
K_2HPO_4	25.00g
Yeast extract	5.00g
Glucose	53.50g
Beef extract	18.75g
Bacto Peptone	31.25g
$(NH_4)_2SO_4$	10.00g

Table 6. Influent feed concentration with glucose feed

$(NH_4)2{\cdot}H_2O$	24.11 mg/l
Glucose	249.60 mg/l
K_2HPO_4	60.29 mg/l
NH_4Cl	108.50 mg/l
Yeast extract	24.17 mg/l
$CaCl_2$	3.99 mg/l
$MgCl_2{\cdot}6H_2O$	7.11 mg/l
$FeCl_3$	0.094 mg/l
$MnCl_2{\cdot}4H_2O$	0.023 mg/l
$ZnCl_2$	0.016 mg/l
$CuCl_2.2H_2O$	0.0099 mg/l
$CoCl_2.6H_2O$	0.0139 mg/l
$(NH_4)Mo_7O_{24}.4H_2O$	0.01 mg/l
Na_3 Citrate	0.85 mg/l
$Na_2B_3O_7.10H_2O$	0.0058 mg/l

Reactor Operation

The reactors were initially seeded with activated sludge from Hyperion Treatment Plant in Playa Del Rey, CA. To achieve acclimation the reactors were then fed increasing amounts of the toxic compound and periodically seeded with activated sludge from treatment plants that received refinery wastes. Once the activated sludge was acclimated, the reactors were allowed to run at steady state for one to three months before batch testing was done. The final operating parameters are given in Table 7.

Table 7. The operating parameters of the continuous flow reactors.

MCRT	13.8 days
HRT	13.8 hrs
MLVSS	1.5-2.0 g/l
COD(glucose)	253 mg/l
COD(nutrient)	290mg/l
pH	7-8
DO	>3.0 mg/l
Q	24 L/day
F/M	0.22 mg/mg

Sequencing Batch Reactors

Reactors and associated equipment

Three activated sludge sequencing batch reactors were operated simultaneously and used as a source of microorganisms for the experiments. One reactor was fed a succinic acid- anthranilic acid feed, one was fed a salicylic acid feed and one was fed a glucose feed along with high concentrations of naphthalene.

The reactors were constructed of 0.25" plexiglass. Each reactor had a working volume of 5.0 liters. Access for the addition of feed, caustic, toxic compounds, air and pH probes was through holes in the cover of the reactors. A hole on the side of the reactor was used to withdraw mixed liquor.

Air was added through a single diffuser stone located at the bottom of the reactor and provided oxygen for the microbial activity as well as turbulence for the mixing process. The air flow rates ranged from 0.28 to 0.42 m^3/hr and were monitored independently in each reactor by the use of rotometers. The dissolved oxygen was maintained at a level above that expected to limit aerobic heterotrophs.

Feed Addition

In this system concentrated feed was manually added on a daily bases. Enough feed was added to maintain a F/M (food:microorganism) ratio of 0.2-0.3 mg/mg. In the reactor maintained on salicylate the influent concentration of salicylate was 571 mg/l. In the reactor maintained on 2 aminobenzoic acid and succinate the influent concentrations of 2 aminobenzoic and succinic acid

were 210 mg/l and 420 mg/l, respectively. The composition of the feed which was added to each reactor is given in Table 8 and 9.

Table 8. Concentrated succinate/2-aminobenzoic acid feed composition

H_2O	1000ml
Trace mineral soln	2ml
K_2HPO_4	25.00g
Yeast extract	10.00g
2-aminobenzoic acid	35.00g
Succinate	70.00g
$(NH_4)_2SO_4$	20.00g

Table 9. Concentrated salicylate feed composition

H_2O	1000ml
Trace mineral soln	2ml
K_2HPO_4	25.00g
Yeast extract	10.00g
Salicylate	100.00g
$(NH_4)_2SO_4$	20.00g

Reactor Operation

The batch reactors were started up with activated sludge from continuous flow reactors which were acclimated to naphthalene. For about two weeks after start-up the batch reactors were also seeded daily with 100 ml of activated sludge from the acclimated continuous flow reactors to ensure the presence of a stable naphthalene degrading microbial population. They were then operated for a minimum of one month, receiving only the substrate and a small amount of naphthalene (0.04 mg/l) before testing began. The final operating parameters can be found in Table 10.

Table 10. The operating parameters for the batch reactors.

HRT	1.43 days
MLVSS	1.5-2.0 g/l
pH	6-8
DO	>3 mg/l
COD(succinate,anthranilic acid)	1176mg/l
COD(salicylic acid)	1438 mg/l
F/M	0.2-0.3 mg/mg
Settling period	1 hr
Draw period	5 min
Fill period	5 min
React period	22 hrs 50 min
MCRT(succinate, anthranilic)	13.9 days
MCRT(salicylate)	28 days

Enrichment Reactor System Operation

Once the sequencing batch reactor maintained on salicylate was established and showed good removal of naphthalene after of 3 months of operation, it was then operated as an enrichment reactor in an enrichment reactor system. The enrichment reactor system consisted of a continuous feed reactor which is periodically inoculated with activated sludge form a sequencing batch reactor enriched in microbes capable of degrading a specific compound.

Three of the continuous feed reactors were re-established using activated sludge from hyperion, one test reactor and two controls. All three reactors had the same operating parameters and were fed a glucose/nutrient broth feed. One test continuous feed reactor and one of the control continuous feed reactor were fed an influent of 0.0057 mg/l naphthalene. After 6 weeks of operation the test reactor was seeded, daily, with 300 ml of waste activated sludge from the enrichment reactor fed salicylate. The enrichment reactor had a MLVSS concentration of approximately 3 gm/l. After one month of seeding the rates of removal of naphthalene for the test reactor and the controls was measured and compared.

Reactor Maintenance

The following steps were taken to ensure the integrity of the system throughout the experimental period.

On a weekly basis:

- The lines were cleaned with a dilute solution of bleach followed by a rinsing of at least two liters of water. This prevented erratic

flow rates and the depletion of the feed solution from the presence microbial growth in the feed lines.

- The tank was washed out to remove all apparent growth.
- The over flow lines were cleaned. This prevented blockage and flooding.
- The flow rates on all feed pumps were measured and adjusted.
- The feed bottles were cleaned.
- The pH probes were cleaned with 0.1N HCl solution. This greatly extended the life of the pH probes.
- A MLVSS was done on all reactors.

Other maintenance included:

- The cylinders containing soda ash solution were washed out once every two weeks.
- The pH meters were calibrated daily.
- The air flow was checked and monitored daily.
- The walls or the reactors cleaned to remove fixed growth daily.

Experimental Procedures

Batch Assays

Batch assays to determine biodegradation rates of isophorone, xylenol, trimethylphenol, and cresol were performed with samples of activated sludge in 150 ml bottles. The procedure is as follows:

i. For each data point 100 ml aliquots were collected from the experimental and control reactors, and placed in separate bottles.

ii. Each bottle was then centrifuged and the supernatant discarded.

iii. One hundred ml of an aqueous solution containing appropriate amounts of the compound being tested and glucose feed solution was added to each bottle.

iv. The bottles were then incubated and aerated for the appropriate time intervals.

v. At the end of an incubation period the reaction was stopped by the addition one drop (0.1ml) of 1+1 H_2SO_4 solution in H_2O.

vii. The final volume was then measured and in some cases adjusted. The sample was then refrigerated until the cells could be separated form the supernatant by centrifugation.

viii. The cells and/or supernatant were then extracted.

For the batch assays in which 2,4 xylenol, 2,3 xylenol and o-cresol were tested for mineralization alone and combined in a mixture, single containers of activated sludge were used. For each compound tested, two flasks or beakers were incubated with the appropriate amount of toxic compound and feed solution, one containing acclimated activated sludge and one containing unacclimated sludge. At periodic intervals 25 ml of sample were removed from the beaker or flasks, acidified and immediately extracted.

Batch assays to determine the degradation rate of naphthalene were done in 60 ml serum bottles. The procedure is as follows:

i. For each analysis 30 ml aliquots were collected from the experimental and control reactors and placed in 60 ml serum bottles.

ii. Each bottle was then centrifuged and the supernatant discarded.

iii. Thirty ml of a aqueous solution containing only naphthalene and a phosphate buffer solution (to maintain the ph at 7.0-7.3) was added to each bottle.

iv. The bottles were immediately sealed and placed on a shaker table for the duration of the timed test.

v. At the end of the incubation period the bottles were centrifuged.

vi. With a long needle, 5 ml of supernatant was removed from the bottles and run though Bond Elut (C8), eluted with acetylnitrile and measured using the spectrophotometer and the gas chromatograph.

vii. Cell extractions were done at various intervals.

Analytical Procedures

Extraction Techniques

Xylenols, cresols, and trimethylphenol were extracted using bonded phase silica sorbents. CH (cyclohexyl) bonded phase sorbent (Bond Elute from Analytichem International, Harbor City, CA) was used in this project. To prepare the sample for extraction, the pH was adjusted to 1-2 and 5% wt/vol of NaCl was added to enhance recovery. The procedure used for the extraction itself is the same as that previously described by Chaldek (1984).

Naphthalene and phenanthrene were extracted using the bonded phase silica sorbents C8 (octyl). The use of a buffer in the feed solution maintained the pH of the sample at 6.5-7.5 which was required for a neutral extraction (Chaldek 1984). The elution solvent for naphthalene, xylenols, and cresols was acetylnitrile. The elution solvent for phenanthrene was methanol which resulted in a sample which was more compatible with HPLC analysis.

Isophorone was concentrated using liquid/liquid extraction (US EPA, 1979). Three successive extraction using 5 ml of dichloromethane to 25 ml sample were made. Excess water remaining in the dichloromethane fraction after extraction was removed using anhydrous sodium sulfate. The solvent was then filtered with a 45 um filter and its volume reduced using roto-evaporator.

Isophorone and Xylenols which were adsorbed onto the surface of the suspended solids in the activated sludge were extracted using a modified method from Warner (1980). 100 ml aliquots were removed from each reactor and centrifuged. After removing the supernatant, 1 ml of distilled water was

added to the centrifugation vial to facilitate cell removal. Using a syringe 5 ml of cells were removed and placed in a screw top test tube. The cells were then extracted with three successive portions of dichloromethane. Each extraction involved shaking for one minute followed by a three minute centrifugation. The dichloromethane layer was removed from the bottom of the test tube using a syringe with a long needle. The extract was dried and concentrated by using the same methods as discussed in the liquid/ liquid extraction for Isophorone.

Naphthalene and other polyaromatic hydrocarbons adsorbed onto the surface of suspended solids in the activated sludge were extracted using a modified lipid extraction method taken from Bligh and Dyer (1959). In this procedure the volumes of chloroform, methanol, and water, before and after dilution, were kept in the proportions 1:2:0.8 and 2:2:1.8, respectively. The activated sludge was considered to be approximately 100% water. The procedure is as follows:

i. The activated sludge was centrifuged and the supernatant discarded.

ii. Chloroform and methanol, were added to the cells so that the proportions of the three components were 1:2:0.8 respectively. The mixture was homogenize for 2 min.

iii. Chloroform was added and the mixture was then homogenized for 30 sec. Then water was added and the mixture was homogenize for another 30 sec. The final ratio of chloroform, methanol and water was 2:2:1.8 respectively.

vi. The mixture was then centrifuged and the volume of the chloroform layer measured. The rotovap was used to reduce the volume if necessary.

vii. Measurements were made using the spectrophotometer and gas chromatograph.

Detection Techniques

Gas Chromatography

The gas chromatography was done on a Varian Vista 6000. A fused silica Supelcowax 10 capillary column was used for isophorone, xylenol and trimethylphenols . For Naphthalene a fused silica Altech RSL 200 capillary column was used. The temperature programs are as follows:

Compound	Xylenol/Cresol	Isophorone	Naphthalene
Injector	250°C	250°C	250°C
Detector	280°C	280°C	300°C
Initial temp.	55°C	90°C	56°C
Hold	1 min	1 min	6 min
Delta T	10°C/min	8°C/min	20°C/min
Final Temp	200°C	170°C	130°C

Naphthalene was also successfully analyzed on the gas chromatograph using an isothermal program. All the parameters are the same as above except that the oven temperature was set at 120°C for the entire run. This method produced a more consistently reproducible peak area than the method using the temperature program.

Spectrophotometric Methods

Naphthalene was also quantified in acetylnitrile using a spectrophotometer which could measure adsorption in the UV range. Naphthalene adsorbed at both 276nm and 222nm. At 276nm a linear response was observed for concentrations ranging from 1.5 to 35 mg/l. At 222 nm a linear response was observed for concentrations ranging from 0.05 to 1.5 mg/l.

HPLC Methods

Quantitative analysis of phenanthrene was done using a HPLC with a 250mm x 4mm reverse phase column (BIO-SIL ODS-55). Runs were done under isocratic conditions at a flow rate of 1.0 ml/min. The mobile phase consisted of a 1:10 water:methanol solution. The detector on the HPLC was an ultraviolet spectrophotometer set at 254nm. Under these conditions a linear response was observed for the range of phenanthrene concentrations from .002mg/l to 2.0 mg/l.

RESULTS

Acclimation of Activated Sludge

In three separate experiments, activated sludge continuously exposed to the compounds isophorone, 2,4 xylenol, or naphthalene became acclimated to these compounds in a one to three month period (Table 11). Activated sludge from wastewater plants that handle a large amount of oil refinery wastes was periodically added until acclimation was achieved. The acclimated activated sludge was then run at a steady state influent concentration for at least one month to develope a stable microbial population.

Table 11. Acclimation of activated sludge to specific toxic compounds.

Compound	Beginning Conc. (mg/l)	Highest Conc. (mg/l)	Stabilization Conc. (mg/l)	Time (mos.)
Isophorone	0.952	117.73	75.3	2-3
2,4 Xylenol	0.49	132.70	96-100	1-2
naphthalene*	0.0656	26.70	26.70	3-4

The results of tests for adsorption of the compound to the activated sludge (Table 12) added further evidence to the belief that the primary mechanism of removal was biodegradation.

Table 12. Adsorption of the specific toxic compounds to the surface of the activated sludge.

Compound	Abs.(mg/g AS)	Initial Conc.	% Adsorbed
2,4 xylenol	0.710	99.62 mg/l	0.7
naphthalene	0.58	18.69 mg/l	3.0
isophorone	ND	100 mg/l	0.0
phenanthrene	0.039,0.037	0.4525 mg/l	8.4

Using gas chromatography with a FID detector and using a GC/MS it was shown that in the continuous flow reactors the effluent concentrations of these compounds were reduced to ug/l concentrations (Table 13) and no stable intermediates of the degradation pathway remained.

Table 13. Removal of the specific toxic compounds from acclimated activated sludge.

Compound	Influent (mg/l)	Effluent (ug/l)
Isophorone	85.0	< 1.72
2,4 Xylenol	53.3	< 8.97
Naphthalene	26.7	< 0.0714

Removal of Isophorone

In time series batch tests, activated sludge acclimated to isophorone reduced a feed solution containing 67.8 mg/l isophorone to non-detectable levels in 4 hours (Figure 7). The error bars shown represent the absolute concentration difference measured in replicate extractions performed in the development of the analytical procedure for each compound (see Appendix I). The appearance and disappearance of the tentatively identified intermediate 5,5 dimethyl-1,3 cyclohexanedione was found to coincided with the removal of isophorone (Figure 8).

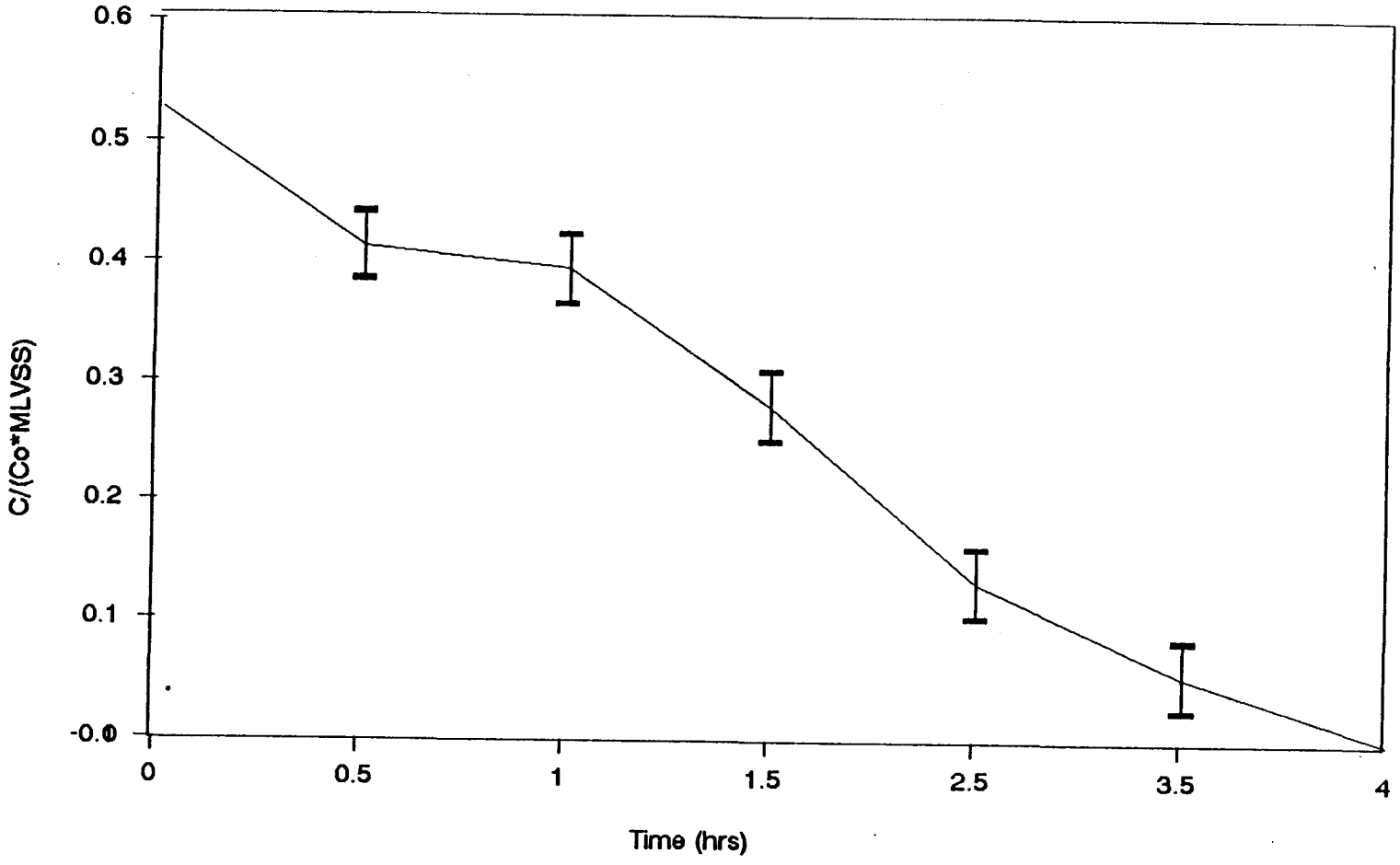

Fig. 7. Degradation of isophorone in acclimated activated sludge.

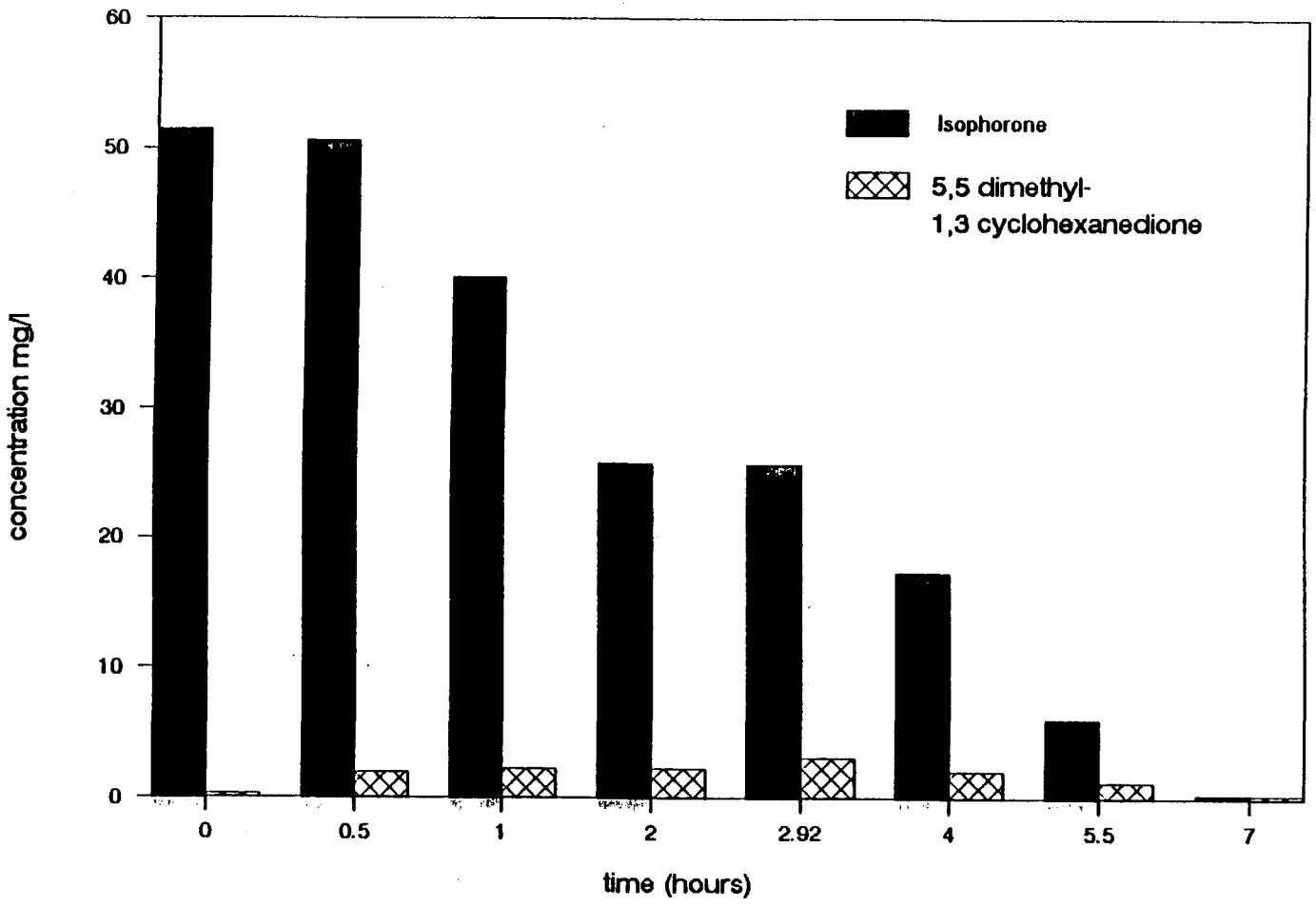

Fig. 8. Degradation of isophorone and the simultaneous formation and removal of an intermediate of the degradation pahtway.

Removal of Xylenols, Cresols and Thrimethylphenol

In time series batch tests, activated sludge acclimated to 2,4 xylenol reduced a feed solution containing 90.3 mg/l 2,4 xylenol to non-detectable levels in 2 hours (Figure 9). Controls which used unacclimated activated sludge showed very little removal during this time period. The appearance and disappearance of the tentatively identified intermediate, 2-methoxy-4-methyl phenol coincided with the appearance and removal of xylenol (Figure 10).

Figure 11 shows that the reaction rate, while zero-order with respect to concentration, at high substrate concentrations is most likely first order with respect to MLVSS concentration for the degradation of 2,4 xylenol. This graph shows the concentration of 2,4 xylenol after approximately one hour of incubation divided by its' initial concentration of 90.3 mg/l 2,4 xylenol at different % MLVSS. Using 2,4 xylenol acclimated activated sludge at 2.1 g/l MLVSS approximately 64% of the 2,4 xylenol was destroyed after 1 hour and at 1.05 g/l MLVSS approximately 28% was destroyed.

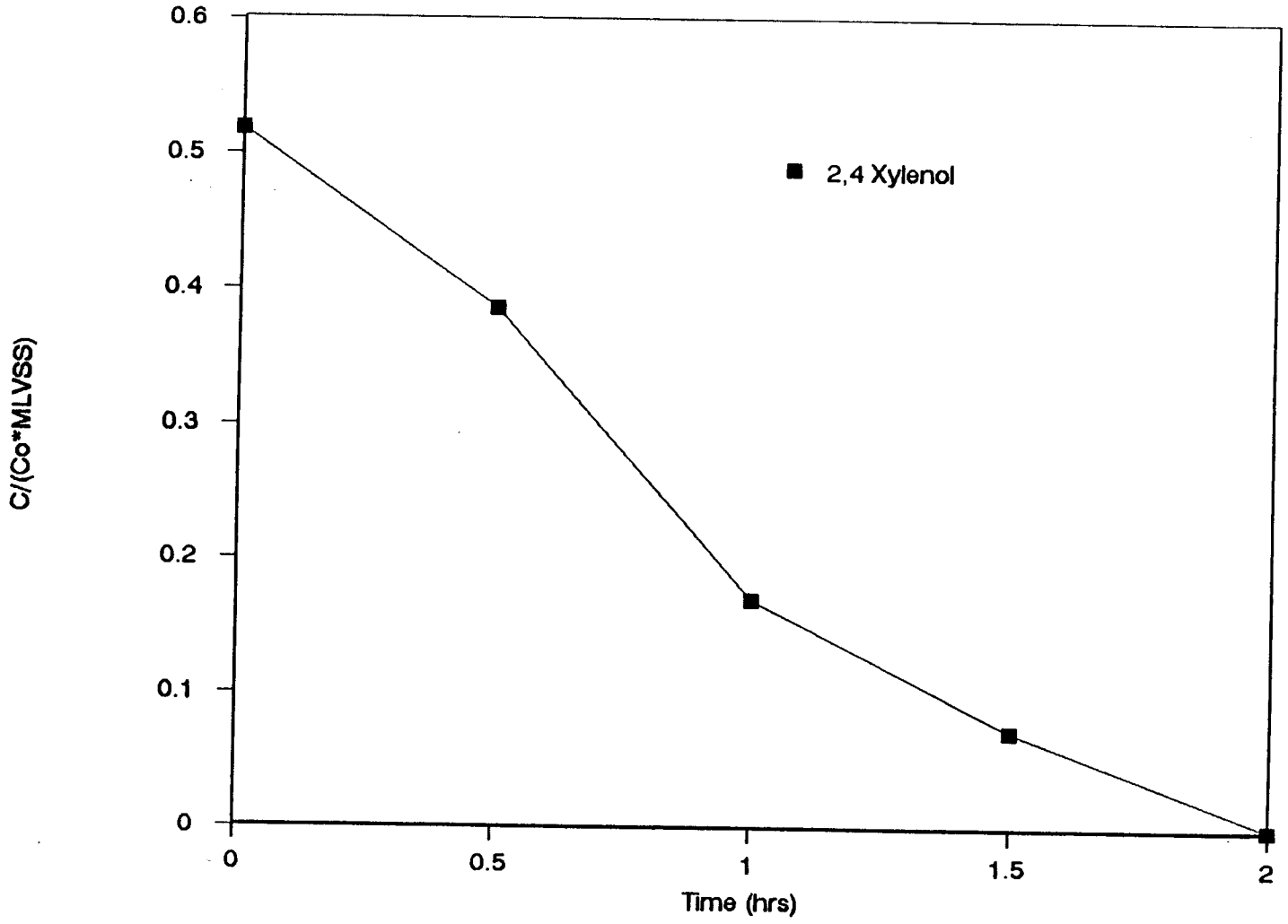

Fig. 9. Degradation of 2,4 xylenol in acclimated activated sludge

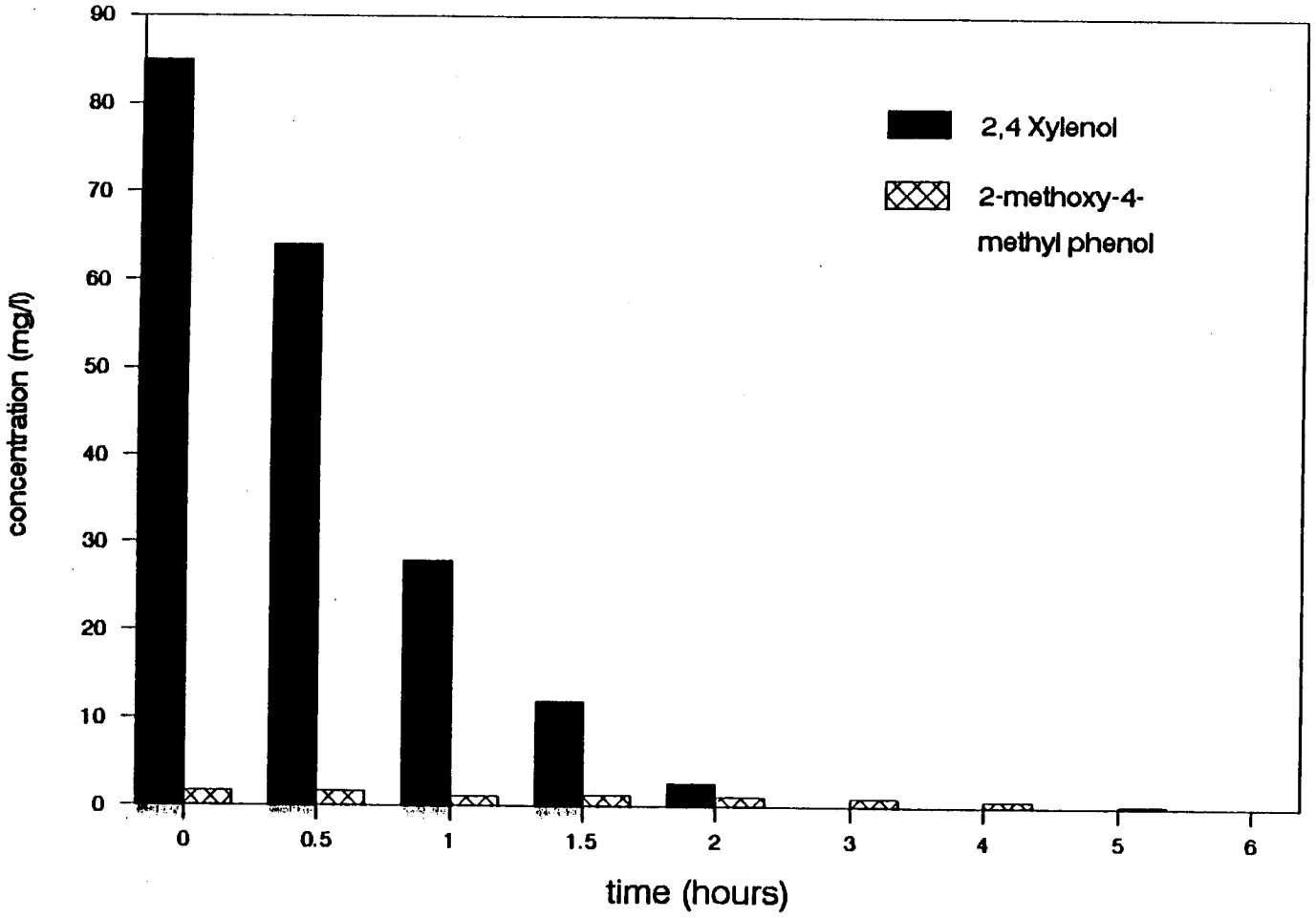

Fig. 10. Degradation of 2,4 xylenol with the simultaneous appearance and removal of an intermediate of the degradation pathway.

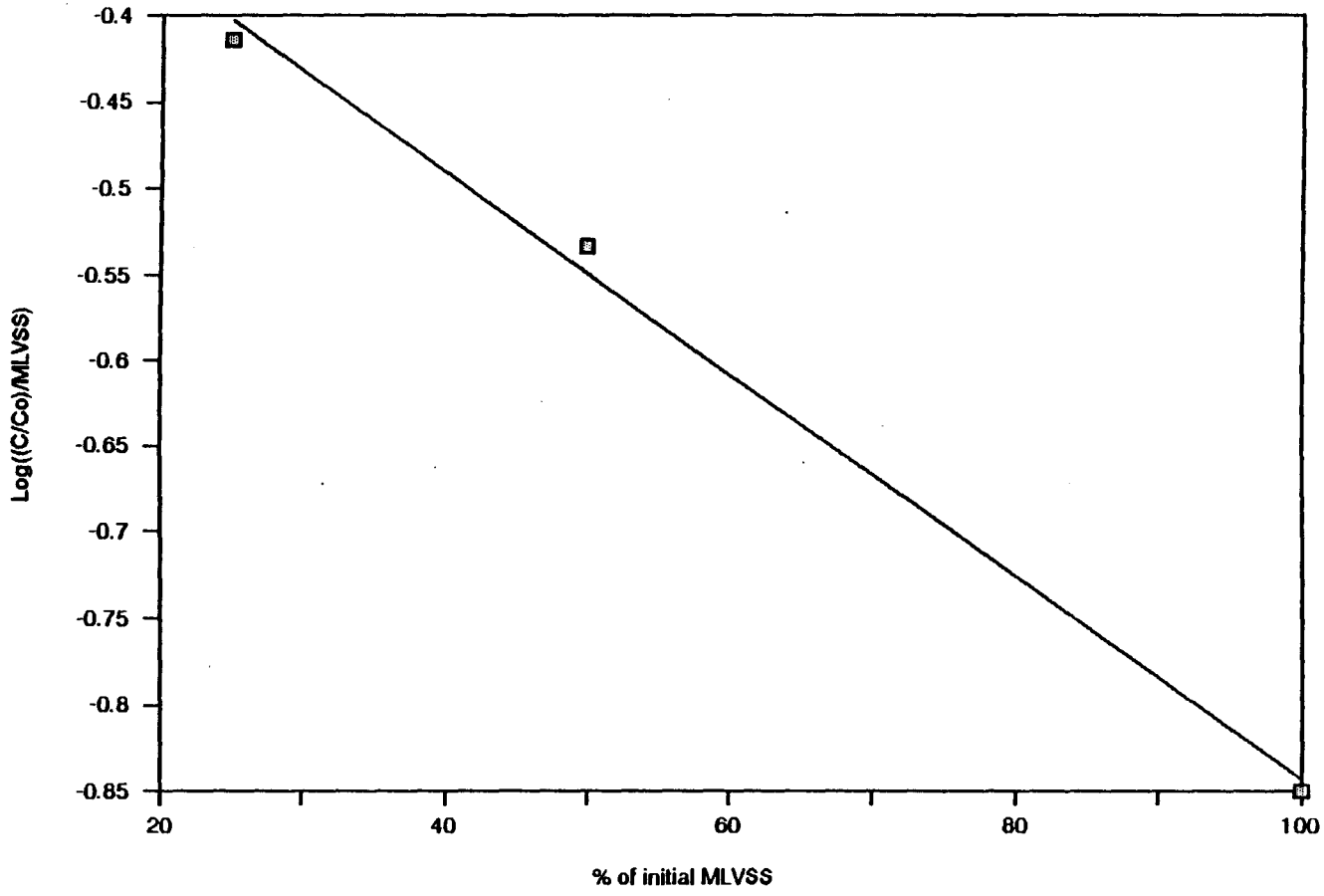

Fig. 11. Degradation of 2,4 xylenol in one hour at different MLVSS concentrations.

Activated sludge acclimated to 2,4 xylenol was tested with isomers of xylenol, cresol, and 2,4,6-trimethylphenol. A summary of the data can be seen in Table 14 and Figure 12. All isomers of xylenol which have methyl groups in the 2 or 4 position, and all isomers of cresol and 2,4,6 trimethylphenol were degraded by the acclimated activated sludge. 2,6 xylenol, o-cresol, and m-cresol degraded the slowest and 3,5 xylenol did not biodegrade in any batch assay experiments, even those which lasted 14 hours.

Table 14. Utilization Rates for xylenol isomers,cresols and 2,4,6 trimethylphenol by activated sludge capable of degrading 2,4 xylenol to CO_2 and H_2O.

Compound	(Co-C)/(MLVSS*T) (mg/gm·hr) Minimum	Maximum
2,4 xylenol	14.77	15.37
2,3 xylenol	1.07	1.74
2,5 xylenol	0.78	1.93
3,5 xylenol	0.0	0.0
2,6 xylenol	0.09	0.54
3,4 xylenol	0.99	4.16
2,4,6 tri-methyl phenol	0.54	0.64
m-cresol	0.05	1.15
o-cresol	0.13	0.72
p-cresol	0.74	3.70

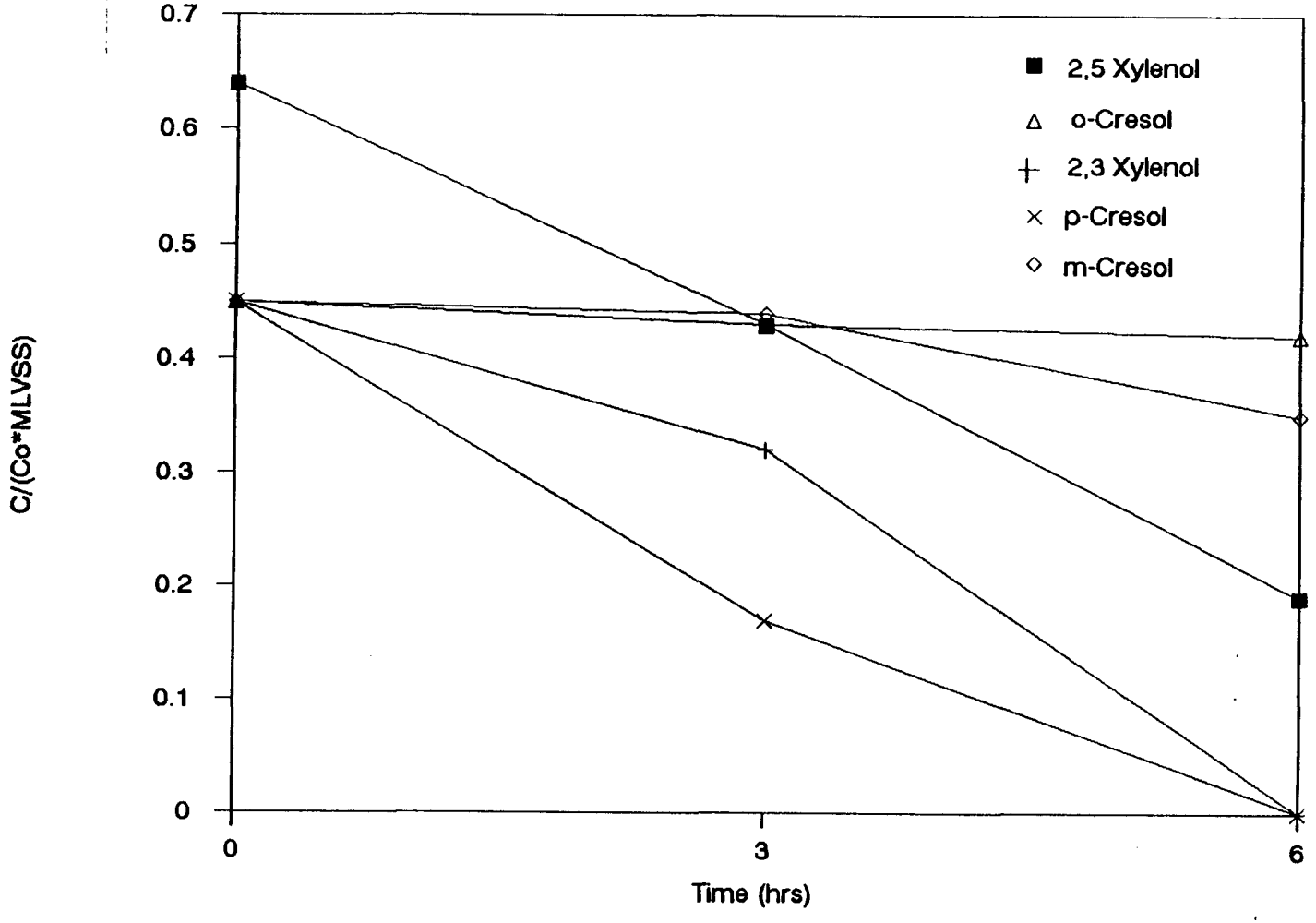

Fig. 12. Degradation of xylenols and cresols in activated sludge acclimated to 2,4 xylenol.

The compounds 2,4 xylenol, 2,3 xylenol and o-cresol were tested for biodegradability in a mixture using activated sludge that was previously acclimated to 2,4 xylenol (Figure 13). As would be expected in a batch assay 2,4 xylenol disappears first after which 2,3 xylenol and o-cresol begin to degrade.

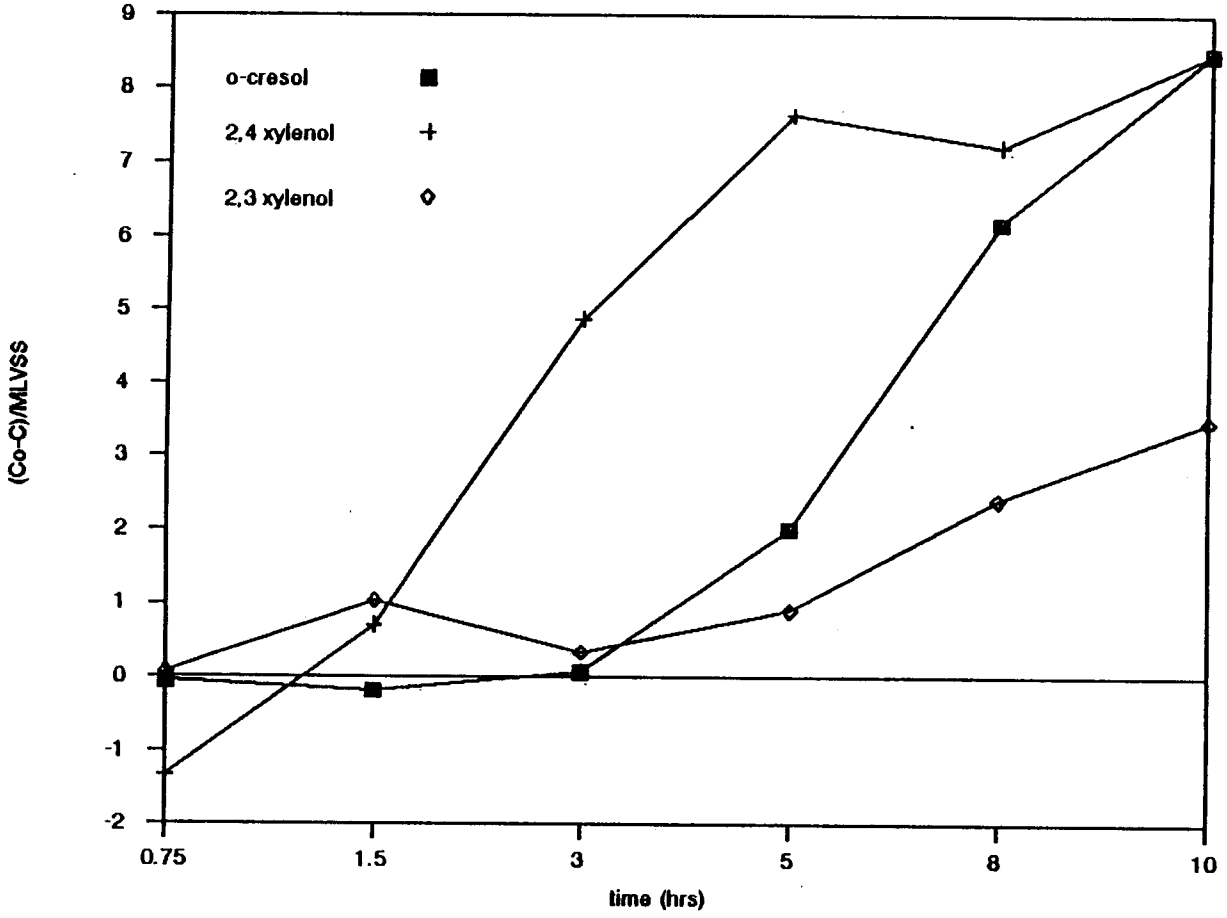

Fig. 13. Removal of a mixture of 2,4 xylenol, 2,3 xylenol, and o-cresol in activated sludge acclimated to 2,4 xylenol.

Removal of Naphthalene

Continuously Fed Reactors

In time series batch tests activated sludge acclimated to naphthalene reduced a feed solution containing 14 mg/l naphthalene to non-detectable levels in less than 1.8 hours (Figure 14). Error bars represent absolute concentration difference as in the case of figure 7. Controls using unacclimated activated sludge and run under the same experimental conditions showed an average removal of only 2 percent during the period of incubation.

The reaction rate for naphthalene was also found to be first order with respect to MLVSS concentration (Figure 15). The initial concentration of naphthalene in the feed solution was 21 mg/l. Using activated sludge acclimated to naphthalene at 2.24 g/l MLVSS approximately 75% of the naphthalene was destroyed after 1.167 hrs; at 0.56 g/l MLVSS approximately 13% of the naphthalene was destroyed after 1.167 hrs.

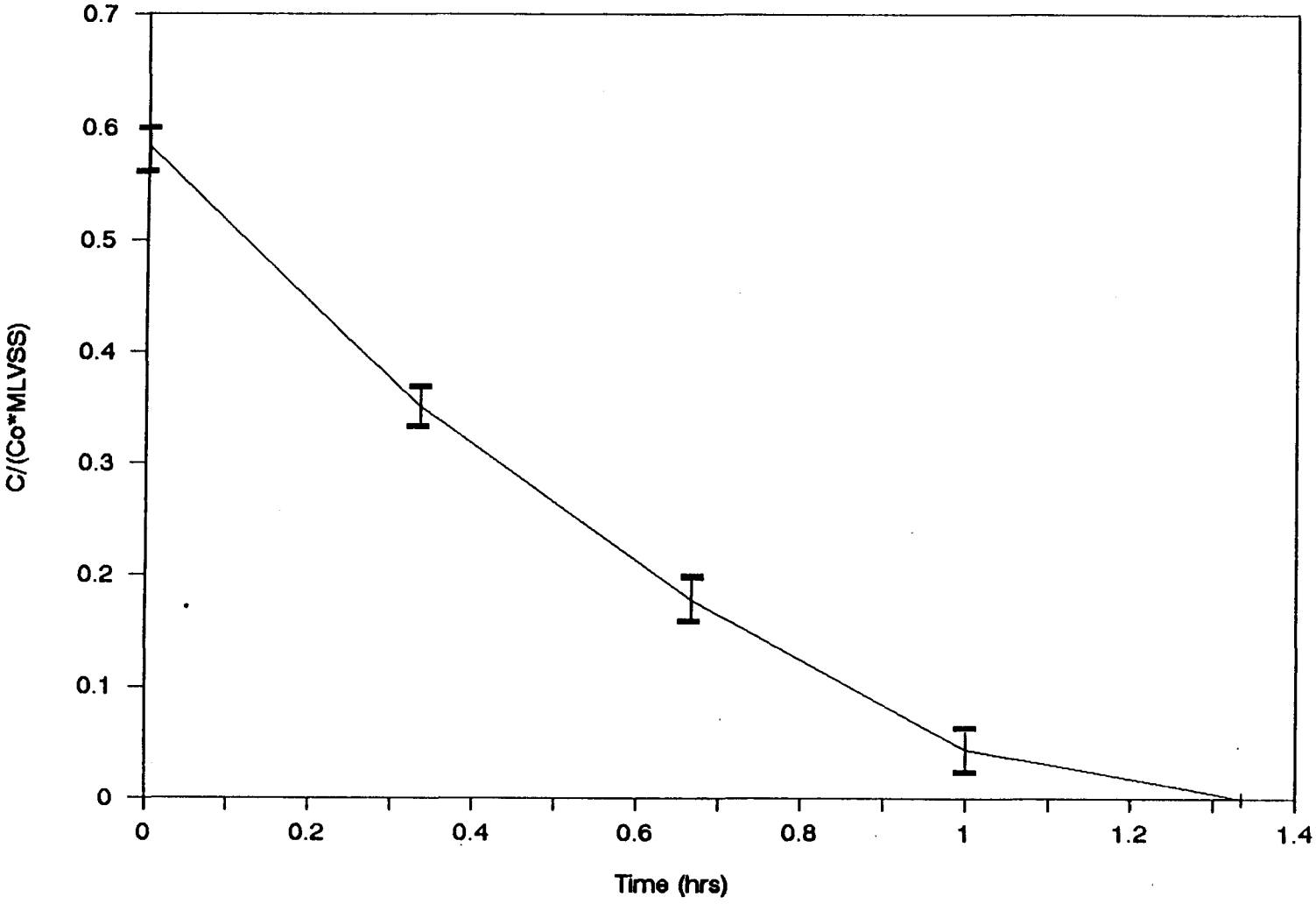

Fig. 14 Degradation of naphthalene in acclimated activated sludge.

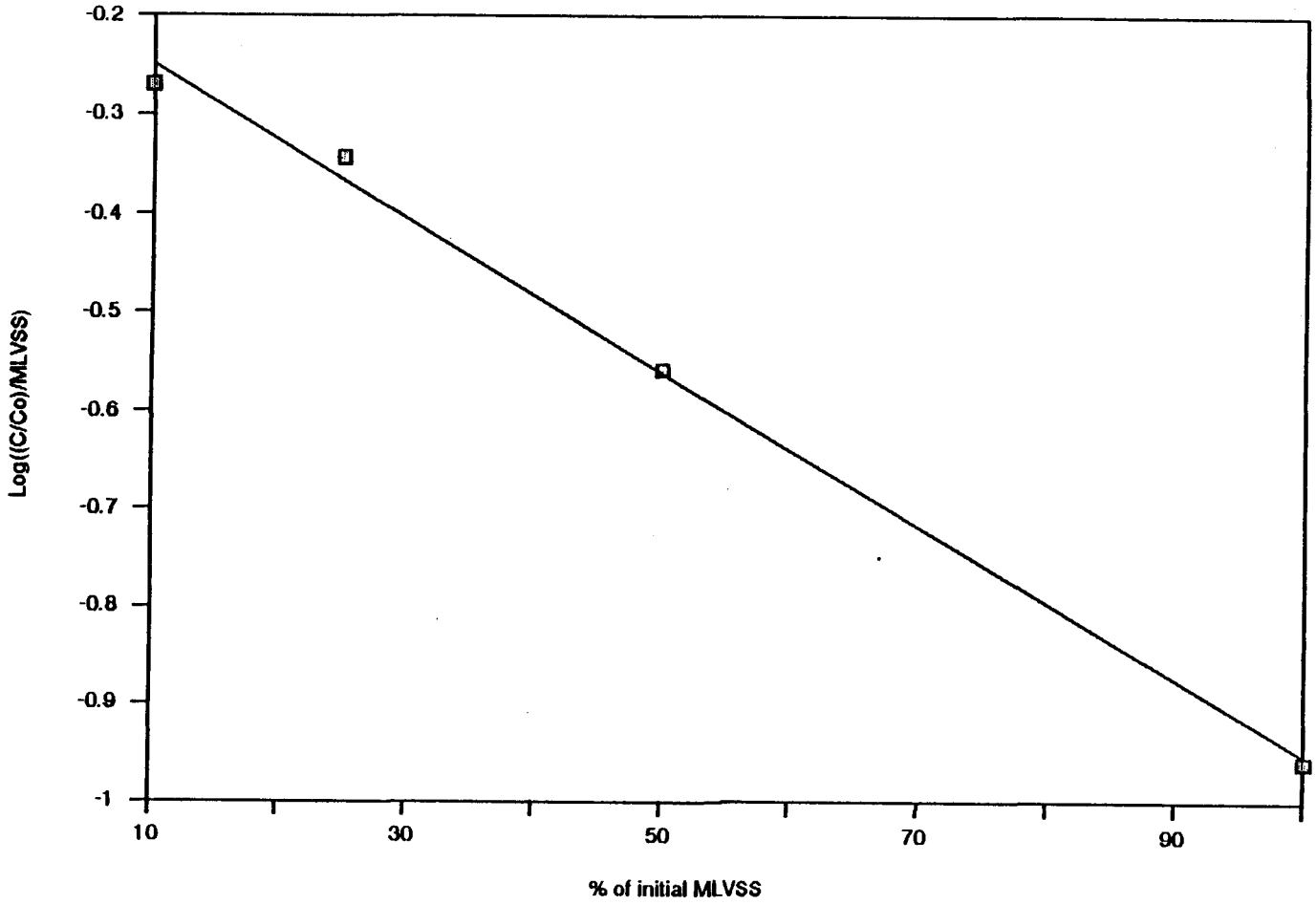

Fig. 15. Degradation of naphthalene in a period of 1.17 hours at different MLVSS concentrations.

Enrichment Sequencing Batch Reactors

Time series batch tests for the removal of naphthalene were done on the sequencing batch reactors maintained on 2 aminobenzoic acid plus succinate feed or the salicylate feed and an influent of 0.320 m the continuous flow reactor maintianed on naphthalene can be seen on Figure 16.

The three test are plotted together for ease of comparison. Batch tests on the continuous fed reactor recieving an influent containing 27.7 mg/l naphthalene showed greater than 99% removal in less than 1.5 hours. Batch tests using activated sludge acclimated to naphthalene and maintained on salicylate for over 3 months showed approximately 99% removal in approximately 4 hours. Activated sludge acclimated to naphthalene and maintained on anthranilic acid + succinate for 3 months showed good removal of naphthalene in less than 7 hours. Both reactors degraded naphthalene in a reasonable time period.

The influent concentration of naphthalene on both batch reactors was reduced to 0.16 mg/l for 6 more months, everything else remained the same. The reactor maintiained on salicylate still showed good removal of naphthalene in 4 hours. The degradation curve of the reactor maintained on 2 aminobenzoic acid plus succinate was very hard to reproduce.

The exact effect of the 0.32 mg/l influent of naphthalene on the reactors fed salicylate or 2 aminobenzoic acid was not determined in this study. Since future tests on the reactor fed 2 aminobenzoic acid did not show any significant removal of naphthalene in 6 hours it is possible that all removal of naphthalene in this reactor is a consequence of the naphthalene in the influent feed. Further evidence of this can be seen in figure 18. In this batch test the control

continuous feed reactor, which was maintianed on a glucose/nutrient broth, had a total influent naphthalene concentration of 0.136 mg/l over a 24 hour period. The rate of removal demonstrated in this reactor is very similar to that seen in the sequencing batch reactor maintianed on 2 aminobenzoic acid plus succinate.

Activated sludge acclimated to naphthalene and maintained on naphthalene or salicylate was tested for its ability to degrade phenanthrene (Figure 17). The activated sludge fed naphthalene degraded phenanthrene as expected. The reactor maintained on salicylate did not reduce the concentration of phenanthrene to any significant degree greater than the controls.

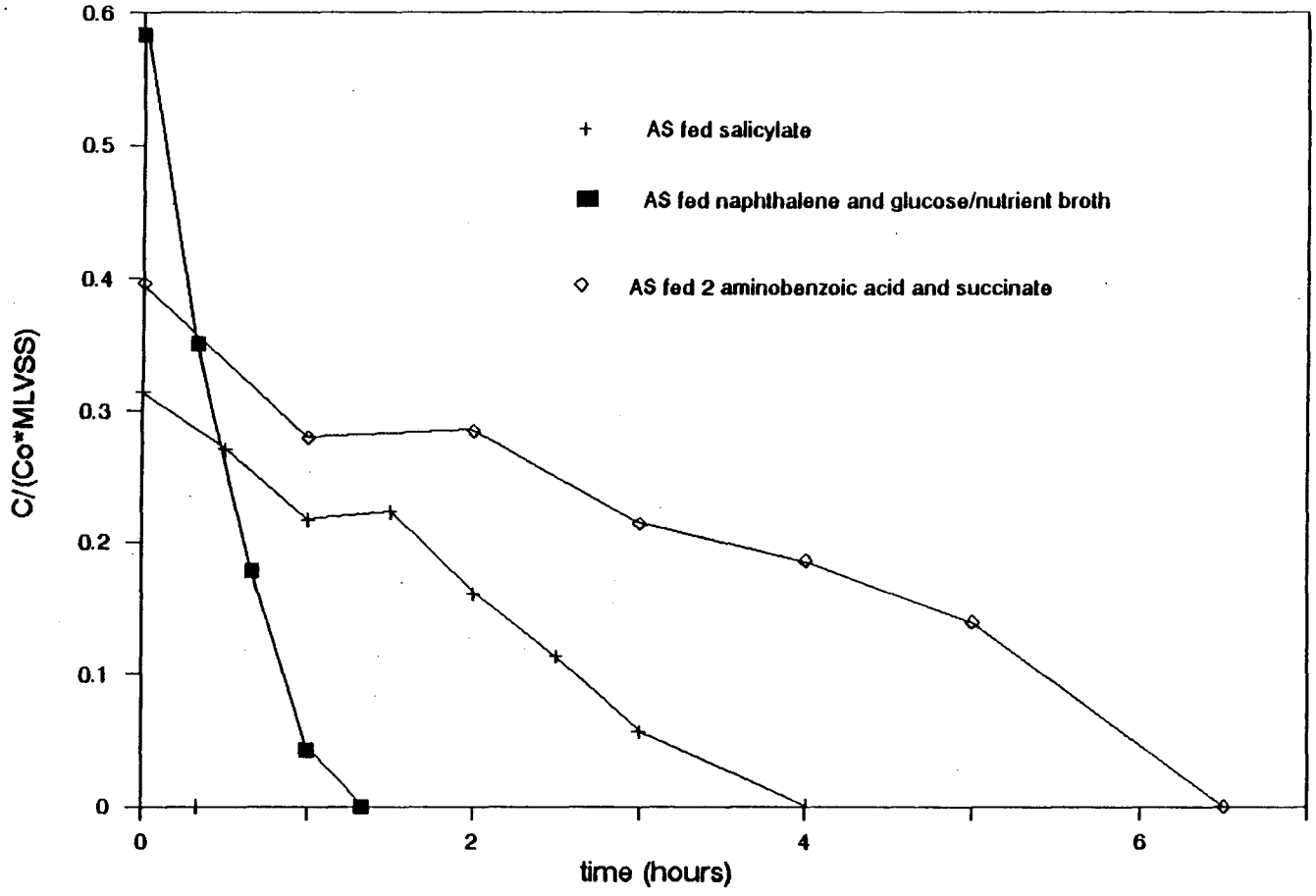

Fig. 16. Degradation of naphthalene by activated sludge fed either salicylate, 2 aminobenzoic acid plus succinate, or naphthalene and glucose/nutrient broth as a primary substrate.

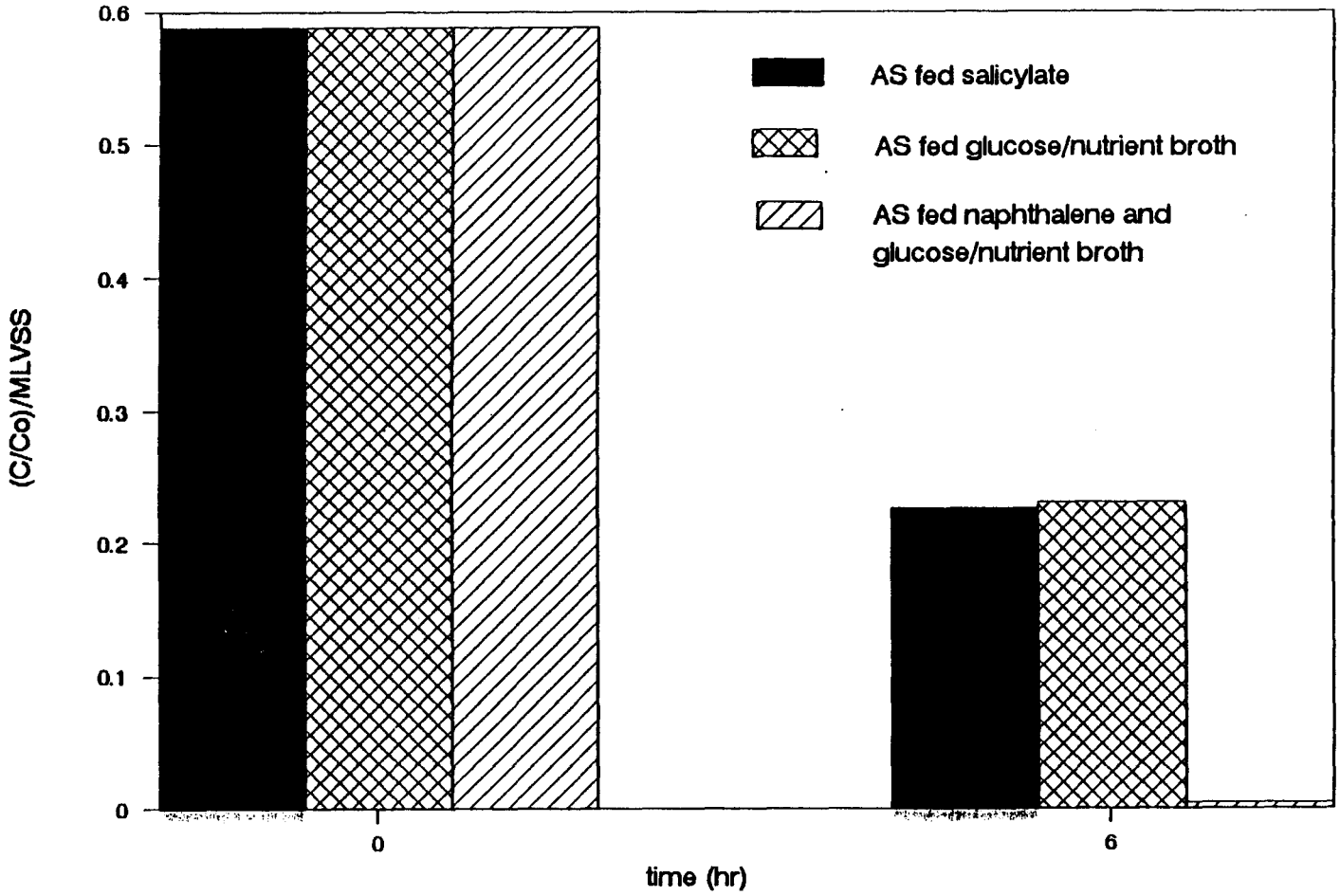

Fig. 17. Degradation of phenanthrene by activated sludge maintained on different substrates.

Enrichment Reactor System

A continuously fed reactor which was fed an influent containing the glucose/nutrient broth feed solution and 5.7 ug/l naphthalene was periodically given activated sludge from the enrichment reactor maintained on salicylic acid for a period of 2 months. At the end of this time it was measured for its ability to degrade naphthalene using time series batch assays. The results were compared with time series tests on the two continuously fed reactors used as controls; one receiving an influent containing glucose/nutrient broth feed solution and 5.7 ug/l naphthalene and one receiving an influent containing glucose/nutrient broth only. Neither of the controls received activated sludge from the enrichment reactor maintained on salicylate. The results are shown in Figure 18. The reactor which received the enrichment activated sludge showed a significantly enhanced ability to degrade naphthalene compared to the reactor fed only low levels of naphthalene. The control reactor fed only glucose feed showed no ability to degrade naphthalene.

The above experiment was repeated with the only change being that none of the reactors received naphthalene. After approximately 3 months they were tested for their ability to degrade naphthalene. None of the reactors degraded naphthalene to a significant degree in a period of 6 hours.

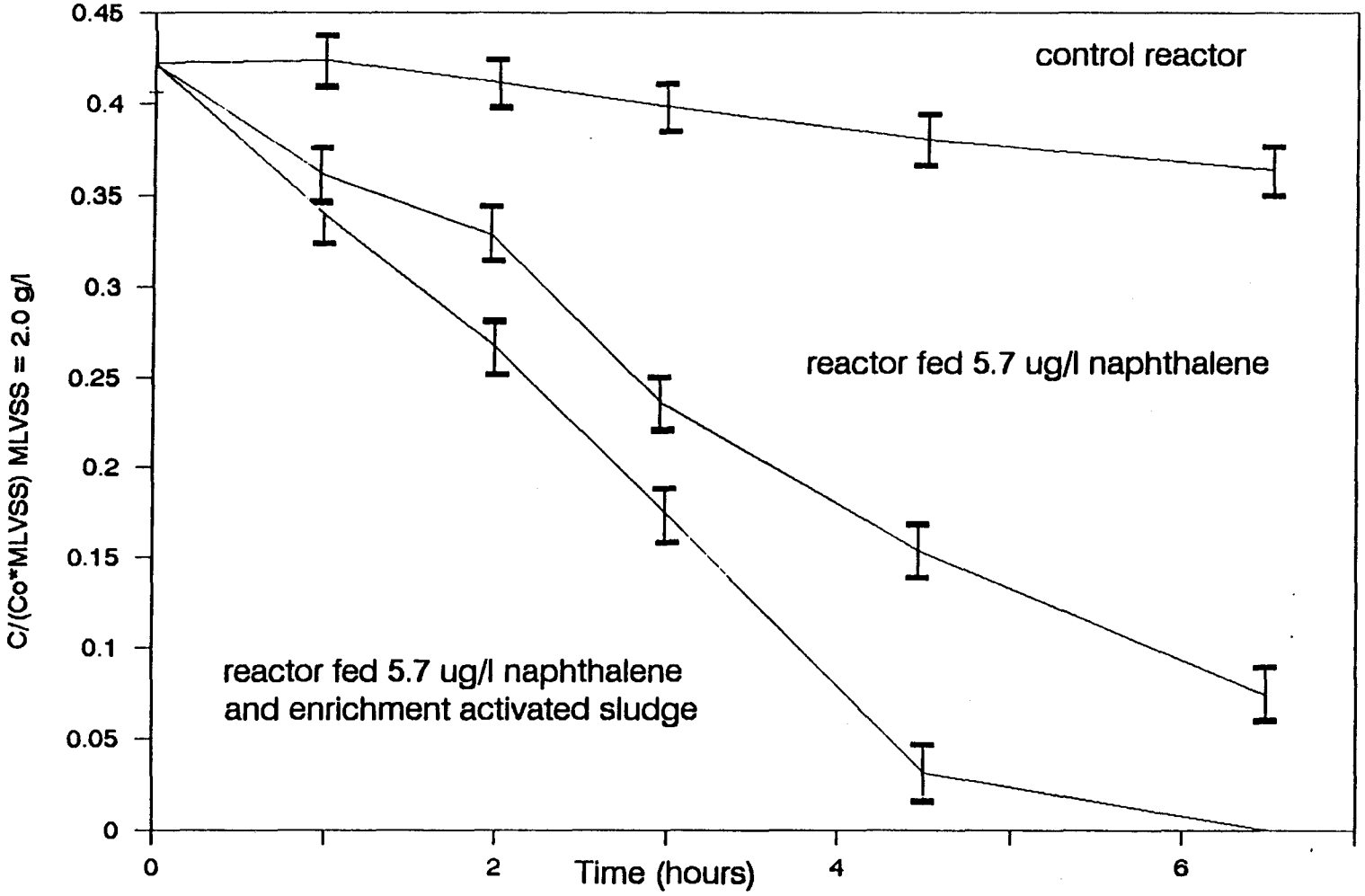

Fig. 18. Degradation of naphthalene by a CFSTR given activated sludge from enrichment reactor.

Temperature

Temperature was shown to have a very significant effect on the ability of the activated sludge to degrade naphthalene. Time series tests for the degradation of naphthalene run at 10-12^{o}C exhibited no significant degradation of naphthalene, whereas times series tests, using the same source of activated sludge, run at 18-23^{o}C exhibited good rates of degradation.

Volatilization

The effects of K (the decay rate) on the mechanism of volatilization of naphthalene, 2,4 xylenol, and isophorone was modeled using previously described equations; steady state conditions were assumed. The minimum K values were estimated, using methods found in Metcalf and Eddy (1979), from the timed series tests done in this study. The Henry's constants used in the model were taken from the literature or calculated using equations found in Lyman et al. (1982). The calculated Henry's constant is equal to the vapor pressure of the compound divided by the solubility. Assumptions here are that the data used must be for the same temperature and physical state. Calculated values are only approximations. The Henry's constants and minimum K values can be found Table 15.

Table 15. Henry's' constants and estimated K values used for modeling. (McKay 1981)[a], (Petrasek, 1983)[b], (Calculated)[c]

Compound	Henery's Constants ($atm \cdot m^3/mol$)	Minimum $K = k/K_s$ (hr^{-1})
Naphthalene	$(4.3 \times 10^{-4})^a$	0.4
2,4 Xylenol	$(5.9 \times 10^{-7})^b$	0.65
Isophorone	$(5.76 \times 10^{-6})^c$	0.11

The modeling was done using operating parameters for both the bench scale reactors used in this study (Figures 19, 21 and 23) and for a typical domestic wastewater plant (Figures 20, 22 and 24). The wastewater plant has an influent volume of 1000 m3/hr (6.5 MGD) with a 6 hour hydraulic retention time. In these figures the remaining mass of compound which is not volatilized or biodegraded is assumed to leave the reactors untreated.

Gas flow rates, which can range from 500 m3/hr for the highest efficiency subsurface aeration system to 12,000 m3/hr for the lowest efficiency spiral roll system (Stenstrom 1988), was set at 12,000 m3/hr.

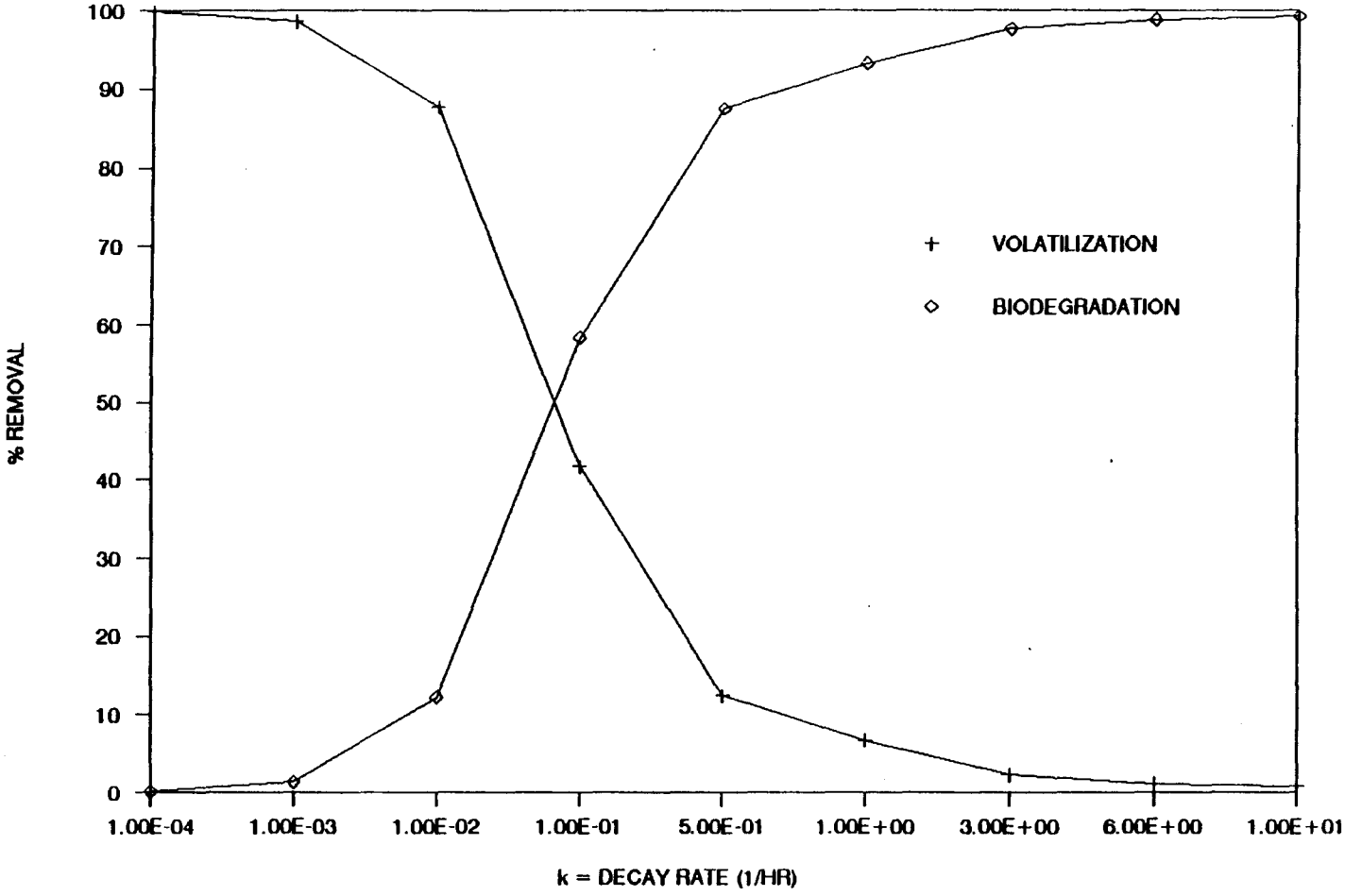

Fig. 19. The effect of K on the volitalization of Isophorone in in bench scale reactors.

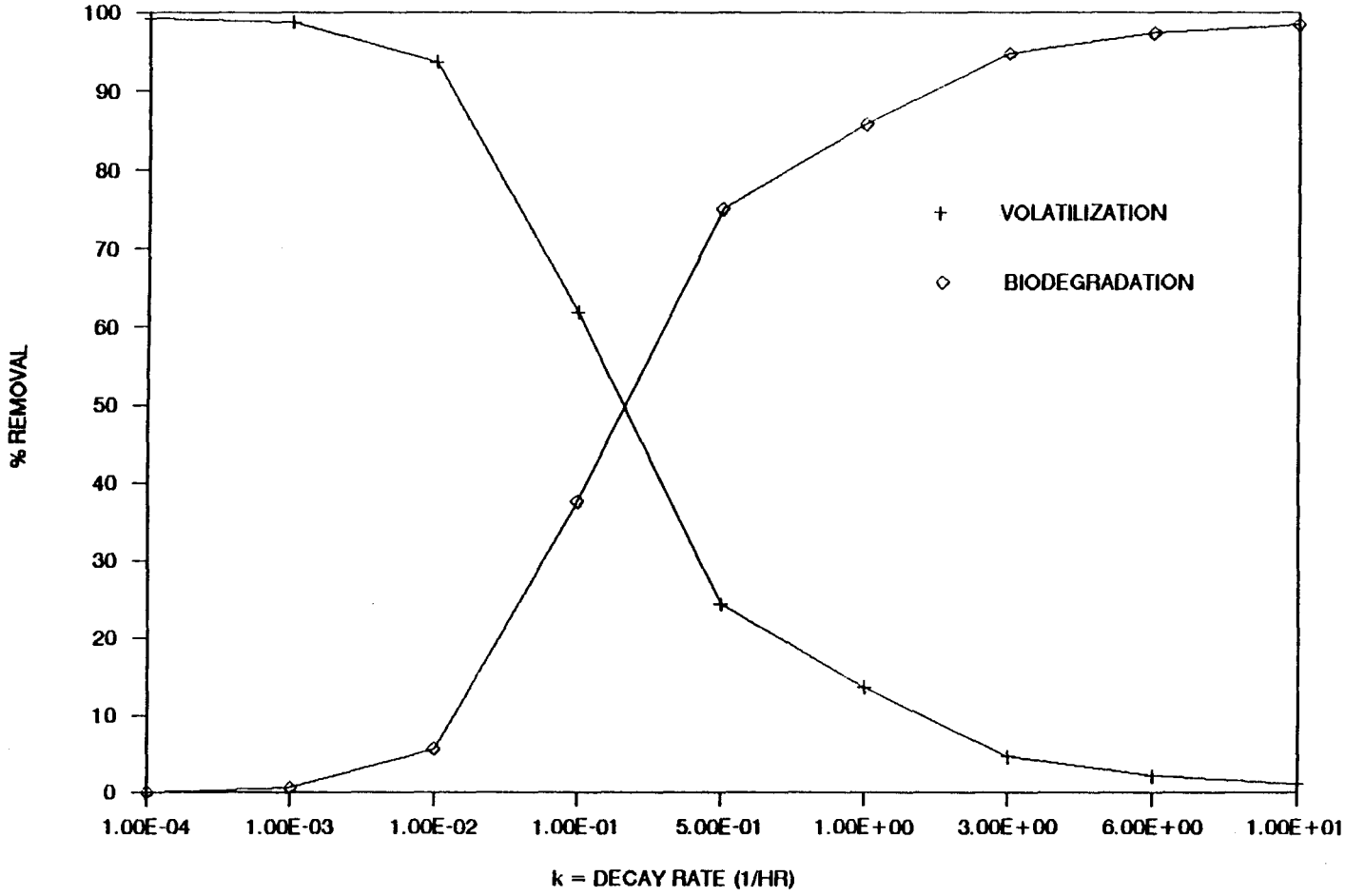

Fig. 20. The effect of K on the volitalization of isophorone in a typical wastewater treatment plant.

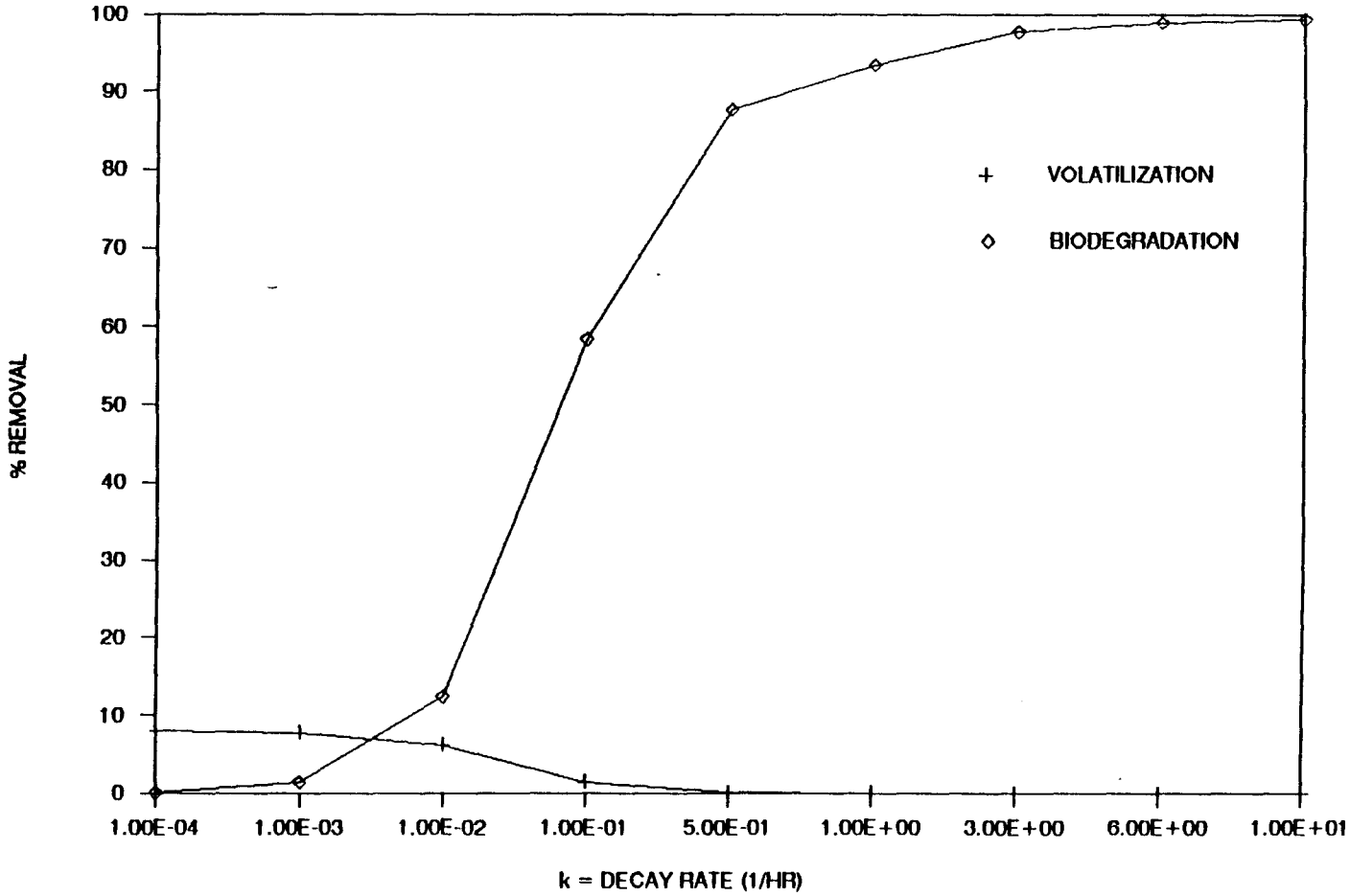

Fig. 21. The effects of K on the volitalization of 2,4 xylenol in bench scale reactors.

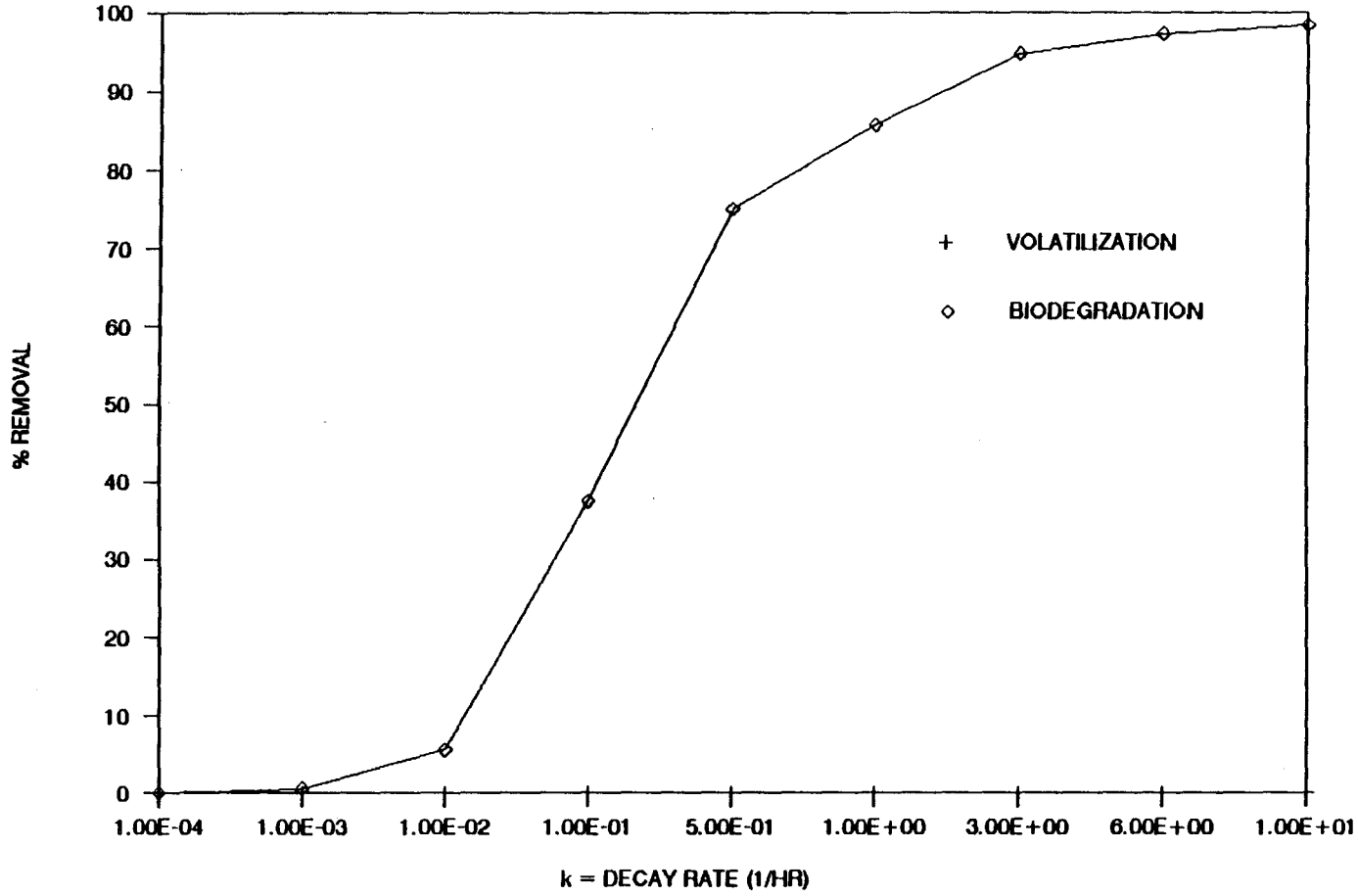

Fig. 22. The effect of K on Volitalization of 2,4 xylenol in a typical wastewater treatment plant.

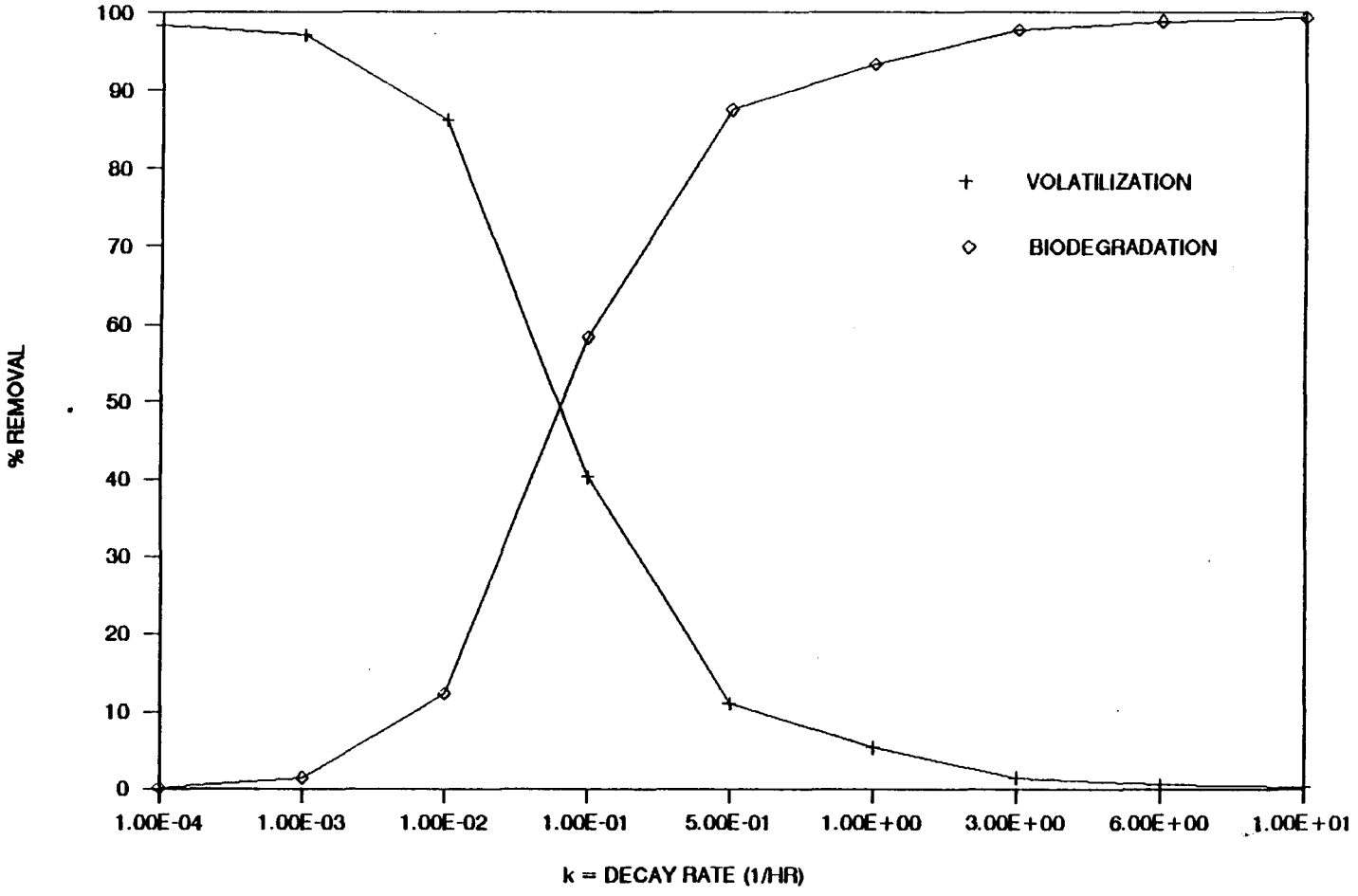

Fig. 23 The effect of K on the volitalization of naphthalene in naphthalene in bench scale reactors.

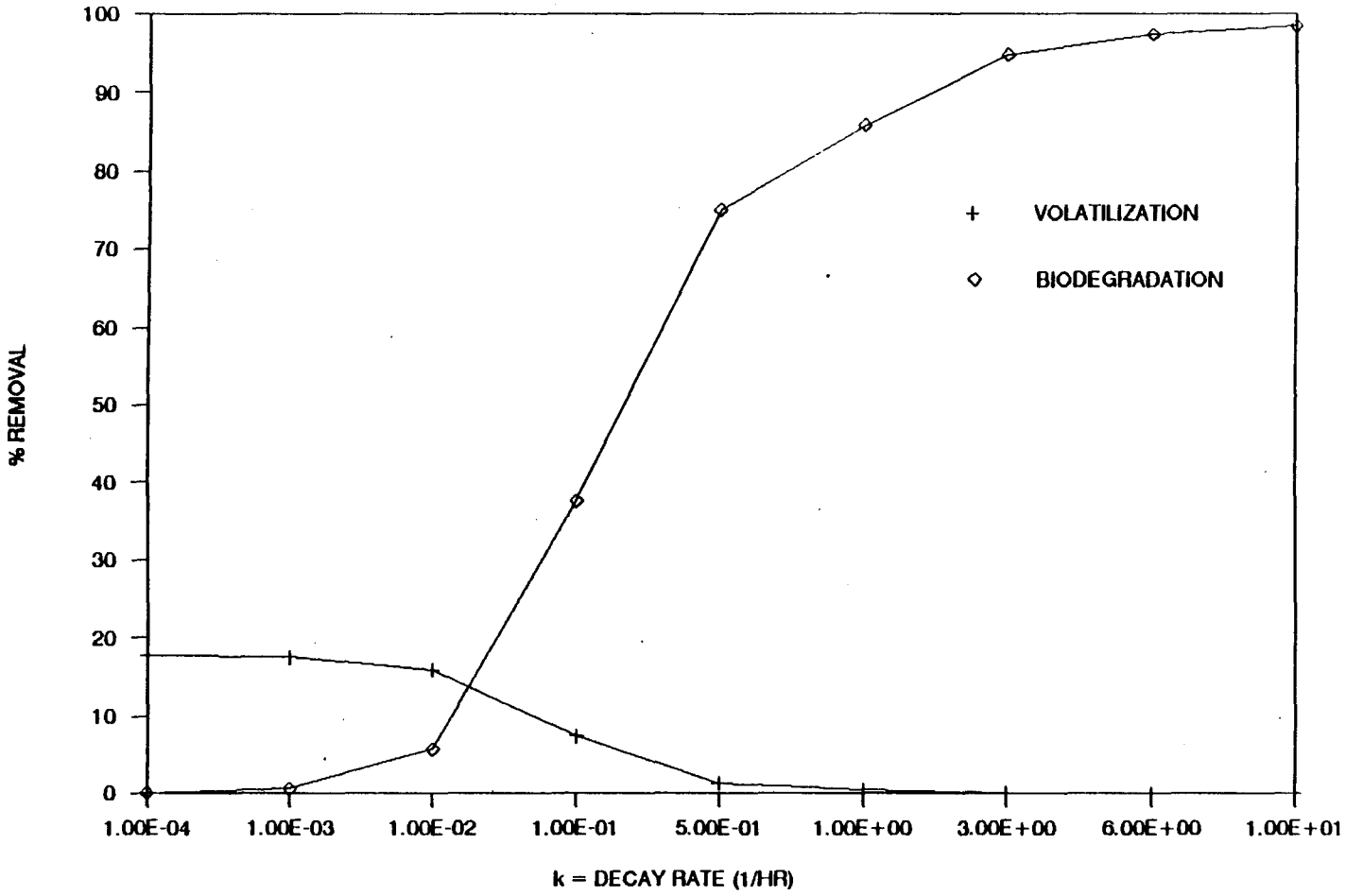

Fig. 24. The effects of K on the volatilization of naphthalene in a typical wastewater treatment plant.

DISCUSSION

Removal by Biological Treatment

Activated sludge which is continuously exposed to a toxic compound can, in many cases, develop the necessary bacterial population capable of producing the enzymes for the degradation of the target compound. This was the case with isophorone, 2,4 xylenol, and naphthalene.

Factors that effect degradablility are not only solubility, volatility and adsorbability but the presence of that compound in nature. Aerobic bacteria have the ability to catalyze the early steps in the degradation of a toxic resulting in the formation of compounds that can enter common pathways such as the Krebs cycle. The probability of the occurrence of an organism capable of producing enzymes which can catalyze the early steps of a degradation pathway increases with the increasing abundance of that compound in nature. The heterotrophic population present in an activated sludge treatment reactor, which is highly diverse and constantly being inoculated by microbes from many sources, is very likely to develop the ability to mineralize any compound which is prevalent in nature and to which it has continuous exposure.

The compounds in this study were mineralized by the activated sludge process because they are some-what soluble, are only slightly volatile, and do not adsorb to any significant degree to the activated sludge. These compounds are also commonly found in nature, i.e. the xylenols, cresols and naphthalene are found in coal, oil, and plant and animal pigment.

Isophorone

The results of the study by Trudgill (1984), in which they concluded that removal of isophorone was by mechanisms of cometabolism or commensalism from a mixed culture, most likely has a direct application to the activated sludge process which was acclimated to isophorone. In time series tests of the degradation of isophorone there was always a period of transient cessation of the utilization of isophorone. This is most likely due to product inhibition of the initial oxidation reaction; the product here is 5,5 dimethyl-1,3 cyclohexane dione. The lag phase of the growth of the organisms which utilize 5,5-dimethyl-1,3-cyclohexane dione allows for the build up of this intermediate. Once efficient removal of 5,5-dimethyl-1,3-cyclohexanedione begins the removal of isophorone resumes. See Figure 6.

Xylenols, Cresol, and Thimethylphenol

From the data presented on Table 14 it is apparent that of all the structural analogues of 2,4 xylenol tested the ones with a methyl in the 2 or the 4 position were degraded at a faster rate than those that did not have methyl groups in these positions. Compounds that have a methyl group in the 4 position showed the greatest maximum rate of degradation. The preference of these organisms for compounds with methyl groups in the 4 position would be expected if the dominant pathway of degradation was that depicted in Figure 1.

These results are similar to those found in pure culture studies by Chapman et al. (1968, 1971) in which cells grown with 2,4-xylenol readily oxidize 4-hydroxy-3-methylbenzoate, p-cresol, p-hydroxybenzoate, protocatechuate, and 3,4-xylenol. M-cresol and 3,5-xylenol are also oxidized but at a slower rate. It can be assumed that the broad specificity of the early enzymes of xylenol degradation found in these pure cultures are also present in the mixed cultures of activated sludge.

In Chapman's studies an accumulation of the corresponding carboxylic acids from compounds that were oxidized but not directly involved in the degradation pathway of 2,4-xylenol developed. In contrast activated sludge acclimated to 2,4-xylenol showed no such accumulation. This was verified by gas chromatographic analysis. Although the isomers can cause induction of the enzymes needed for the initial steps of the reaction, the complete mineralization of the isomers is dependent on the presence of other microorganisms in the activated sludge.

In summary, activated sludge maintained on 2,4-xylenol and glucose/nutrient broth feed solution will become enriched in microorganisms capable of not only degrading 2,4-xylenol but other isomers of 2,4-xylenol, cresols and trimethylphenols. The rate of the removal of any specific compound is dependent on the location of the methyl groups on the phenol ring.

Acclimation of the activated sludge to 2 or 3 isomers of xylenol may further enhance the microbial diversity in the activated sludge resulting in the production of enzymes for all three different pathways involving protocatecuic acid, gentisic acid and catechol. The net result of this acclimation would be an

activated sludge which can degrade a wider range of compounds at a faster rates.

In the batch test where 2,4-xylenol, 2,3-xylenol and o-cresol were added to the activated sludge simultaneously the removal of 2,4-xylenol was almost complete before any significant removal of o-cresol and 2,3-xylenol took place (Figure 12). A phenomena called sequencing may be occurring here. In this case the microbes degrade the compound which give it the most energy (ATP) using easily inducible enzymes first; once this source is oxidized the other compounds are more readily utilized.

Phenanthrene removal by AS

Results showing that activated sludge which was acclimated and maintained on naphthalene readily degraded phenanthrene but the activated sludge acclimated to naphthalene and maintained on salicylate did not degrade phenanthrene (Figure 15.) indicate that the pathways of these compounds are separate. Although some research indicates that microorganism capable of degrading polyaromatic hydrocarbons produce enzymes of broad specificity (Bauer 1988) in the case of phenanthrene and naphthalene the evidience from the literature and these studies indicated that there are two separate pathways for the degradation of these compounds. In support of this, are studys using phenanthrene degrading organism from soil (Barnsley 1983) including pseudomonas and aeromonas species (Kiyohara et al. 1978).

The presences of separate pathways for these compounds may be why the activated sludge which was acclimated to naphthalene and maintained on

salicylate over a 3 month period degraded naphthalene (Figure 16) but did not degrade phenanthrene (Figure 17) to any significant degree. During the period of incubation when the cells received salicylate the enzymes for the initial steps in the degradation of naphthalene were induced thus preserving the genetic information for naphthalene removal whereas the genetic information for phenanthrene removal was lost due to lack of use.

Enrichment Reactors

The sequencing batch enrichment reactors which were maintained on salicylate or succinate plus 2 aminobenzoic acid and 0.16 mg/l naphthalene influent concentration were capable of degrading naphthalene at reasonable rates for a long period of time because these substrates not only served as an energy source for the microbes capable of degrading naphthalene but may have preserved the genetic integrity of the microbial population involved by induction. In support of this are studies of pseudomonas species which produce enzymes specifically involved in naphthalene metabolism when exposed to not only naphthalene but salicylate and 2 aminobenzoic acid as well (Shamsuzzaman 1974, Barnsley 1976). Activated sludge which was acclimated to naphthalene and maintained on glucose/nutrient broth feed only soon lost its ability to degrade naphthalene.

The reactor fed succinate plus 2-aminobenzoic acid did not preform as well as the reactor fed salicylate and all removal of naphthalene in this case may be due to the 0.32 mg/l influent of naphthalene. One reason for its poor performance is because succinate functioned only as an energy source, leaving

a smaller amount of the inducing compound, 2-aminobenzoic acid, in the feed solution. Due to the heterogeneous nature of activated sludge the microbes which can survive on succinate but not naphthalene may have proliferated resulting in the diluting out of the number of microbes that can degrade naphthalene. Salicylate, being a direct intermediate in the degradation pathway, would favor the microbial population capable of utilizing naphthalene by serving as their energy source as well maintaining the genetic integrity of the microbes.

Naphthalene Removal by the Continuous Feed Reactors Inoculated with Biomass From the Enrichment Reactor

The enhanced naphthalene removal by the continuous feed reactor inoculated with enrichment cells from the sequencing batch reactor maintianed on salicylate may have been due either to an increased cell population capable of utilizing naphthalene and/or the transmission of plasmids (Zuniga 1981, Heitkamp 1987). Exposure of naphthalene to cells capable of oxidizing it may not always increase the total number of heterotrophic microorganisms, but may selectively increase the hydrocarbon-degrading microbial population. Although enhanced mineralization rates for naphthalene were a function of cell concentration in batch testing using activated sludge acclimated and maintained on naphthalene (Figure 14), the enrichment of activated sludge using biomass form the enrichment reactor may be a function of plasmid transmission as much as cell concentration.

Results from a time series batch test using activated sludge from a continuous fed reactor, which was dosed with activated sludge from the sequenceing batch reactor maintained on salicylate but recieved no naphthalene in its influent, imply that the added enrichment cells were unable to enhance the reactors ability to degrade naphthalene under these conditions. The most obvious explanation is that the presence of the naphthalene in the influent of the continuously fed reactors, even in small amounts, most likely serves to maintain the viability of the added enrichment reactor cells.

In this project the enrichment reactors as well as the continuous feed reactors, excluding the controls, were fed a small background of naphthalene. All the reactors fed naphthalene on a continuous basis degraded naphthalene.

The data presented implies that simply adding acclimated activated sludge or cells of any kind, i.e. freeze dried bacteria, to a continuously fed reactor will not necessarily enhance that reactors ability to degrade the target compound(s). Addition of the toxic compound(s) to be removed is also necessary if the viability of the added cells is to be maintained between the times at which influent loading of the toxic compound to the plant occurs. The advantage of the enrichment reactor is that the amount of toxic compound which needs to be added to maintain a certain rate of removal is much less if added in conjunction with enrichment reactor activated sludge than if only the compound itself was added.

Activated sludge from an enrichment reactor will most probably function better than freeze dried microbial products when added to the activated sludge reactors in a wastewater treatment plant. The enrichment reactor cells are already in a viable state and are acclimated to the influent wastewater of that specific plant at the time of addition. The genetic diversity in enrichment reactor activated sludge is also greater than in most freeze dried cell products. As a consequence of these factors the enrichment reactor activated sludge will have a greater probability of degrading to completion a wider range of compounds as well as maintaining its viability during fluctuating influent wastewater conditions.

Exactly how much of the toxic compound is needed to maintain the viability of the enrichment cells has not been determined by this study. It is

possible that a much smaller amount added at less frequent intervals than that used here will be sufficient to maintain a reasonable rate of removal.

Volatilization

The level of volatilization for 2,4-xylenol and Isophorone in a municipal waste water plant is negligible as expected due to the low Henry's Law constant for this compound. Volatilization losses become significant when the Henry's Law constant becomes 10^{-2} to 10^{-3} ($atm \cdot m^3/mol$) (Stenstrom 1988).

Volatilization for naphthalene is some what higher. At the minimum K value calculated from the data in this study there is a 5% loss due to volatilization in a typical waste water treatment plant; in the bench scale reactors this can become as high as 20%.

From the model it is apparent that at high decay rates volitalization becomes insignificant for these compounds. This inverse relationship between naphthalene biotransformation and stripping has been observered in previous studies (Blackburn 1987).

SUMMARY AND CONCLUSION

With continuous exposure of activated sludge to a toxic compound, such as 2,4-xylenol, isophorone or naphthalene, there will develop a population of microbes capable of degrading the particular compound. What makes activated sludge so amenable to acclimation is its large gene pool because of the presence of different species of microbes, and the constant influx of new microbes from the influent.

If the microbial population capable of degrading a specific compound produces enzymes of broad specificity, as was the case with 2,4-xylenol, structural analogues of that compound may be degraded as well. The data presented here indicate that the types of analogues degraded, to what extent, and at what rate, are dependent on the enzymes involved in the degradation pathway of the compound to which the activated sludge was originally acclimated.

In activated sludge the genetic information necessary for the production of enzymes used in the degradation of certain toxic compounds may be located on plasmids as in the case of naphthalene and possibly phenanthrene. These precepts are supported by Blackburn (1987) who showed a positive correlated between the catabolic genotype commonly associated with naphthalene degradation, found on plasmids, with the catabolic activity in a biological treatment system operating on an industrial waste water.

The microbes in the activated sludge process will retain genetic information when that information is being used, as in the production of enzymes. This production of enzymes is usually induced because the microbe is

using the target compound as an energy source. The results of this study indicate that the induction of these enzymes by salicylate as well as a small background of naphthalene may be an effective way to encourage the acclimated microbes to retain their genetic information and consequently their ability to degrade naphthalene., although conclusive evidence as to this cause and effect was not within the scope of this study.

In the enrichment reactor system activated sludge, enriched in a microbial population capable of degrading the target compound, was produced and used to enhance the ability of the main waste treatment system to degrade toxic compounds. This enrichment activated sludge was maintained on a low level of naphthalene and a substrate that takes advantage of specific biochemical or genetic aspects of the microbial population involved in the removal of the toxic compound. The results of other researchers, in which the genetic and biochemical aspects of the degradation of these compounds or classes of compounds were determined, were instrumental in the development of these enrichment reactors.

In the enrichment system the enrichment reactor itself can be operated independently from the main process. It can be designed so that operating parameters such as the mean cell retention time, hydraulic retention time, mixed liquor volatile suspended solids concentration, and the influent feed concentration (and composition) can be adjusted to best accommodate the microbial population in the enrichment reactor itself. Its operation can be optimized to assist the main plant. An ideal reactor for this application is a sequencing batch reactor because of its versatility, low maintenance

requirements, and its ability to handle shock loading. A schematic of a wastewater plant with an enrichment reactor on line is shown in Figure 25.

The results of this study have important implications for the future treatment of hazardous waste in POTW's. The development of the enrichment reactor concept could result in the construction of new POTW's or a modification of existing POTW's to greatly enhance their ability to handle dilute toxic wastes. The final results of implementing this process could take the form of a large savings to industry resulting from reduced cost of pre-treating dilute wastes, protection of POTW's from upsets caused by uncontrolled discharges into the sewer system, and protection of the receiving waters from effluent containing untreated toxic waste.

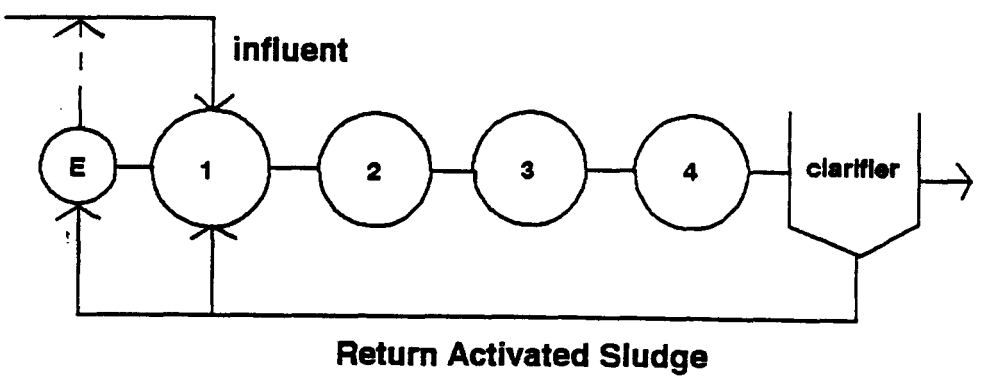

Fig. 25. Schematic of wastewater plant with and enrichment reactor on line.

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APPENDIX 1

The data for determining the best extraction methods and efficiencies from various extraction methods used to isolate isophorone, 2,4 xylenol and naphthalene from water.

Extraction of Isophorone, percent recovery data:

Type of extraction	initial conc	vol extract	% recovery
bondelut(s)(C8)	8.9	25 ml	86.73
liq/liq*	89.52	25 ml	98.0
liq/liq*	89.52	25 ml	85.0
liq/liq*	89.52	25 ml	96.7
liq/liq*	89.52	25 ml	78.7

* liq/liq solvent was $CHCl_2$; no NaCl was added

Extraction of 2,4 xylenol using bondelute (CH):

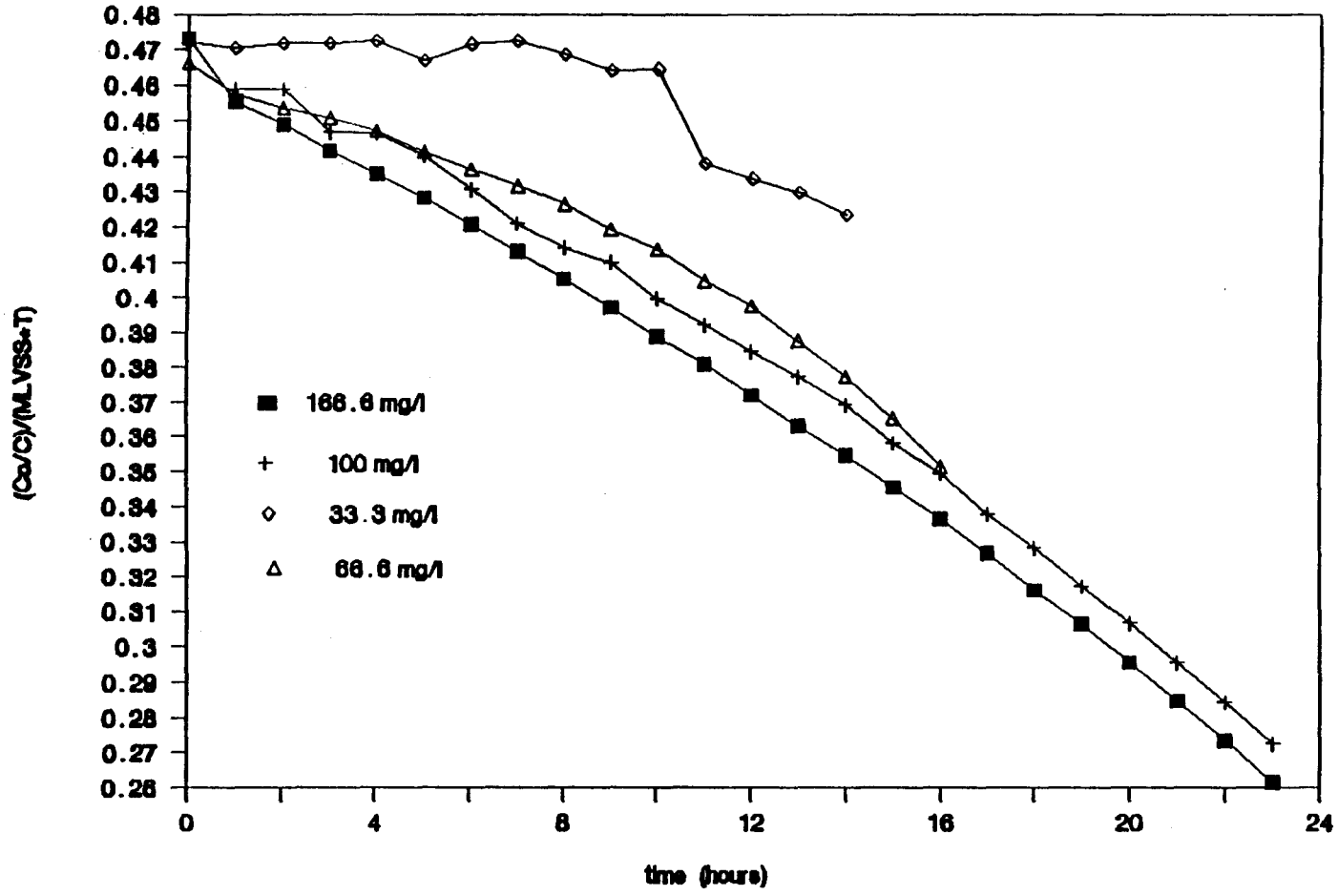
original conc.(mg/l)	volume extracted	% NaCl	% recovery
9.96	25 ml	0	67.0
91.47	25 ml	0	59.8
9.79	25 ml	0	79.58
9.77	25 ml	0	119.14
0.1	25 ml	0	82.0
0.1	25 ml	0	82.0
99.6	25 ml	5	0.51
99.6	25 ml	10	79.0
99.6	25 ml	15	81.0
99.6	25 ml	20	40.0
99.6	10 ml	5	87.0
99.6	10 ml	15	81.5
99.6	10 ml	20	90.0

Extraction of Naphthalene using bondelute (C8)

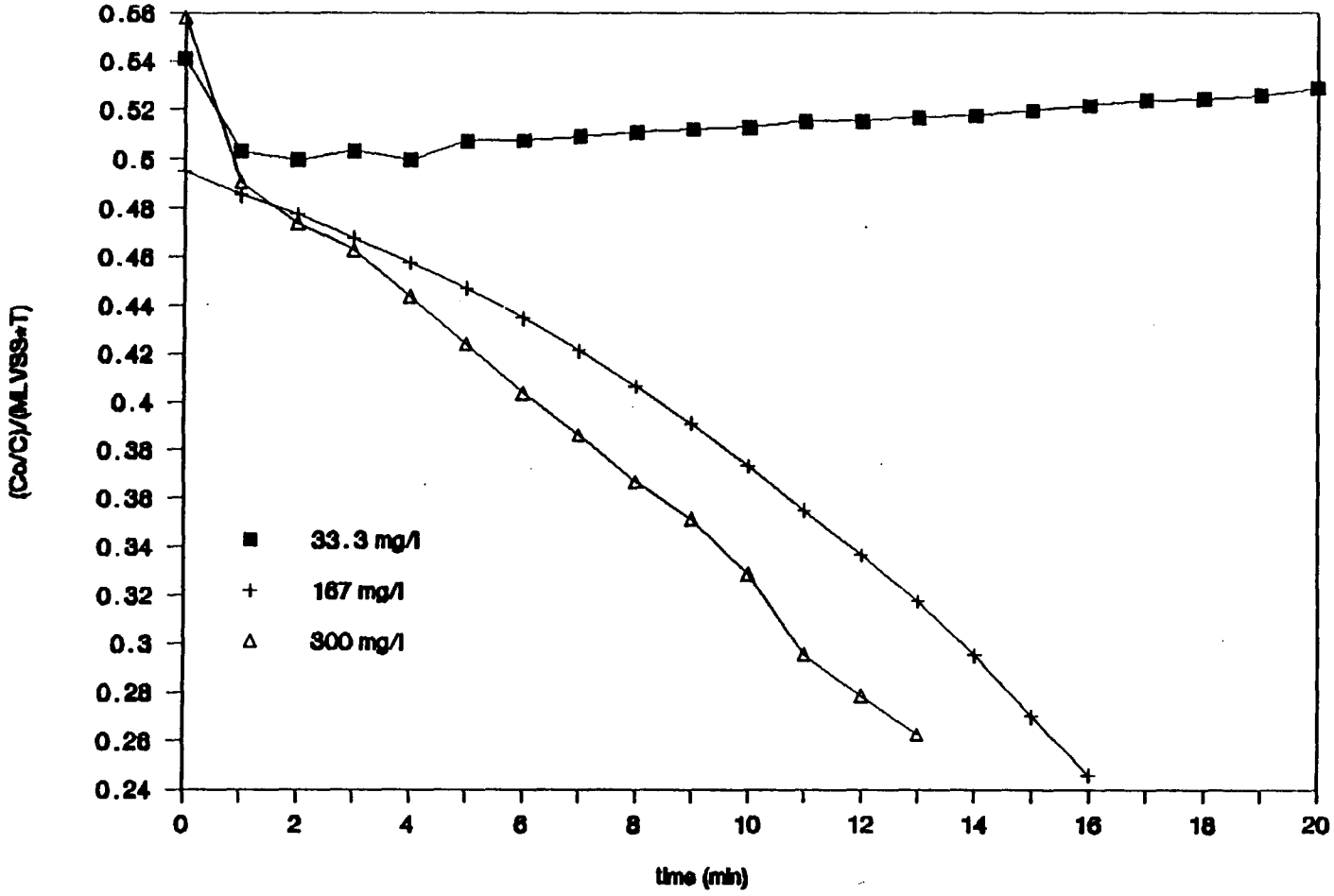
original conc. (mg/l)	volume extracted	% NaCl	% recovery
26.64	5 ml	0	78.77
27.30	5 ml	0	86.3
27.46	5 ml	0	86.84
15.96	5 ml	0	88.0
15.96	5 ml	0	92.0

APPENDIX 2

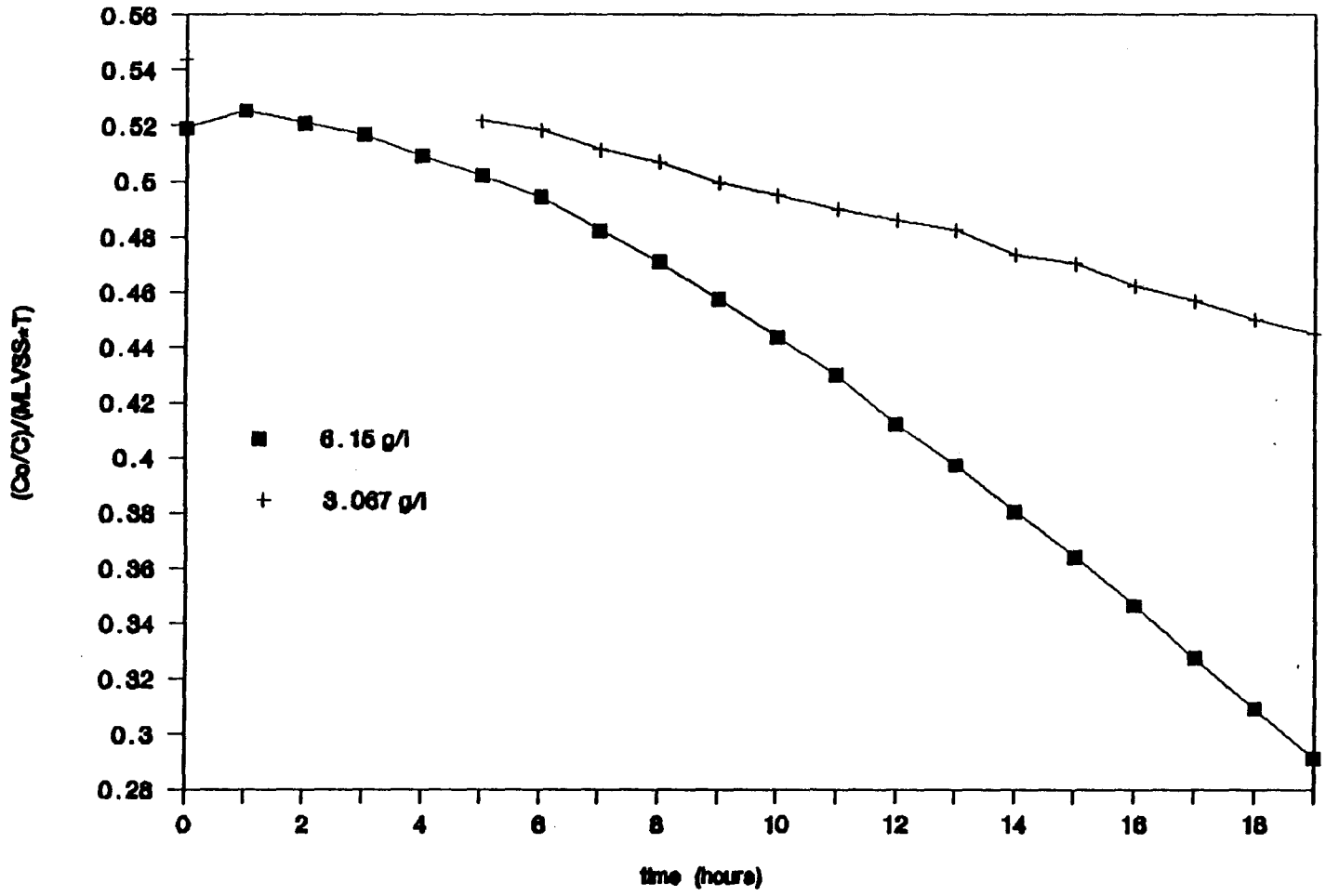
Dissolved oxygen studies to determine the effect of specific toxic compounds on unacclimated and acclimated sludge.



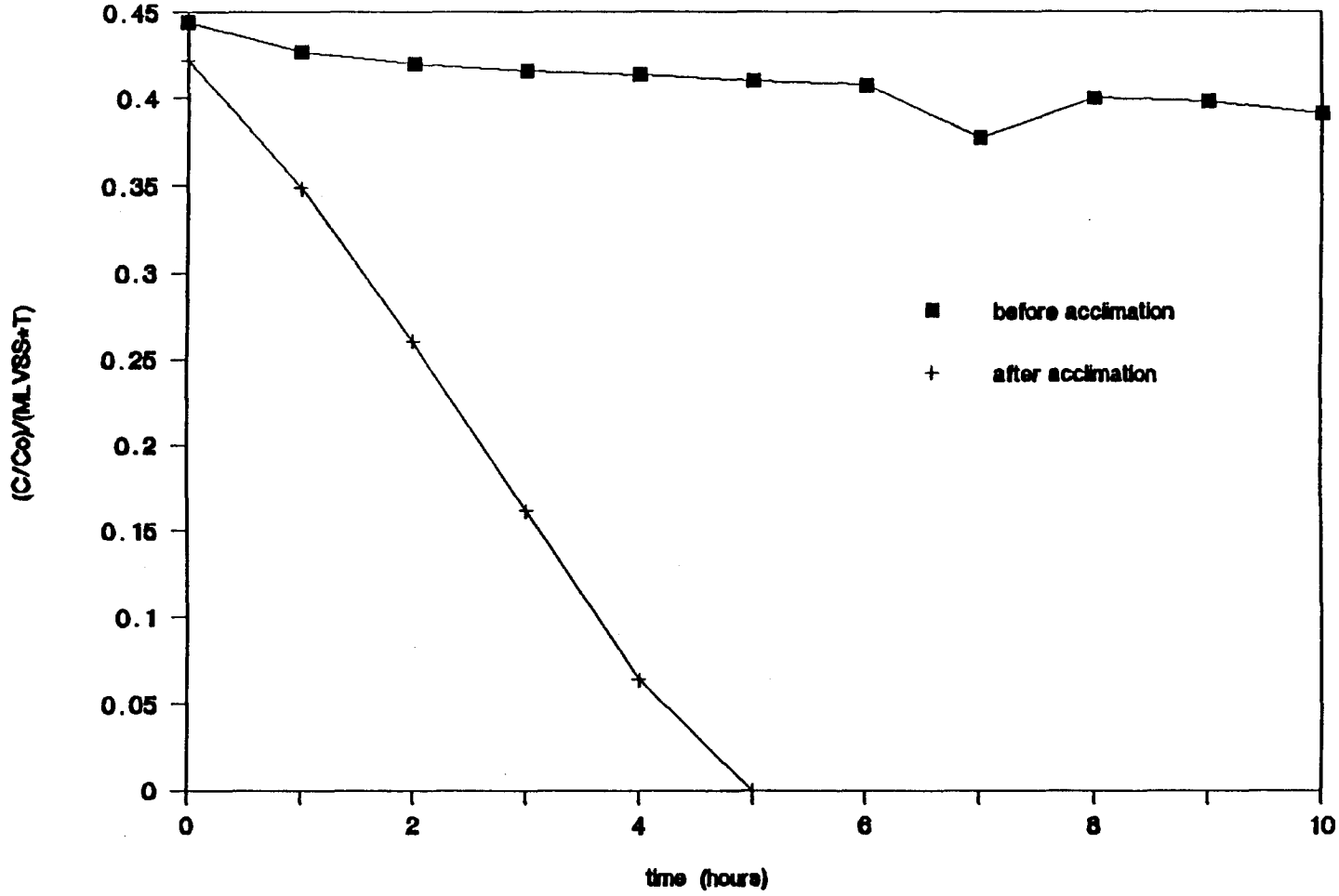
Effect of 2,4 D on dissolved oxygen uptake in unacclimated activated sludge .



Effect of 2,4,5-D on dissolved oxygen uptake in unacclimated activated sludge.



Effect of isophorone on the dissolved oxygen uptake in unacclimated activated sludge.



Effect of 2,4 xylenol on dissolved oxygen uptake in acclimated and unacclimated activated sludge.

Dissolved oxygen uptake of the activated sludge from the enrichment reactors after they have been fed the substrates. This is compared to the dissolved oxygen uptake of the activated sludge from the control reactor.

Substrate	D.O. uptake control	D.O. uptake enrichment reactors
Succinate +		
2-aminobenzoate	0.18 mg/min	2.96 mg/min
Salycilate	0.14 mg/min	1.14 mg/min