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# ABSTRACT

## BIODEGRADATION OF PAH CONTAMINATED SOIL AND SLUDGE USING NON-IONIC SURFACTANTS

**by**
**Seema Narula**

Polycyclic aromatic hydrocarbons (PAHs) are one of the most prevalent environmental pollutants contaminating a large number of industrial and Superfund sites. Low solubility and sorption to solid surfaces limit biodegradation rates of PAHs in the environment. Bioremediation of these compounds have been previously tested with partial success. In the present study, aerobic biodegradation of three PAHs (fluorene, phenanthrene, and pyrene) has been studied in shaker flasks, batch fermenter, and bioslurry reactor, in the presence of non-ionic surfactants. A mixed bacterial culture derived from both a refinery sludge and an activated sludge was used as the seed population in the degradation studies. A non-ionic surfactant, Makon 10 (at a concentration above the CMC), was selected for most of the studies, based on screening a number of surfactants in solubilization and respirometric experiments. PAH biodegradation was examined in both the presence and absence of an initially clean soil, as well as in the presence of a real PAH-contaminated refinery sludge. The results obtained from batch experiments indicated an increase of 2 to 3 orders of magnitude in the solubility of the tested PAHs, in the presence of surfactant. Furthermore, provided the surfactant concentration was maintained in the reactor to overcome mass transfer effects, biodegradation of all three PAHs proceeded to the detection limit, even in the presence of soil or sludge.

# BIODEGRADATION OF PAH CONTAMINATED SOIL AND SLUDGE USING NON-IONIC SURFACTANTS

by
Seema Narula

A Dissertation
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

Department of Chemical Engineering, Chemistry and Environmental Science

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Dedicated to my parents and my husband

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## TABLE OF CONTENTS


Chapter | Page

1 INTRODUCTION ..... 1

2 LITERATURE REVIEW ..... 3

2.1 Solubilization of PAHs ..... 4

2.2 Biodegradation of PAHs ..... 6

2.2.1 Research involving Commercial Surfactants ..... 9

2.2.2 Research involving Natural Biosurfactants ..... 14

2.3 Summary of Literature ..... 15

3 OBJECTIVES ..... 16

4 MATERIALS AND METHODS ..... 17

4.1 Selection of PAH Compounds ..... 17

4.2 Selection of Surfactants ..... 17

4.3 Selection of Soil ..... 18

4.4 Sludge Characteristics ..... 18

4.5 Preparation of Chemical Solutions ..... 18

4.5.1 Fluorene, Phenanthrene and Pyrene Standards ..... 18

4.5.2 Surfactant Solutions ..... 18

4.6 Determination of PAHs ..... 19

4.7 Inoculum and Growth Medium ..... 19

4.7.1 Inoculum ..... 19

4.7.2 Growth Medium ..... 20

4.8 Dissolved Oxygen and pH Measurement ..... 20

## TABLE OF CONTENTS
## (Continued)


**Chapter** **Page**

4.8.1 Dissolved Oxygen Concentration ........ 20

4.8.2 pH Measurement ........ 21

4.9 Selection of Temperature for the Study ........ 21

5 EXPERIMENTAL APPARATUS ........ 22

5.1 Shaker Flask ........ 22

5.2 Respirometer ........ 22

5.3 Batch Fermenter ........ 23

5.4 Slurry Reactor ........ 23

6 EXPERIMENTAL PROCEDURE ........ 25

6.1 Solubilization Experiments ........ 25

6.2 Respirometric Experiments ........ 25

6.2.1 Respirometric Studies for Biodegradation of Surfactants ........ 25

6.2.2 Respirometric Studies to Assess Inhibition of Makon 10 on Microorganisms ........ 26

6.2.3 Respirometric Studies with Sludge ........ 26

6.3 Aerobic Biodegradation of PAHs by Mixed Bacterial Culture ........ 27

6.3.1 Aqueous Phase Biodegradation Studies ........ 27

6.3.1.1 Shaker Flask without Surfactant ........ 27

6.3.1.2 Shaker Flask with Surfactant ........ 27

6.3.1.3 Shaker Flask with Surfactant and Soil ........ 28

6.3.1.4 Shaker Flask with Sludge ........ 28

## TABLE OF CONTENTS
## (Continued)


**Chapter** **Page**

6.3.2 Batch Fermenter Studies with Sludge ........ 29

6.3.3 Bioslurry Reactor Studies ........ 29

6.4 Sorption Experiments ........ 29

6.5 Desorption Experiments ........ 30

7 RESULTS AND DISCUSSION ........ 31

7.1 PAH Solubility Enhancement ........ 31

7.2 Results of Sorption Experiments on Pequest Soil ........ 32

7.3 Results of Respirometric Experiments ........ 33

7.3.1 Biodegradability of Surfactants ........ 33

7.3.2 Effect of Surfactant Concentration on Microorganisms ........ 34

7.3.3 Respirometric Studies with Sludge ........ 34

7.4 Aerobic Biodegradation of PAHs as Sole Carbon Source ........ 34

7.4.1 Initial Results of Shaker Flask Experiments ........ 34

7.4.1.1 Shaker Flasks without Surfactant ........ 35

7.4.1.2 Shaker Flasks with Surfactant ........ 35

7.4.1.3 Soil-water Slurry Phase Studies with Surfactant ........ 36

7.4.1.4 Biodegradation of PAHs in Sludge ........ 37

7.4.2 Biodegradation of PAHs in Batch Fermenter ........ 37

7.4.3 Biodegradation of PAHs in Bioslurry Reactor ........ 37

7.4.3.1 Mass Balance in the Slurry Reactor ........ 38

7.4.3.2 Evaluation of Oxygen-Transfer Coefficient ($K_La$) ........ 40

## TABLE OF CONTENTS
## (Continued)


**Chapter** **Page**

8 CONCLUSIONS AND RECOMMENDATIONS .......... 42

8.1 Conclusions .......... 42

8.2 Recommendations .......... 43

APPENDIX A TABLES .......... 44

APPENDIX B FIGURES .......... 59

REFERENCES .......... 87

## LIST OF TABLES

Table Page

2.1 Literature summary ... 45

4.1 Surfactants used in the study ... 46

4.2 Soil Characteristics ... 46

4.3 Sludge Characteristics ... 47

4.4 Composition of Growth Medium ... 47

7.1 Solubility of model compounds in Makon 10 ... 48

7.2 Oxygen utilization (in mg) by microbial consortia in the presence of 0.3% surfactant ... 49

7.3 Inhibition of Makon 10 on microbial growth ... 50

7.4 Oxygen utilization (in mg) by microbial consortia in the presence of 20% refinery sludge ... 52

7.5 Biodegradation of model PAH compounds without surfactants ... 53

7.6 Biodegradation of PAHs with 0.01% Makon 10 ... 53

7.7 Biodegradation of PAHs with 0.3% Makon 10 ... 54

7.8 (a) Biodegradation of fluorene in soil-water slurries (soil/solution ratio of 1:5); (b) Biodegradation of phenanthrene and pyrene in soil-water slurries (soil/solution ratio of 1:5) ... 55

7.9 Biodegradation of PAHs in 20% sludge with 0.3% Makon 10 ... 56

7.10 Biodegradation of PAHs in Bioflo IIC fermenter with 20 % sludge and 0.3% Makon 10 ... 56

7.11 Biodegradation of PAHs in bioslurry reactor with 20% sludge and 0.3% Makon 10 ... 57

7.12 DO uptake ... 58

## LIST OF FIGURES


**Figure** **Page**

1.1 Distribution of PAH and surfactant in soil/aqueous system.....60

4.1 (a) Calibration curve for fluorene at low concentrations.....61
(b) Calibration curve for fluorene at high concentrations.....62

4.2 (a) Calibration curve for phenanthrene at low concentrations.....63
(b) Calibration curve for phenanthrene at high concentrations.....64

4.3 (a) Calibration curve for pyrene at low concentrations.....65
(b) Calibration curve for pyrene at high concentrations.....66

5.1 Respirometer Bottle.....67

5.2 Batch Fermenter.....68

5.3 Bioslurry Reactor.....69

7.1 Solubility of model PAHs in presence of Makon 10.....70

7.2 Adsorption isotherm of fluorene at various concentrations of Makon 10.....71

7.3 Adsorption isotherm of fluorene at various concentrations of Makon 10 below CMC.....72

7.4 Adsorption isotherm of phenanthrene at various concentrations of Makon 10.....73

7.5 Adsorption isotherm of pyrene at various concentrations of Makon 10.....74

7.6 Cumulative oxygen utilization during surfactant degradation in respirometer.....75

7.7 Inhibition of Makon 10 concentration on microbial growth.....76

7.8 Cumulative oxygen utilization during biodegradation of PAHs.....77

7.9 Biodegradation of PAHs in growth medium without surfactant.....78

7.10 Biodegradation of PAHs in presence of 0.01% Makon 10.....79

7.11 Biodegradation of PAHs in presence of 0.3% Makon 10.....80

## LIST OF FIGURES
**(Continued)**

**Figure** **Page**

7.12 (a) Degradation of fluorene in 20% soil and 0.3% Makon 10 ........ 81
(b) Degradation of phenanthrene and pyrene in 20% soil and 0.3% Makon 10 ........ 82

7.13 Biodegradation of PAHs in 20% sludge and 0.3% Makon 10 ........ 83

7.14 Aqueous phase biodegradation of PAHs in 20% sludge with 0.3% Makon 10 in a batch fermenter ........ 84

7.15 Biodegradation of PAHs in 20% sludge in the presence of 0.3% Makon 10 in a bioslurry reactor ........ 85

7.16 Oxygen uptake by microorganisms ........ 86

# CHAPTER 1

# INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants found in soil, fresh water and marine environments. They present a serious risk to human health, because many of these compounds are carcinogenic. They are classified as priority pollutants and are present at a number of industrial as well as Superfund sites (EPA/540/2-91/007, 1991), particularly in the vicinity of coal gasification facilities and petroleum refineries. Bioremediation technologies hold great promise as economical and permanent solutions for this group of compounds.

PAHs are difficult to treat primarily because of their minimal water solubility and strong adherence to soil. Despite their proven biodegradability, strong adsorption to soil makes them unavailable to microorganisms. In addition, their toxicity at high concentrations, and nutrient and mass transfer problems in soil, limit the biodegradation of PAHs.

The use of surfactants can substantially enhance the bioavailability, and therefore the biodegradability of PAHs. Surfactant molecules consist of a hydrophilic part which is preferentially associated with the aqueous phase, and a hydrophobic part which is preferentially associated with surfaces (thus reducing surface tension). The inward orientation of the hydrophobic ends can create aggregates called "micelles" when the surfactant concentration exceeds a critical level ("critical micelle concentration", or CMC). Formation of micelles (Figure 1.1) allows for the partitioning of PAHs into the

hydrophobic pseudophase of the micellar core. This phenomena greatly increases the total concentration of PAHs above their aqueous solubility limit.

PAHs can exist in several forms: they can be solubilized in surfactant micelles, dissolved in the surrounding solution, sorbed directly on the soil, or sorbed in association with sorbed surfactants.

Higher concentrations of surfactant can actually inhibit biodegradation of PAHs because of: (1) preferential use of the surfactant as a substrate; (2) toxic effects of the surfactant; or (3) attachment of PAH to surfactant adsorbed on soil surfaces.

Refinery sludges have proven to be resistant to biodegradation under natural field conditions. One option is to excavate the sludge and treat it in a bioslurry reactor. The basic mechanisms which determine the performance of such a process are poorly understood, and as a result design and operation of slurry reactors is based on trial and error and experience rather than proven scientific principles.

This research focuses on surfactant enhanced biodegradation generally, and on decontamination of refinery sludges in particular. Various surfactants were tested to enhance the solubility and biodegradation of model PAH compounds (i.e., fluorene, phenanthrene, and pyrene). Degradation of PAHs was also studied for a simulated PAH-contaminated soil.

# CHAPTER 2

# LITERATURE REVIEW

The main barriers for implementing bioremediation in soil are: (a) irreversible adsorption of contaminants which makes them unavailable to microorganisms; (b) toxicity of the contaminants to the microbial consortia; (c) unsuitable pH for proper microbial growth; (d) oxygen mass transfer limitations; (e) temperature limitations; and (f) nutrient limitations.

Because of their hydrophobicity, PAHs occur in the environment mainly attached to particles. PAHs are strongly sorbed to soil or sediments. As a consequence, remediation of hydrophobic organic contamination in soil-water or sludge systems is often dependent on desorption of the contaminant from the soil surface and subsequent incorporation of the pollutant into the bulk aqueous phase. Surfactants are used to increase the solubility of PAHs.

The use of surfactants to enhance the bioremediation of contaminated environments has been of considerable interest in recent years. Research studies have reported both enhancement and inhibition of biodegradation in the presence of surfactants, as explained later in this chapter. Functioning as emulsifiers or solubilizers, surfactants can serve to increase surface area and aqueous concentration of poorly soluble compounds, and thus potentially improve their accessibility to microorganisms. Many bacteria under the right conditions produce biosurfactants and bioemulsifiers to aid in their attack on specific compounds. Commercial surfactants can also serve to enhance the transport of immiscible hydrocarbons into solution, but have on occasion proven to be

inhibitory to microorganisms. The mechanisms behind occurrences of surfactant enhancement and inhibition of microbial degradation are not well understood. In an effort to better understand the factors involved, a search of the literature addressing this subject has been conducted, as presented in the following sections.

## 2.1 Solubilization of PAHs

Experimental studies by Edwards et al[1]. indicated that apparent solubilities of PAH compounds such as naphthalene, phenanthrene, and pyrene were proportional to the concentration of non-ionic surfactants above the CMC.

Janiyani et al.[2] showed that synthetic surfactants released hydrocarbons in the aqueous phase from an oily sludge. They observed an increase in soluble chemical oxygen demand with increase in concentration of surfactant and contact time (mixing).

Volkering et al.[3] observed that PAHs in the micellar phase may not be readily available to microorganisms due to a shell of surfactant around the PAHs. As soluble PAH is depleted in the aqueous phase, PAHs leak out from the micelles in a mass transfer dependent process. They observed the inhibition of phenanthrene mineralization in the presence of certain nonionic surfactants (Triton X-100, Tergitol NPX, Brij 35, and Brij 30). Furthermore, the associated toxicity of some surfactants may contribute to inhibition of PAH degradation. They also observed that non-ionic surfactants at concentrations below the CMC had no effect on the dissolution of naphthalene, whereas the presence of micelles resulted in higher levels of solubility.

Guha et al.[4] reported the ability of non-ionic surfactants (Triton N101, Triton X100, Brij 30, and Brij 35) to solubilize hydrophobic contaminants into their micelles.

They found that for three of the surfactants tested (Triton N101, Triton X100, and Brij 30), the micellar-phase bioavailable fraction of phenanthrene decreased with an increasing surfactant concentration above CMC, because of mass transfer effects between the micelle and the microorganism.

In 1994, Edwards and his co-workers reported desorption of hydrophobic organic compounds from soil by solubilization, in the presence of non-ionic surfactants[5]. They showed that in a soil/aqueous system with surfactant, the hydrophobic organic compound is distributed at equilibrium among three separate phases: a sorbed phase, an aqueous phase, and a micellar pseudophase.

Yeom et al.[6] evaluated a coal tar-contaminated soil from a manufactured gas plant site for the solubilization of PAHs using nonionic polyoxyethylene surfactants at dosages greater than CMC. Longer periods were required to reach equilibrium at higher surfactant dosages. They developed an equilibrium model to predict the solubilization of PAHs from coal tar-contaminated soils for given properties of the soil, surfactant, and PAHs. The model predicted solubilization of PAHs reasonably well at low surfactant dosages. However, at high surfactant dosages (supra-CMC), the model failed to reliably predict solubilization. They presumed that mass transfer effects limited the attainment of equilibrium during their experiments.

Yeom et al. studied the effect of nonionic polyoxyethylene surfactants on the solubilization rate of individual polycyclic aromatic hydrocarbons from a weathered, coal tar-contaminated soil obtained from a manufactured gas plant (MGP) site[7]. The release of PAHs from the MGP soil exhibited a non-equilibrium behavior. The rate of PAH solubilization was reported to be significantly enhanced by the surfactants. They

concluded that surfactants enhanced PAH release from the test soil mainly by increasing the diffusivity of PAHs (due to swelling of the soil organic matrix), while the increase in solubility resulting from partitioning of PAHs into the micellar pseudophase played a secondary role.

## 2.2 Biodegradation of PAHs

The bioavailability of sorbed organic chemicals is a deciding factor in the applicability of bioremediation processes to contaminated sediments and soils. Sorption of pollutants may prevent contact between the microbes and the contaminant, or it may simply maintain the aqueous phase contaminant concentrations at levels too low to support growth.

Auger et al.[8] investigated two factors accounting for the variability of bioremediation when surfactants are used to increase the bioavailability of PAHs: (1) surfactant toxicity; and (2) the link between microbial metabolism and mass transfer from a solid phase. They found the nonionic surfactants to be nontoxic. They observed that microbial growth was limited by the dissolution of naphthalene once the aqueous phase naphthalene was depleted. They reported that increasing the bioavailability by increasing the interfacial surface area, introducing convective mass transfer, and adding surfactant were all found to reduce growth rate. Their results suggested that different mismatches between solubilization/mass transfer and metabolic capacity might be among the factors responsible for variable bioremediation outcomes.

Chandra et al.[9] studied the biodegradation and desorption of fluorene in estuarine sediment-water slurries. Adsorption of fluorene to sediments with 1.4% organic carbon

was characterized with a linear isotherm. They showed desorption to be both completely reversible and rapid. They further evaluated the rate and extent of fluorene removal in systems containing a fluorene-degrading culture. They reported the rapid degradation of fluorene (after a lag phase) to levels below the detection limit. They concluded that the rate of fluorene disappearance in a biologically active system was controlled by microbial degradation rates and was not limited by desorption.

Mihelcic et al.[10] observed microbial degradation of naphthol, naphthalene, and acenaphthene under aerobic, anaerobic and denitrifying conditions in soil-water systems. They reported that PAHs used in their study were degraded to non-detectable levels under aerobic and denitrifying conditions. Under anaerobic conditions, naphthol was found to be degradable in 15 days, whereas naphthalene and acenaphthene showed no significant degradation over a long period of time. They further showed that low molecular-weight, unsubstituted PAHs were amenable to microbial degradation in soil-water systems under denitrifying conditions.

Bouchez and his co-workers[11] studied the interactions of various PAHs (naphthalene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene) during their biodegradation in pairs, with one PAH at least being used as a carbon source. Inhibition phenomena were often observed, but synergistic interactions were also detected. Naphthalene was found to be toxic to the microorganisms. They noticed that mixed cultures were able to overcome inhibition phenomena, including the toxic effects of naphthalene.

Prince et al.[12] studied the bioremediation of sludge containing waste produced during the refining of lubricant oils in shake flasks with indigenous and other bacterial

sources and nutrient supplementation. They found that the indigenous bacteria were able to degrade the PAHs (naphthalene, phenanthrene, and pyrene) present at some locations at the site. They reported a lack of sufficient nutrients to sustain a sufficient level of microbial growth, particularly at high sludge loading.

Volkering et al.[13] demonstrated that bacterial growth on crystalline or adsorbed PAHs can result in a linear increase in biomass concentration. They likewise found that desorption of substrate from the surface limited microbial growth.

Ye and his co-workers[14] investigated the ability of *Sphingomonas paucimobilis* to metabolize a variety of high molecular weight polynuclear hydrocarbons. This organism was able to degrade several four- and five-ring PAHs varying in molecular size, shape, and chemical structure. They recommended further studies to examine whether *S. paucimobilis* can degrade PAHs in soil from a coal gasification site.

Erickson et al.[15] investigated loss of PAHs while attempting to bioremediate soils from a manufactured gas plant site. They reported that addition of free naphthalene and phenanthrene to the soils resulted in rapid loss of added chemicals, while the concentrations of indigenous chemicals were unchanged. The PAHs in these soils were thus reported to be unavailable for microbial degradation.

Guha et al.[16] developed a model to describe the biodegradation of bioavailable micellar-phase substrate. The hypothesis on which the model was based considered the following steps: (a) the contaminant is transported by filled micelles from the bulk solution to the proximity of the cells; (b) the exchange of the filled micelles with the hemi-micellar layer around the cell delivers the contaminant to the cell; (c) the contaminant diffuses into the cell and is biodegraded. The biodegradation kinetics were

explained in terms of a series of mass-transfer processes, which led to an equation similar in form to the Monod equation. The bioavailable fraction of the micellar-phase substrate was independent of the biomass concentration, and was a function of the surfactant concentration, the polyoxyethylene chain length, and the biomass surface characteristics.

### 2.2.1 Research involving Commercial Surfactants

Efroymson and Alexander[17] observed that naphthalene initially dissolved in a hydrocarbon solvent was mineralized by an *Arthrobacter* strain that did not produce biosurfactants and appeared to be predominantly attached to the solvent. The addition of 0.1% Triton X-100 (greater than CMC) enhanced the rate and extent of naphthalene mineralization, indicating that the surfactant was not toxic at the concentration used. Cell counts from the aqueous phase were greater in the presence of the surfactant, indicating that surfactant prevented the bacteria from adhering to the solvent-water interface.

Laha and Luthy[18] investigated the effects of nonionic surfactants on the biodegradation of phenanthrene in soil-water systems. The surfactants consisted of Brij 30 (dodecylethoxylate, C12E4), Tergitol NP-10 (nonphenylethoxylate, C8PE9.5), and Triton X-100 (octylphenylethoxylate, C8PE9.5), and the bacteria were a mixture of PAH-degrading organisms. At concentrations below the CMC, no significant enhancement or inhibition of phenanthrene degradation was observed as compared to the surfactant-free control, whereas supra-CMC levels resulted in virtually complete inhibition of biodegradation. Possible reasons considered for the inhibition included: (1) toxicity of the surfactant or solubilized hydrocarbon at high concentration; (2) preferential metabolism of the surfactant; (3) lowering of the available substrate concentration in

aqueous solution due to micellization; and (4) interference with microbial membrane processes. Reasons (1) and (2) were experimentally ruled out, and chemical modeling indicated that reason (3) was not a significant concern. Thus, it was hypothesized that the inhibition might be due to interference with substrate transport into the cell, or to reversible physical-chemical interference with enzyme activity and other membrane proteins involved in hydrocarbon degradation. Additional work was suggested to assess the specific mechanisms of surfactant interference on biodegradation.

Laha and Luthy[19] furthered their investigation of the effects of surfactants on the biodegradation of phenanthrene in soil-water systems by expanding to a larger group of commercial, nonionic surfactants. The surfactants used consisted of Brij 30, Triton X-100, Tergitol NP-10, Tween 20, Tween 80, Brij 30/Brij 35 mix, Neodol 25-3/Neodol 25/9 mix, CHAPS and octylglucoside. CHAPS and octylglucoside have relatively high CMCs and low aggregation numbers. As in their previous results, sub-CMC surfactant doses were non-enhancing, and supra-CMC doses for all surfactants tested resulted in complete inhibition of phenanthrene degradation. At sub-CMC levels, only one surfactant, octylglucoside, exhibited an inhibitory response. Results of partitioning experiments for surfactants and phenanthrene between aqueous and hexane phases indicated that exit rates of phenanthrene from micelles should be sufficiently high as to not limit microbial degradation rates. Reversible interference of micellar surfactants with cell membrane processes was considered to be the most likely reason for the observed results.

Mueller et al.[20] used the nonionic surfactant Tween 80 to enhance the solubility and biodegradation of fluoranthene by P. paucimobilis. This was the first report of the primary utilization of a PAH containing four or more rings by a pure microbial culture.

Biodegradation of this poorly soluble chemical was enhanced by surfactant addition up to 2 g/L (well above the CMC) without exhibiting toxicity to the isolate.

Guerin and Jones[21] studied the mineralization of phenanthrene by a Mycobacterium species isolated from sediment and identified by making cell wall lipid comparisons with various genera. A series of nonionic surfactants (Tweens) were used to solubilize the hydrocarbon substrate for the biodegradation assays. All of the surfactants enhanced the aqueous solubility of phenanthrene, and thus potentially the bioavailability of the substrate. None of the Tween surfactants served as growth substrate when present as a sole carbon source.

Aronstein et al.[22] studied the effects of nonionic surfactants at sub-CMC concentrations on the desorption and biodegradation of sorbed aromatic compounds in soil. Low levels of surfactant were chosen: (1) to avoid leaching contaminants into underlying aquifers; (2) because of possible inhibitory effects of the surfactant above the CMC; and (3) for economic considerations in potential field applications. Enhanced mineralization of phenanthrene by a mixed soil consortium was observed for some of the surfactants tested, in both low and high TOC soils.

Aronstein and Alexander[23] further studied the effects of low levels of Alfonic 810-60 and Novel II on desorption and biodegradation of phenanthrene in batch assays using an aquifer sand (0.4% organic matter). Both surfactants significantly enhanced desorption and biodegradation of phenanthrene by indigenous microbiota at surfactant concentrations of 10 and 100 mg/L (both above CMC), but not at lower concentrations. Enhanced partitioning of the PAH to the aqueous phase due to the influence of the surfactants was suggested as the reason for enhancements in biodegradation.

Tiehm[24] used the anionic surfactant SDS and a series of nonionic surfactants to investigate the influence of surfactants on the biodegradation of PAHs. All of the surfactants were capable of solubilizing phenanthrene to varying degrees, and only SDS served as a preferred substrate in the presence of PAH for a mixed population of microorganisms (cultured on phenanthrene). In the presence of SDS, inhibition of phenanthrene degradation increased with an increase in surfactant concentration, and was virtually complete at supra-CMC levels. Using mixed microbial populations, phenanthrene and fluoranthene were biodegraded after solubilizing in a series of eight nonionic surfactants. Utilization of fluorene or pyrene under the same test conditions was dependent on the nonionic surfactant being used, with some surfactants demonstrating an apparent toxicity.

Tiehm et al.[25] studied the effect of two nonionic surfactants (Arkopal N-300 and Sapogenat T-300) on bioavailability of a series of PAHs in manufactured gas plant soil. Both surfactants enhanced the mass transfer rate of sorbed PAH into the aqueous phase. Solubilized PAH were found to be available for biodegradation. Reduction of PAH content of the contaminated soil was obtained in all cases.

Robichaux and Myrick[26] investigated the effects of various commercial dispersants on the biodegradation of weathered, crude petroleum by a mixed microbial culture obtained from a treatment plant's aeration basin. Each dispersing agent was a heterogeneous mixture of surfactants in organic solvents. Increasing the concentration of dispersants was shown to either increase or decrease oxygen uptake rates depending on the dispersant used, suggesting that toxic effects on microorganisms may be of concern with some dispersants.

Roch et al.[27] investigated the possible adverse effects of surfactant addition on the biodegradation of two hydrophobic compounds, biphenyl and phenanthrene. Several of the surfactants tested were found to be toxic to the test bacteria and prevented the biodegradation of biphenyl and phenanthrene at concentrations below the critical micelle concentration.

Liu et al.[28] evaluated the effects of aqueous, micellized nonionic surfactants on the microbial mineralization of naphthalene. They observed that surfactant concentrations above the CMC were not toxic to the microorganisms, and that the presence of surfactant micelles did not inhibit mineralization of naphthalene. Naphthalene was reported to be solubilized by micelles of Brij 30 or Triton X-100 in liquid media and was bioavailable and degradable by the mixed bacterial culture.

Tsomides et al.[29] studied the effect of commercial surfactants on the bioremediation of PAH-contaminated sediments. Phenanthrene degradation was found to be inhibited by all nonionic surfactants studied, except Triton X-100. They suggested that the inhibition of phenanthrene mineralization may be due to the preferential microbial utilization of surfactant over phenanthrene, or due to toxic effects of the surfactants. They noticed a decrease in free aqueous surfactant concentration due to sorption of the surfactant to the sediment.

Churchill et al.[30] examined the effect of three nonionic surfactants (Triton X-45, Triton X-100, and Triton X- 165) on the rate of biodegradation of phenanthrene by pure bacterial cultures. All the surfactants dramatically increased the apparent aqueous solubility of phenanthrene, which led to enhanced biodegradation rates by two *Pseudomonas saccharophila* strains.

Ortega-Clavo et al.[31] studied the influence on biodegradation of varying the rates of partitioning of phenanthrene from nonaqueous-phase liquids to water. They observed that concentrations of the nonionic surfactant Alfonic 810-60 that increased partitioning also inhibited biodegradation.

Jahan et al.[32] studied the influence of nonionic surfactants on the biodegradation of poorly soluble organic compounds in soil and water. They reported that the solubility of phenanthrene was enhanced by the presence of micelles. Sorption of phenanthrene to soil was enhanced significantly in the presence of the surfactants. They reported that low surfactant concentrations promoted mineralization of phenanthrene without inhibitory or toxic effects. Their study indicated that surfactant selection for in-situ bioremediation purposes depends on a number of factors, mainly its hydrocarbon solubilizing power, sorptive properties, low toxicity to bacteria, and fate in the environment.

### 2.2.2 Research involving Natural Biosurfactants

Zhang et al.[33] studied the effect of biosurfactants on the dissolution, bioavailability, and biodegradation of phenanthrene in a series of batch studies. Two forms of the biosurfactant, a monorhamnolipid and a dorhamnolipid, were tested. It was found that both surfactants increased the solubility and enhanced the rate of phenanthrene biodegradation. Monorhamnolipid was more effective than dirhamnolipid for solubilization; however, overall rates of mineralization were essentially the same. Phenanthrene within monorhamnolipid micelles was apparently less bioavailable than phenanthrene within dirhamnolipid micelles. Therefore, the effect of a surfactant on

biodegradation is a combination of the solubilizing power of the surfactant, and the bioavailability of the substrate within the surfactant micelles.

## 2.3 Summary of Literature

The literature results for three model PAHs chosen for this study are summarized in Table 2.1.

# CHAPTER 3

# OBJECTIVES

The objectives of this research were to:

(1) screen potential surfactants for enhancement of bioavailability and biodegradability of three model PAHs: fluorene, phenanthrene, and pyrene.

(2) examine the performance of batch fermenter and bioslurry reactor in degrading the model PAHs in the presence of surfactants and artificially contaminated soil (from Pequest, NJ).

(3) Examine the performance of batch fermenter and bioslurry reactor in degrading the model PAHs in the presence of a real contaminated refinery sludge.

# CHAPTER 4

# MATERIALS AND METHODS

## 4.1 Selection of PAH Compounds

Three model PAH compounds - fluorene, phenanthrene and pyrene - selected for the study are listed in Table 2.1, along with their relevant physical properties. These compounds are often present in PAH contaminated soil or sludge, and are representative of two (fluorene)- and three-ring (phenanthrene and pyrene) PAHs. Some information relating to their solubility and biodegradability is available in the literature (see Chapter 2), and more research was done in the present work, to investigate the effects of a non-ionic surfactant, Makon 10, on the solubility and biodegradation of the model PAHs.

## 4.2 Selection of Surfactants

Commercially available surfactants selected for the study are listed in Table 4.1. These surfactants are all biodegradable, in order to avoid any further contamination of treated soil or sludge. Studies by Tiehm et al.[25] indicated that ionic surfactants were not effective when applied to soil. In the case of cationic surfactants, negatively charged soil particles attract the oppositely charged part of the surfactant molecule, thereby inducing a strong adsorption of surfactant molecules onto the soil particles. On the other hand, anionic surfactants were also not effective when applied to soil, due to the repulsion between the soil particles and surfactant molecules, thereby minimizing the dissolution rate. Therefore, non-ionic surfactants were chosen to avoid significant adsorption or dissolution-limiting effects.

There is a substantial body of information available in the literature on the application of most of these surfactants in biodegradation processes (see Chapter 2).

## 4.3 Selection of Soil

A clean soil from Pequest, NJ was chosen as a model soil in this study because of its similarity to a model soil developed by the US EPA, Edison Laboratory. Soil characteristics are given in Table 4.2.

## 4.4 Sludge Characteristics

Experiments were also performed using a PAH-contaminated refinery sludge obtained from a New Jersey facility. The sludge characteristics are given in Table 4.3.

## 4.5 Preparation of Chemical Solutions

### 4.5.1 Fluorene, Phenanthrene and Pyrene Standards

To prepare stock standards for PAH compounds, carefully weighed quantities were solubilized in known volumes of HPLC grade acetonitrile solution using a magnetic stirrer for 48 hours. The stock solutions were later diluted with acetonitrile to obtain various standards of the model PAH compounds.

### 4.5.2 Surfactant Solutions

The surfactant solutions were prepared in distilled water by dissolving a known amount, and stirring for 24 hours with a magnetic stirrer.

## 4.6 Determination of PAHs

The model PAHs were quantified by high-pressure liquid chromatography (HPLC), using a model 9012 solvent pump, model 9095 autosampler, and model 9065 diode array detector (Varian Instruments Co., Palo Alto, CA) at room temperature. The column was PAH 5U, 4.6 x 150 mm (Whatman Inc., Clifton, NJ). A gradient mixture of acetonitrile and DI water at a flow rate of 1 ml/min was used as the mobile phase. The initial mixture of 43% acetonitrile (57% water) was increased to 90% acetonitrile (10% water) over a 20 minute period. The detector was set at 205 nm for fluorene, 249 nm for phenanthrene, and 239 nm for pyrene. Calibration curves were prepared for fluorene, phenanthrene and pyrene and are shown in Figures 4.1, 4.2, and 4.3, respectively. Equilibration time of 10 minutes was provided between runs to void the system of residual contaminants.

This procedure was arrived at by conducting a series of trial-and-error experiments to optimize peak separation and detection. By this method, the PAH detection limits were: 80 ppb of fluorene, 100 ppb of phenanthrene, and 150 ppb of pyrene. Only the disappearance of PAH was determined in this research. The production of any metabolic products (other than $CO_2$) was not investigated.

## 4.7 Inoculum and Growth Medium

### 4.7.1 Inoculum

Microbial consortia utilized to perform biodegradation experiments were isolated from a 1:1 mixture ratio of the refinery sludge (containing approximately 453 ppm total PAHs) and an activated sludge obtained from the Linden, NJ POTW (which contained no detectable PAHs). A series of three dilutions were prepared to reduce the sludge

concentration and enrich the microbial populations. The dilutions were prepared in the following manner. Approximately 100 ml growth media were each placed into three 250 ml shaker flasks. The first shaker flask was dosed with approximately 2 ml of mixed sludge solution. The shaker flask was maintained at 30 °C for 3 days. Microbial growth was confirmed in the shaker flask by increased turbidity. This culture functioned as the seed for a second shaker flask prepared in a similar manner with 2 ml from the first flask plus 100 ml of growth medium. This in turn provided the seed to a third shaker flask. The microorganisms in the third shaker flask were used as inoculum in all experiments.

### 4.7.2 Growth Medium

The growth medium used in all experiments had the composition listed in Table 4.4. The pH of the growth media was in the range of 7.0 to 7.2.

## 4.8 Dissolved Oxygen and pH Measurement

### 4.8.1 Dissolved Oxygen Concentration

To monitor the dissolved oxygen (DO), an Ingold oxygen measurement system (Ingold Electrode Inc., Wilmington, MA) was used in conjunction with a dissolved oxygen meter (New Brunswick Scientific, NJ; Model No. DO-50). To calibrate the DO meter, the probe was immersed in deionized water contained in a 14 liter Microferm fermenter (working volume 10 liters). Air was bubbled for 4 hours through the water at a rate of 3 liters/min, with continuous stirring at 200 rpm. The temperature was maintained at 32.2 °C. The DO meter was then set at 100%. The probe could not be used for continuous monitoring, since biomass grew on the DO membrane if the probe was left in the reactor

for long periods of time. Therefore, to measure DO in the slurry reactor, the probe was periodically inserted in the uppermost liquid part of the reactor and DO was recorded. According to the DO instruction manual, at 32.2 °C, 100% saturation corresponds to 7 mg/liter of dissolved oxygen. Other measurements were made by considering a linear relationship over the DO meter dial gauge as specified by the manufacturer (New Brunswick Scientific, NJ; Model DO-50). The DO electrode was always stored in 1% KCl solution as recommended by the manufacturer.

### 4.8.2 pH Measurement

The pH was measured directly using an Orion pH electrode (Model 95-56) connected to an Orion Expanded Ion-Analyzer (Model EA 920). Standard buffers of pH 4.0 and 7.0 from Orion were used for calibration.

## 4.9 Selection of Temperature for the Study

In this study, the temperature was selected on the basis of literature reports[19,25] and no experiments were performed to determine the optimal temperature. An operating temperature of 30°C was selected for the shaker flask and batch fermenter studies. To simulate an industrial sludge tank condition, room temperature was used for the bioslurry reactor study.

# CHAPTER 5

# EXPERIMENTAL APPARATUS

Initial screening experiments were conducted in 250 ml shaker flasks, followed by a 500 ml respirometer, a 5 liter batch fermenter, and finally a 10 liter slurry reactor. All experiments were conducted in a batch mode.

## 5.1 Shaker Flask

Initial aerobic biodegradation studies were carried out in 250 ml Erlenmeyer flasks (Curtin Matheson Scientific Co., Houston, TX) with a liquid content of 100-150 ml. The mouths were closed with sterilized tissue paper. An incubator equipped with gyratory shaker (Gallencamp, Serial # SG 93-01-420, New Brunswick Scientific Co., New Brunswick, NJ) was used to mix the solution in the flasks at 140 rpm. The temperature in the shaker was maintained at 30°C for all the experiments conducted unless otherwise indicated.

## 5.2 Respirometer

A 500 ml respirometer (N-CON System Inc., Larchmont, NY) was used to study the metabolism of PAHs and surfactants at different concentrations. The Comput-Ox computerized respirometer consists of three main components: (1) reactor monitoring/data collection system, (2) reactor control unit (RCU)/waterbath, and (3) auxillary cooler/circulation unit. Standard reactors are constructed of borosilicate glass. KOH pellets, used to absorb the carbon dioxide produced during degradation process, are

placed in a holder attached to the underside of the cap (as seen in Figure 5.1). During aerobic metabolism, the microorganisms consume oxygen present in the reactor and produce carbon dioxide, and thus a vacuum would be created inside the reactor. The solenoid valve opens by sensing the vacuum, and releases a measured amount of oxygen (monitored by the data collection system) to bring back the pressure in the reactor. Thus, the procedure is entirely automated.

## 5.3 Batch Fermenter

A 5 liter batch fermenter (New Brunswick Scientific Co., NJ, Model: Bioflo II C) with built in aeration, heating, and stirring systems was used for aerobic degradation of PAHs in batch mode. A schematic of the fermenter is shown in Figure 5.2.

Aeration was controlled automatically to maintain a certain level of dissolved oxygen in the suspension. Temperature was maintained by circulating water through the jacket. There are also four peristaltic pumps to feed different solutions (e.g., acid, base, nutrients, antifoaming agents, etc.).

## 5.4 Slurry Reactor

The 10 liter bench-scale slurry reactor is shown in Figure 5.3.

The reactor is composed of two main sections connected by a flange: an upper cylindrical section, and a conical bottom section (with an air distribution ring). The conical bottom section reduces dead space in the reactor, and the air bubbles provide both oxygen and a degree of agitation. The reactor had dedicated ports for seed inoculation and biomass replenishment, surfactant dosage for desorption, and nutrient addition. In

addition, there were sample ports provided at different locations in the reactor, and a bottom drain. An external agitator was also used to achieve maximum agitation of the sludge-water slurry, thereby minimizing the possibility of settling of the sediments and hence ensuring homogeneity of the slurry under operation. Because of the low vapor pressure of PAHs, exhaust emissions from the batch fermenter and bioslurry reactor were not monitored.

# CHAPTER 6

# EXPERIMENTAL PROCEDURE

## 6.1 Solubilization Experiments

Solubilization experiments were performed to estimate the aqueous phase concentration of PAH compounds at different concentrations of surfactants. Batch tests for solubilization were performed at room temperature (approximately 25 °C). Stock solutions of 0.0075, 0.01, 0.06, 0.1, 0.5, 0.75, and 1.0 % (weight/volume)* for Makon 10 were placed in 40 ml vials with an excess of each contaminant (fluorene, phenanthrene, and pyrene). The solutions were prepared in deionized water. All the experimental vials were filled to avoid any head space, thereby minimizing volatilization losses. The vials were agitated on an orbital shaker at 8 rpm for approximately 48 hours. These samples were then centrifuged at 12,000 rpm for 6 minutes to separate any undissolved PAH. Duplicate vials were prepared for some of the dilutions, as a check. The PAH concentrations were determined over time in each vial by HPLC. *Note: 1% (w/v) means 1 g surfactant per 100 ml of solution.

## 6.2 Respirometric Experiments

### 6.2.1 Respirometric Studies for Biodegradation of Surfactants

The biodegradability of the all the surfactants listed was assessed using the mixed bacterial culture, by performing respirometric experiments. 300 ml of 0.3% (w/v) surfactant in growth medium were added to 500 ml respirometer bottles. Then 5 ml of mixed bacterial inoculum were added to each bottle. 6-7 pellets of KOH were added to

the KOH pot inside the bottle and the bottles were closed. The oxygen utilization was noted for each surfactant over a period of 185 hours.

### 6.2.2 Respirometric Studies to Assess Inhibition of Makon 10 on Microorganisms

Based on the respirometric study for the surfactant biodegradation, Makon 10 was chosen as a model surfactant (see Chapter 7, Results and Discussion). In order to assess the inhibition or toxicity effects of Makon 10 on microorganisms, additional respirometric experiments were conducted, utilizing the surfactant as sole carbon source. 300 ml of Makon 10 at concentrations of 0.01, 0.1, 0.2, 0.5, and 1.0 % (weight/volume) in growth medium were placed in the respirometer bottles and inoculated with 5 ml microbial culture. One respirometer bottle served as a control which contained 300 ml deionized water and was not inoculated. The oxygen uptake data were recorded over time.

### 6.2.3 Respirometric Studies with Sludge

Respirometric experiments were conducted using 20 grams of sludge in 100 ml of 0.3% (w/v) Makon 10 solution [i.e., 20% (w/v) sludge] to: (i) verify the biodegradation of PAHs and, (ii) assess the toxicity of the sludge on microorganisms.

300 ml DI water were placed in two respirometric bottles and the 20% (w/v) sludge mixture was added to the bottles. One bottle contained 0.1% glucose in addition to the surfactant and sludge. The oxygen utilization was noted as a function of time.

## 6.3 Aerobic Biodegradation of PAHs by Mixed Bacterial Culture

### 6.3.1 Aqueous Phase Biodegradation Studies

Shaker flask studies were conducted to assess the batch-mode degradation of the model PAHs, both in the absence and presence of Makon 10. All the biodegradation studies were performed in 250 ml Erlenmeyer flasks, with working volume of about 150 ml.

**6.3.1.1 Shaker Flask without Surfactant:** First, the PAH solution was prepared by dissolving a known amount of PAH individually in a known volume of growth medium and mixing for 48 hours using a magnetic stirrer. This solution was then filtered using Whatman filter paper (#1). The filtered solution (150 ml) was poured in several 250 ml Erlenmeyer flasks, which were autoclaved at 130 °C. One of the flasks served as a control. In the other flasks, 2 ml of mixed bacterial culture were added, and the flasks were loosely stoppered with sterilized tissue paper to minimize contamination. The pH of all flasks was 7.1. The flasks were kept in a gyratory shaker at 30°C and 200 rpm. Initial samples were taken from each flask in a vial, centrifuged to separate biomass, and then filtered using a 50 μm membrane filter. The filtrate was analyzed for PAHs using HPLC. Each day, samples were withdrawn for HPLC analysis.

**6.3.1.2 Shaker Flask with Surfactant:** Makon 10 was added to PAH solutions in growth medium in order to make a final concentration of 0.01% (below CMC) and 0.3% (w/v) (supra-CMC concentration). This solution was mixed for 48 hours using a magnetic stirrer, in order to achieve the maximum solubilization of the PAHs. Then the

solution was filtered using #1 Whatman filter paper under vacuum, to eliminate any unsolubilized PAHs. This filtered solution was then autoclaved at 130 °C and inoculated with 2 ml bacterial culture. The experimental flasks were incubated in a gyratory shaker at 200 rpm, and samples were withdrawn periodically and analyzed via HPLC.

**6.3.1.3 Shaker Flask with Surfactant and Soil:** Biodegradation of the model compounds in the presence of Pequest soil and surfactant (Makon 10) was assessed. The soil was sieved and only particles that passed through No. 12 mesh were used for the experiments. The soil was artificially contaminated by placing a known amount of soil in a petri dish and adding a PAH-acetonitrile solution to cover the soil. Then the petri dish was placed in a fume hood overnight to evaporate the solvent (i.e., acetonitrile). Once the solvent was evaporated, contaminated soil (20%) was placed in Erlenmeyer shaker flasks and 150 ml of growth medium solution with 0.3% Makon 10 were added to it. The flasks were agitated on an orbital shaker for 48 hours to achieve maximum desorption of PAH from the soil into the aqueous phase. These flasks were then inoculated with 2 ml bacterial culture. Samples were taken periodically to assess the biodegradation rate of PAHs.

**6.3.1.4 Shaker Flask with Sludge:** The biodegradation of the three target compounds in the sludge was investigated in 0.3% Makon 10. 150 ml of growth medium with 0.3% Makon 10 were added to 20% (w/v) sludge mixture and stirred for 48 hours. Then the

solution was inoculated with 2 ml of mixed culture, and samples were taken periodically. These experiments were conducted in duplicate.

### 6.3.2 Batch Fermenter Studies with Sludge

The batch fermenter vessel was cleaned with soap solution and dried by purging with compressed air. 2 liters of 0.3% Makon 10 in growth medium were poured in the reactor vessel. 20% sludge was added to the reactor. The temperature was 30°C, and the impeller speed was 200 rpm. The contents of the reactor vessel were allowed to mix for 48 hours, and then about 5 ml of mixed bacterial inoculum was added to the reactor through the inoculation port at the top. Samples were withdrawn at different times through the sampling port, and analyzed by HPLC.

### 6.3.3 Bioslurry Reactor Studies

The slurry reactor vessel was cleaned with soap and dried by purging with compressed air. The reactor was charged with 4 liters of 0.3 % Makon 10 in growth medium. 20% sludge was added to the reactor. The impeller speed was 200 rpm. The contents of the reactor were agitated for 48 hours, and then about 10 ml of inoculum were added to the reactor through the inoculation port. Samples were withdrawn at different intervals and analyzed by HPLC.

## 6.4 Sorption Experiments

The sorption experiments were performed according to the US EPA protocols for the sorption of environmental pollutants on soils (Annual book of ASTM Standards, E 1195-87). The equilibration time of the model PAHs was initially estimated by recording the

concentration of solutes in fixed time intervals. It was assumed that the system had reached equilibrium when the rate of change of solute concentration between two consecutive measurements in a twelve hour interval was within 5%. An equilibrium time of about 48 hours was obtained from these preliminary studies. The soil-to-solution ratios that generated acceptable isotherms in the range of surfactant dosages were also determined by preliminary experiments, and it ranged from 0.1% to 1% (w/v). Some isotherms were also generated at surfactant concentrations below CMC. The Pequest soil was used. Different soil weights were transferred into 40 ml vials. Then 25 ml of surfactant-contaminant solution were added to each vial, and the vial was sealed with a teflon cap. For each surfactant concentration, several vials were prepared in duplicate and the experiments were repeated for different initial concentrations of surfactant. Two extra vials were prepared, one with soil and uncontaminated water, and one with surfactant-contaminant solution without soil, to serve as controls. The vials were placed on a tumbler and allowed to equilibrate for 48 hours. The concentrations of the model compounds in solution were determined by HPLC.

### 6.5 Desorption Experiments

These experiments were conducted to determine any adsorption of PAHs on sludge or soil particles during biodegradation. About 40 ml suspension mixture was collected in a 40 ml vial from the reactor at the end of each experiment and centrifuged to separate the solids from the suspension. Supernatant was removed, and 5 ml acetonitrile was added to the vial and shaken for 4-5 hours to desorb any PAHs. Samples taken from the vial were analyzed by HPLC.

# CHAPTER 7

# RESULTS AND DISCUSSION

## 7.1 PAH Solubility Enhancement

Ideally, a surfactant should increase the aqueous solubility and soil desorption of the contaminants, it should have low affinity for the soil, and it should be biodegradable but with degradation rates lower than the substance it solubilizes. The following results are part of a screening procedure for the identification of solubility and biodegradation enhancers of PAHs that satisfy the aforementioned criteria.

The model surfactant Makon 10 substantially increases the solubility of the model compounds, with the solubility increasing linearly with surfactant concentration above the CMC of 0.005%. The solubilization results are summarized in Table 7.1, and plotted in Figure 7.1. It was observed that the solubility of fluorene increased from 2.3 mg/l (with 0.05% surfactant) to 95.5 mg/l (when 1% surfactant was used). Similarly, the aqueous phase concentrations of phenanthrene and pyrene increased to 250 mg/l and 188 mg/l respectively, with 1% Makon 10 (the solubility of pyrene in water without addition of any solubility-enhancers is 0.14 mg/l). Therefore, the solubility of PAHs was increased by 2-3 orders of magnitude.

Solubilization results were not attempted with Makon 10 concentrations above 1% because of the following: (1) the respirometric experiments indicated an inhibition effect for non-ionic surfactant concentrations of 1%, thereby indicating the optimal surfactant concentration to be below 1%; (2) the economical viability of process scale-up with addition of higher concentrations of surfactant is questionable; and (3) higher

surfactant concentrations cause foaming in commercial reactors, with entrainment of the slurry.

### 7.2 Results of Sorption Experiments on Pequest Soil

Sorption experiments were performed according to the US EPA protocols to determine the adsorption isotherms of the three PAHs in the presence of surfactants on the Pequest soil (2.1% TOC). The experiments were performed with Makon 10 at concentrations above the CMC, and are plotted in Figures 7.2, 7.4, and 7.5. The sorption isotherm is also plotted for fluorene at below CMC (Figure 7.3). It is apparent from these results that the sorption characteristics of the soil change substantially as the concentration of the surfactant increases, and that changes in contaminant solubility influences the overall sorption process. As the concentration of the surfactant increases, the adsorption becomes less favorable and the isotherms approach zero slope. For all three PAHs, there was no adsorption at or above 0.3% Makon 10. Therefore, 0.3% Makon 10 was chosen to perform the biodegradation experiments with soil and sludge, in order to minimize the adsorption of PAHs onto the soil. Moreover, there was no inhibition at 0.3% Makon 10 to microbial growth.

The interactions between soil particles, surfactant micelles, and aqueous phase are not as yet well understood. However, sorption studies of the compounds and surfactants of interest can reveal useful information on the behavior of the system. Various studies have documented the importance of surfactant sorption on the mobilization of contaminants in the subsurface (Edwards, Luthy). Since surfactants themselves adsorb on the soil, they increase the organic carbon content and they can, in some instances,

increase the soil sorption capacity. Depending on the soil characteristics and application of surfactants, the release rates of the contaminants can either increase or decrease due to complex sorption effects.

## 7.3 Results of Respirometric Experiments

### 7.3.1 Biodegradability of Surfactants

All the surfactants listed in the study are biodegradable, as determined using the mixed bacterial culture in respirometric experiments. The surfactants were added to growth media to make a final concentration of 0.3% (w/v) as sole carbon source for microbial metabolism. Oxygen utilization was noted for each surfactant and the experiment was conducted for 185 hours. The data are presented in Table 7.2 and Figure 7.6. The comparative rates of biodegradation were assessed based on the amounts of oxygen consumed for microbial metabolism. These results were in general agreement with those presented by the manufacturers, except for Tergitol NP-10 which was not biodegradable within the duration of this study. The maximum oxygen utilization was observed for Brij 30, which exhibited no lag phase. Significant oxygen consumption was also observed with Novel II, Ninol 40-CO, Adsee 799, and Triton X-100. A longer lag-phase was observed when Adsee 799 was used as sole substrate.

Makon 10 was chosen as the model surfactant since it was biodegradable, but at a slower rate than other surfactants tested.

### 7.3.2 Effect of Surfactant Concentration on Microorganisms

In order to assess the inhibition effects of surfactant concentration on the microorganisms, respirometric experiments were conducted as described in Chapter 5, utilizing Makon 10 as the sole carbon source (at concentrations of 0.01, 0.1, 0.2, 0.5, and 1.0% weight/volume). The results are shown in Table 7.3 and Figure 7.7. In all cases, Makon 10 was biodegradable after a lag phase of approximately 10 hours, with no observable inhibition effects.

### 7.3.3 Respirometric Studies with Sludge

Respirometric studies were conducted (with 0.01% glucose, and without glucose) to test the biodegradation of PAHs by microorganisms in homogenized sludge samples. Table 7.4 and Figure 7.8 show the oxygen uptake by microorganisms in 20% (w/v) slurries, which confirmed that the sludge did not have any toxic effects on microbial growth. As expected, the oxygen uptake rate was higher in the presence of 0.01% glucose.

## 7.4 Aerobic Biodegradation of PAHs as Sole Carbon Source

Biodegradation experiments for aerobic degradation of PAHs as sole carbon source by mixed bacterial consortia were conducted in shaker flasks, the batch fermenter, and bioslurry reactor, as described in Chapter 6.

### 7.4.1 Initial Results of Shaker Flask Experiments

Four different preliminary experiments were conducted to explore the effects of surfactants on the degradation of PAHs. Initially, the biodegradation of PAHs as sole

carbon source by the mixed bacterial consortia was investigated, both in the absence and presence of Makon 10.

**7.4.1.1 Shaker Flasks without Surfactant:** The biodegradation of fluorene, phenanthrene, and pyrene was investigated in the absence of Makon 10. This experiment was conducted in duplicate flasks and an additional pair of flasks without inoculum was prepared and served as control. The results are illustrated in Table 7.5 and Figure 7.9. The data indicate no significant change in PAH concentrations after eight days of incubation, which is confirmation of their lack of bioavailability in the absence of surfactant.

**7.4.1.2 Shaker Flasks with Surfactant:** Experiments conducted in duplicate with 0.01% (below CMC) and 0.3% (above CMC) Makon 10 under identical conditions have shown significant degradation of the contaminants, as presented in Tables 7.6 and 7.7, respectively. The concentration-time profiles for the three PAHs, with 0.01% and 0.3% Makon 10, are plotted in Figures 7.10 and 7.11, respectively. A 95% reduction in fluorene and 90% reduction in phenanthrene concentration with 0.01% Makon 10 was achieved within 24 hours of incubation in the test flasks. Pyrene could not be detected in the aqueous phase in the presence of 0.01% Makon 10 (the detection limit was 0.15 ppm). But with 0.3% Makon 10, significant reduction in pyrene concentration from 27.2 ppm to 0 ppm was observed in 15 days of incubation. A slight increase in pyrene concentration was observed within the first day of incubation. This increase in pyrene concentration

was also seen in experiments where higher concentrations of surfactant were used and suggests a lag between pyrene availability and degradation. A comparison of the results presented in Figures 7.9, 7.10, and 7.11 reveals that higher degradation rates are attained in the presence of surfactant. Visual observation of shaker flasks dosed with surfactant indicates a high concentration of the biomass. This observation can be attributed to the fact that the surfactant provides an additional carbon source to the inoculum as well as increasing PAH availability.

**7.4.1.3 Soil-water Slurry Phase Studies with Surfactant:** Biodegradation studies were also conducted using the Pequest soil with 2.1% total organic carbon (TOC). The results obtained for this soil, using 0.3% Makon 10, a 1:5 soil-to-solution ratio (w/v) [i.e., 1 gram soil per 5 ml solution], and initial concentrations of: 11.5 ppm fluorene, 1.2 ppm phenanthrene, and 1.8 ppm pyrene in the aqueous phase, are presented in Table 7.8 and Figure 7.12. Compared to earlier experiments in the absence of soil, using 0.3% Makon 10 and model PAHs, the biodegradation rates with soil follow the same pattern, except that the initial aqueous concentrations of PAHs are lower in the presence of soil. This decrease in the initial concentration of PAHs could be attributed to one or more of the following reasons: (1) surfactant may sorb onto the surfaces of the soil particles, and hence be unavailable for PAH solubilization; (2) sorbed surfactant can enhance the capacity of the solid to act as a sorbent for PAHs, thereby reducing the PAH concentration in the aqueous phase; (3) direct sorption of PAHs on to soil organic matter may result in lower concentrations in the aqueous phase. The biodegradation data for the

soil revealed that all three model PAHs were below their detectable limits after 10 days of incubation.

**7.4.1.4 Biodegradation of PAHs in Sludge:** Biodegradation experiments were performed in the shaker flasks with 1:5 sludge-solution ratio, in the presence of 0.3% Makon 10. The results for the three model PAHs are presented in Table 7.9 and Figure 7.13. Significant degradation was achieved within seven days of incubation. The slight decline in PAH concentrations in the controls could be due to some biological contamination during the course of the experiment.

### 7.4.2 Biodegradation of PAHs in Batch Fermenter

The results of a typical experiment for the biodegradation of PAHs using 0.3% Makon 10 and 20% (w/v) sludge are given in Table 7.10 and Figure 7.14. Bacterial growth medium was used in the experiments. The pH was maintained between 6.9-7.0, and the temperature at 30°C. No PAH was detected in the reactor after seven days, which again may be due to a mass transfer limitation in these batch experiments.

### 7.4.3 Biodegradation of PAHs in Bioslurry Reactor

Experiments were conducted in the bioslurry reactor to determine the biodegradation rate and oxygen mass transfer coefficient. A 1:5 sludge to water ratio was used with 0.3% Makon 10. The results for this experiment are presented in Table 7.11 and Figure 7.15. On the $17^{th}$ day, 0.3% Makon 10 was again added to the reactor and the concentration of

all three PAHs increased in the aqueous phase. On day 24, nutrients were added to the reactor and degradation of the PAHs proceeded. No PAHs were detected after 30 days. On day 32, another dose of 0.3% Makon 10 was added, but no further detection of PAHs was observed. The results were comparable to the experiments in the Bioflo reactor. Clearly, this process is strongly mass transfer dependent, and the continuous presence of surfactant is needed to maintain a high desorption rate. At the end of these experiments, a mass balance was conducted for each PAH.

**7.4.3.1 Mass Balance in the Slurry Reactor:** For the mass balance, a known volume of the slurry was placed in a 40 ml vial from the bioslurry reactor and centrifuged. The supernatant was removed and acetonitrile was added to the vial. The vial was shaken for 10 hours to desorb the PAHs from the solids. A sample was taken from the vial and centrifuged, and the supernatant was analyzed for PAHs using HPLC.

**Mass balance for Fluorene:**

| | |
|---|---|
| Amount of original sludge: | 400 g |
| Fluorene in original sludge: | 38.12 mg |
| Fluorene in aqueous phase with 0.3% Makon 10 at the start of the experiments: | 21.64 mg (5.41 mg/l × 4 l) |
| Fluorene extracted from the sludge after day 15: | 17.20 mg |

$$38.12 \text{ mg} \approx 21.64 \text{ mg} + 17.20 \text{ mg} = 38.84 \text{ mg}$$

| | |
|---|---|
| Fluorene in aqueous phase after added dose of Makon 10 on day 18: | 15.84 mg (3.96 × 4 l) |

| | |
|---|---|
| Fluorene extracted from the sludge after day 30: | 0 mg |

Thus, 102% (38.84/38.12) of the fluorene was accounted for after day 18, and 92% (15.84/17.20) after day 30.

**Mass balance for Phenanthrene**

| | |
|---|---|
| Amount of original sludge: | 400 g |
| Phenanthrene in original sludge: | 32.40 mg |
| Phenanthrene in aqueous phase with 0.3% Makon 10 at the start of the experiments: | 31.96 mg (7.99 mg/l × 4 l) |
| Phenanthrene extracted from the sludge after day 15: | 0 mg |

32.40 mg ≈ 31.96 mg

| | |
|---|---|
| Phenanthrene in aqueous phase after added dose of Makon 10 on day 18: | 0 mg |
| Phenanthrene extracted from the sludge after day 30: | 0 mg |

Thus, 99% (31.96/32.40) of the phenanthrene was accounted for.

**Mass balance for Pyrene**

| | |
|---|---|
| Amount of original sludge: | 400 g |
| Pyrene in original sludge: | 64.98 mg |
| Pyrene in aqueous phase with 0.3% Makon 10 at the start of the experiments: | 51.88 mg (12.97 mg/l × 4 l) |
| Pyrene extracted from the sludge after day 15: | 12.64 mg |

$64.98 \text{ mg} \approx 51.88 \text{ mg} + 12.64 \text{ mg} = 64.52 \text{ mg}$

Pyrene in aqueous phase after added dose of Makon 10 on day 18: 12.84 mg (3.21 × 4 l)

Pyrene extracted from the sludge after day 30: 0 mg

Thus, 99% (64.52/64.98) of the pyrene was accounted for after day 18, and 102% (12.84/12.64) after day 30.

The mass balance studies showed that virtually all PAHs were desorbed from the solids, and approximately 100% biodegradation was achieved.

#### 7.4.3.2 Evaluation of Oxygen-Transfer Coefficient ($K_La$)

The $K_La$ value was determined by considering the uptake of oxygen by microorganisms. The oxygen level is maintained at 3.4 mg/l, and the oxygen is used by the microorganisms as rapidly as it is supplied.

In equation form,

$$dC/dt = K_La(C_s - C) - r_M \tag{7.1}$$

Where

$C$ – is the concentration of oxygen in the solution, mg/l;

$C_s$ – is the saturation concentration of oxygen in solution at room temperature, mg/l;

$K_La$ – is the overall mass transfer coefficient, $s^{-1}$; and

$r_M$ – is the rate of oxygen used by the microorganisms.

Since the oxygen level is maintained constant, dC/dt is zero, and thus

$$K_La = r_M/(C_s - C) \tag{7.2}$$

To calculate the rate of oxygen used by the microorganisms ($r_M$), the dissolved oxygen (DO) uptake was noted as a function of time. The data are given in Table 7.12.

$$\text{Rate } (r_M) = dC/dt = k\,(C_i - C) \qquad (7.3)$$

Where

$C_i$ – is the applied DO concentration of 3.4 mg/l; and

k – is the respiration rate constant.

$\ln(C_i - C)$ versus t was plotted (Figure 7.16) to find the slope, k, which was found to be 0.15 $min^{-1}$.

Therefore, $r_M = 0.15\,(3.4 - 1) = 0.36$ mg/l . min

Now, the overall oxygen transfer coefficient is:

$K_La = r_M/(C_s - C) = 0.36/(9.21 - 3.4) = 0.062\ min^{-1} = 3.72\ h^{-1}$

Where, the saturation concentration at 19.2°C is 9.21 mg/l (assumed the same for distilled water).

# CHAPTER 8

# CONCLUSIONS AND RECOMMENDATIONS

## 8.1 Conclusions

- It was experimentally verified that concentrations of non-ionic surfactants below 1% are capable of large increases, up to two to three orders of magnitude, in the solubility of model PAH compounds (fluorene, phenanthrene, and pyrene).
- The following surfactants were tested: Adsee 799, Alfonic, Brij 30, Makon 10, Ninol 40-CO, Novel II, Tergitol NP-10, and Triton X-100. Except for Tergitol NP-10 which appeared to be non-biodegradable in respirometric experiments, Makon 10 was the least biodegradable (although still significant), and was therefore chosen as the preferred surfactant for further study.
- No inhibition in microbial growth was observed up to 1% (w/v) Makon 10.
- No significant change in PAH concentration was observed after eight days of incubation in the absence of surfactant. In shaker flasks without soil or sludge, nearly complete biodegradation of all three PAHs was achieved after about 15 days with 0.3% Makon 10 (above CMC). In the presence of refinery sludge, the process is clearly mass transfer limited unless the surfactant concentration is maintained at a sufficient level. Nevertheless, with sufficient surfactant, biodegradation of all three PAHs proceeded to the detection level.

## 8.2 Recommendations

- Biodegradation studies should be performed in a continuousp reactor, under both aerobic and anaerobic conditions.
- Studies should be performed with mixed substrates.
- Pilot-scale studies, using industrial real effluents, should be undertaken.

# APPENDIX A

# TABLES

**Table 2.1** *Literature summary*

| Model Compound | No. of Rings | Molecular Weight | Aqueous Solubility @ 20°C | Literature Results | |
|---|---|---|---|---|---|
| | | | | **Enhancement of Biodegradation** | **Inhibition of Biodegradation** |
| Fluorene | 2 | 153 | 1.900 | (10), (12), (13) | |
| Phenanthrene | 3 | 178 | 0.816 | (12), (13), (24)<br>Tweens, above CMC (21)<br>Alfonic 810-60, Novel II, above CMC (23) | Triton X 100, Tergitol NP 10,Brij 35, Brij 30, above CMC (3)<br>Triton X 100, Brij 30, Tergitol NP 10, above CMC (18)<br>SDS, above CMC (24)<br>Below CMC (27)<br>(31) |
| Pyrene | 3 | 202 | 0.148 | (12), (13) | |

**Table 4.1** *Surfactants used in the study*

| Surfactant Name | Manufacturer | Average Molecular Weight |
|---|---|---|
| **Adsee 799** | Witco | 610 |
| **Alfonic** | Vista Chemicals | 354 |
| **Brij 30** | Aldrich Chemicals | 363 |
| **Makon 10** | Stephan Co. | - |
| **Ninol 40-CO** | Stephan Co. | - |
| **Novel II** | Vista Chemicals | 532 |
| **Tergitol NP-10** | Union Carbide | 682 |
| **Triton X-100** | Aldrich Chemicals | 628 |

**Table 4.2** *Soil Characteristics*

| | |
|---|---|
| **Gravel, %** | 0 |
| **Sand, %** | 44 |
| **Silt, %** | 44 |
| **Clay, %** | 12 |
| **TOC, %** | 2.1 |
| **pH** | 5.2 |

**Table 4.3** *Sludge Characteristics*

| | |
|---|---|
| **pH** | 7.11 |
| **Solids Content, %** | 47.1 |
| **Bulk Density, g/ml** | 1.19 |
| **TPH by IR, ppm** | 137,000 |
| **Oil & Grease, ppm** | 222,000 |
| **Total Acid/Base Neutrals, ppb** | 481,000 |
| **Total PAHs, ppb** | 453,000 |
| **Heterotrophs, #/g** | 2.32E+07 |
| **Oil Degraders, #/g** | 3.44E+06 |
| **Phenanthrene, mg/kg sludge** | 241.30 |
| **Pyrene, mg/kg sludge** | 111.12 |
| **Fluorene, mg/kg sludge** | 110.25 |

**Table 4.4** *Composition of Growth Medium*

| Compound | Amount |
|---|---|
| **Sodium Phosphate (Dibasic)** | 11.2 g |
| **Pottasium Phosphate monobasic** | 5.7 g |
| **Ammonium Sulfate** | 500 mg |
| **Magnesium Sulfate** | 100 mg |
| **Manganese Sulfate** | 10 mg |
| **Ferric Chloride** | 5 mg |
| **DI Water** | 1 literr |

**Table 7.1** *Solubility of model compounds in Makon 10*

| Makon 10 (w/v%) | Fluorene (mg/L) | Phenanthrene (mg/L) | Pyrene (mg/L) |
|---|---|---|---|
| 0.00 | 1.90 | 0.82 | 0.15 |
| 0.05 | 2.30 | 7.37 | 17.25 |
| 0.10 | 6.27 | 22.15 | 22.40 |
| 0.50 | 45.82 | 128.15 | 95.00 |
| 0.75 | 69.44 | 166.76 | 150.25 |
| 1.00 | 95.54 | 250.17 | 188.20 |

Table 7.2 *Oxygen utilization (in mg) by microbial consortia in the presence of 0.3% surfactant*

| Time (Hours) | Adsee 799 | Brij 30 | Makon 10 | Ninol 40-CO | Novel II | Tergitol NP-10 | Triton X-100 |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 0 | 2.29 | 0 | 0 | 0 | 0 | 0 |
| 10 | 101.77 | 135.59 | 0 | 278.84 | 28.93 | 0 | 546.45 |
| 15 | 306.18 | 1367.41 | 0 | 671.86 | 482.30 | 0 | 844.31 |
| 20 | 520.86 | 2553.26 | 24.6 | 854.80 | 1417.96 | 0 | 1003.32 |
| 25 | 744.95 | 3651.75 | 91.7 | 994.22 | 2336.75 | 0 | 1148.89 |
| 30 | 970.74 | 4476.81 | 156.56 | 1106.21 | 3043.36 | 0 | 1296.69 |
| 35 | 1160.60 | 5062.89 | 212.48 | 1193.06 | 3648.67 | 0 | 1401.95 |
| 40 | 1324.81 | 5520.26 | 266.16 | 1261.63 | 4159.94 | 0 | 1478.09 |
| 50 | 1596.77 | 6274.11 | 360.10 | 1378.19 | 4999.18 | 0 | 1596.79 |
| 55 | 1720.78 | 6618.86 | 400.36 | 1426.19 | 5363.34 | 0 | 1650.54 |
| 60 | 1837.09 | 6931.44 | 433.91 | 1476.47 | 5696.14 | 0 | 1708.76 |
| 65 | 1946.56 | 7223.33 | 462.99 | 1517.61 | 5990.36 | 0 | 1769.23 |
| 70 | 2045.76 | 7522.11 | 492.06 | 1558.75 | 6243.58 | 0 | 1827.46 |
| 75 | 2134.71 | 7816.30 | 516.67 | 1597.60 | 6441.33 | 0 | 1865.53 |
| 80 | 2211.68 | 8105.89 | 545.74 | 1638.74 | 6607.73 | 0 | 1903.60 |
| 90 | 2335.68 | 8636.71 | 599.42 | 1714.16 | 6930.89 | 0 | 2022.29 |
| 95 | 2380.15 | 8896.37 | 677.61 | 1769.02 | 7133.47 | 0 | 2111.87 |
| 100 | 2412.65 | 9149.13 | 762.70 | 1862.72 | 7333.63 | 0 | 2212.65 |
| 105 | 2435.74 | 9392.70 | 854.41 | 2052.42 | 7531.38 | 0 | 2315.67 |
| 110 | 2455.41 | 9620.19 | 937.16 | 2456.98 | 7717.08 | 0 | 2411.97 |
| 115 | 2473.37 | 9843.08 | 1008.74 | 3229.53 | 7902.77 | 0 | 2492.59 |
| 120 | 2492.19 | 10059.0 | 1071.37 | 3766.65 | 8081.23 | 0 | 2557.53 |
| 125 | 2512.71 | 10265.8 | 1122.81 | 4150.64 | 8242.80 | 0 | 2618.00 |
| 130 | 2532.38 | 10463.5 | 1174.25 | 4438.63 | 8385.06 | 0 | 2673.99 |
| 135 | 2548.63 | 10663.4 | 1207.80 | 4708.34 | 8510.44 | 0 | 2714.30 |
| 140 | 2563.17 | 10867.9 | 1241.35 | 4973.47 | 8655.11 | 0 | 2750.13 |
| 145 | 2572.58 | 11072.4 | 1274.91 | 5227.18 | 8823.88 | 0 | 2788.20 |
| 155 | 2592.25 | 11484.4 | 1342.01 | 5723.17 | 9166.27 | 0 | 2862.11 |
| 160 | 2600.80 | 11679.0 | 1371.08 | 5956.30 | 9327.81 | 0 | 2891.22 |
| 165 | - | 11874.4 | 1397.92 | 6191.72 | 9482.12 | 0 | 2918.09 |
| 170 | - | 12065.1 | 1420.29 | 6427.14 | 9634.03 | 0 | 2942.73 |
| 175 | - | 12253.5 | 1447.13 | 6667.14 | 9785.93 | 0 | 2965.12 |
| 180 | - | 12432.7 | 1467.26 | 6902.56 | 9928.18 | 0 | 2987.52 |
| 184 | - | 12575.2 | 1480.68 | 7092.27 | 10036.6 | 0 | 2996.48 |

Table 7.3 *Inhibition of Makon 10 on microbial growth*

| Time (Hours) | 0.01% | 0.1% | 0.2% | 0.5% | 1.0% |
|---|---|---|---|---|---|
| 0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1 | 0.8 | 0.0 | 8.8 | 0.0 | 0.0 |
| 5 | 13.7 | 0.0 | 23.9 | 0.0 | 0.0 |
| 10 | 26.6 | 0.0 | 37.4 | 0.0 | 14.1 |
| 15 | 55.6 | 13.7 | 62.9 | 24.1 | 66.7 |
| 20 | 141.6 | 82.7 | 125.9 | 85.3 | 194.5 |
| 25 | 334.1 | 145.4 | 220.7 | 175.0 | 372.5 |
| 30 | 400.3 | 159.0 | 249.4 | 232.7 | 560.7 |
| 35 | 452.8 | 209.6 | 262.9 | 320.7 | 733.2 |
| 40 | 512.2 | 269.0 | 341.8 | 413.7 | 801.4 |
| 45 | 578.4 | 364.6 | 447.8 | 572.3 | 865.0 |
| 50 | 671.2 | 452.9 | 541.8 | 678.4 | 900.2 |
| 55 | 745.8 | 528.4 | 631.9 | 736.1 | 930.8 |
| 60 | 793.0 | 596.7 | 733.1 | 768.9 | 969.2 |
| 65 | 850.1 | 633.6 | 822.3 | 787.0 | 930.8 |
| 70 | 892.7 | 654.5 | 937.0 | 806.0 | 969.2 |
| 75 | 930.8 | 687.5 | 1047.0 | 856.8 | 1018.6 |
| 80 | 965.0 | 722.0 | 1119.5 | 912.8 | 1170.8 |
| 85 | 965.0 | 755.7 | 1179.3 | 993.9 | 1274.3 |
| 105 | 968.8 | 759.7 | 1184.9 | 1037.9 | 1340.2 |
| 110 | 968.8 | 760.5 | 1199.2 | 1106.0 | 1391.1 |
| 115 | 1009.1 | 795.9 | 1270.9 | 1163.7 | 1395.8 |
| 120 | 1050.2 | 835.2 | 1339.5 | 1205.1 | 1403.6 |
| 125 | 1095.9 | 863.3 | 1404.8 | 1229.2 | 1453.1 |
| 130 | 1152.2 | 901.1 | 1470.9 | 1265.4 | 1497.0 |
| 135 | 1223.0 | 941.2 | 1535.5 | 1306.8 | 1545.6 |
| 140 | 1314.2 | 982.2 | 1598.4 | 1357.7 | 1601.3 |
| 145 | 1396.5 | 1020.7 | 1658.2 | 1408.5 | 1654.6 |
| 150 | 1431.5 | 1058.5 | 1686.9 | 1463.7 | 1705.6 |
| 155 | 1433.8 | 1101.0 | 1686.9 | 1524.9 | 1758.1 |
| 160 | 1472.6 | 1143.6 | 1706.8 | 1579.2 | 1788.7 |
| 165 | 1506.9 | 1182.9 | 1732.3 | 1632.6 | 1818.5 |
| 170 | 1540.4 | 1220.7 | 1758.6 | 1683.5 | 1879.6 |
| 175 | 1570.8 | 1253.6 | 1781.7 | 1714.5 | 1936.1 |
| 180 | 1601.2 | 1279.3 | 1803.2 | 1737.1 | 1991.8 |

**Table 7.3 (Continued)** *Inhibition of Makon 10 on microbial growth*

| 185 | 1632.4 | 1306.6 | 1823.1 | 1781.7 | 2044.3 |
|---|---|---|---|---|---|
| 190 | 1666.7 | 1335.5 | 1841.4 | 1818.0 | 2092.9 |
| 195 | 1701.7 | 1362.8 | 1856.6 | 1850.7 | 2140.0 |
| 200 | 1741.3 | 1391.8 | 1872.5 | 1884.3 | 2183.1 |
| 205 | 1783.1 | 1419.1 | 1886.1 | 1914.5 | 2222.3 |
| 210 | 1823.5 | 1444.8 | 1898.8 | 1940.4 | 2260.0 |
| 215 | 1860.8 | 1468.1 | 1910.0 | 1967.1 | 2293.7 |
| 220 | 1895.8 | 1490.5 | 1919.5 | 1991.2 | 2325.1 |
| 225 | 1930.0 | 1513.8 | 1929.1 | 2025.7 | 2351.7 |
| 230 | 1962.0 | 1537.9 | 1938.6 | 2049.8 | 2376.0 |
| 235 | 1992.4 | 1560.4 | 1946.6 | 2073.1 | 2398.8 |
| 240 | 2021.3 | 1580.5 | 1953.8 | 2093.8 | 2418.4 |
| 245 | 2051.0 | 1599.0 | 1960.2 | 2110.2 | 2435.6 |
| 250 | 2079.9 | 1617.4 | 1965.7 | 2133.4 | 2450.5 |
| 255 | 2108.9 | 1635.9 | 1971.3 | 2151.5 | 2463.9 |
| 260 | 2137.0 | 1654.4 | 1976.9 | 2170.5 | 2474.8 |
| 265 | 2165.2 | 1672.0 | 1980.9 | 2187.7 | 2484.2 |
| 270 | 2192.6 | 1688.1 | 1984.9 | 2205.0 | 2492.9 |
| 275 | 2220.0 | 1706.6 | 1988.8 | 2228.3 | 2499.1 |
| 280 | 2246.6 | 1725.0 | 1992.0 | 2244.6 | 2505.4 |
| 285 | 2274.0 | 1744.3 | 1995.2 | 2267.9 | 2510.1 |
| 290 | 2299.1 | 1761.2 | 1997.6 | 2280.0 | 2514.0 |
| 295 | 2324.3 | 1774.0 | 1999.2 | 2291.2 | 2517.2 |
| 300 | 2348.6 | 1787.7 | 2000.8 | 2303.2 | 2519.5 |
| 305 | 2373.0 | 1799.7 | 12002.4 | 2312.7 | 2520.3 |
| 310 | 2394.3 | 1809.4 | 2004.0 | 2319.6 | 2522.7 |
| 315 | 2414.8 | 1814.2 | 2004.0 | 2319.6 | 2523.5 |
| 320 | 2436.1 | 1817.4 | 2004.0 | 2319.6 | 2524.2 |
| 325 | 2458.2 | 1822.2 | 2004.0 | 2319.6 | 2524.2 |
| 330 | 2478.0 | 1827.0 | 2004.0 | 2328.2 | 2524.2 |
| 335 | 2499.3 | 1835.9 | 2004.0 | 2345.5 | 2524.2 |

Table 7.4 *Oxygen utilization (in mg) by microbial consortia in the presence of 20% refinery sludge*

| Time (Hours) | Without Glucose | With Glucose |
|---|---|---|
| 0 | 0.0 | 0.0 |
| 5 | 78.5 | 70.9 |
| 10 | 92.2 | 78.2 |
| 15 | 106.9 | 86.4 |
| 20 | 122.4 | 92.7 |
| 25 | 130.6 | 95.4 |
| 30 | 148.9 | 120.0 |
| 35 | 166.2 | 200.0 |
| 40 | 183.6 | 295.4 |
| 45 | 200.9 | 388.1 |
| 50 | 220.1 | 499.9 |
| 55 | 241.1 | 604.5 |
| 60 | 256.6 | 685.4 |
| 65 | 269.4 | 713.5 |
| 70 | 281.3 | 734.4 |
| 75 | 292.2 | 757.2 |
| 80 | 303.2 | 778.1 |
| 85 | 313.3 | 798.1 |
| 90 | 320.6 | 814.4 |
| 95 | 327.0 | 829.9 |
| 100 | 332.4 | 842.6 |
| 105 | 337.9 | 854.4 |
| 110 | 341.6 | 865.3 |
| 115 | 346.1 | 876.2 |
| 120 | 349.8 | 885.3 |
| 125 | 354.3 | 893.5 |
| 130 | 359.8 | 900.8 |
| 135 | 365.3 | 907.1 |
| 140 | 370.8 | 913.5 |
| 145 | 375.4 | 918.9 |
| 150 | 380.8 | 925.3 |
| 155 | 385.4 | 930.8 |
| 160 | 389.1 | 935.3 |
| 165 | 392.7 | 938.9 |
| 170 | 397.3 | 942.6 |
| 175 | 405.5 | 947.1 |

Table 7.5 *Biodegradation of model PAH compounds without surfactants*

| Days | Fluorene (ppb) | Fluorene Control (ppb) | Phenanthrene (ppb) | Phenanthrene Control (ppb) |
|---|---|---|---|---|
| 0 | 75.84 | 123.34 | 486.46 | 575.89 |
| 1 | 69.41 | 124.79 | 483.77 | 501.43 |
| 2 | 86.17 | 150.47 | 463.12 | 495.54 |
| 4 | 78.84 | 162.2 | 419.08 | 490.48 |
| 6 | 54.08 | 130.86 | 383.11 | 523.46 |
| 8 | 46.23 | 125.58 | 373.6 | 532.55 |

Table 7.6 *Biodegradation of PAHs with 0.01% Makon 10*

| Days | Fluorene (ppb) | Fluorene Control (ppb) | Days | Phenanthrene (ppb) | Phenanthrene Control (ppb) |
|---|---|---|---|---|---|
| 0 | 1734.56 | 1972.62 | 0 | 1849.58 | 1545.08 |
| 1 | 207.30 | 1870.20 | 1 | 591.63 | 1590.48 |
| 2 | 136.29 | 1960.22 | 2 | 492.43 | 1478.71 |
| 3 | 100.62 | 1732.20 | 5 | 316.53 | 1377.00 |
| 6 | 0 | 1625.02 | 6 | 263.12 | 1321.94 |
| | | | 7 | 184.03 | 1291.00 |
| | | | 8 | 0.00 | 1290.74 |
| | | | 9 | 0.00 | 1279.86 |

**Table 7.7** *Biodegradation of PAHs with 0.3% Makon 10*

| Days | Fluorene (ppm) | Fluorene Control (ppm) | Phenanthrene (ppm) | Phenanthrene Control (ppm) | Pyrene (ppm) | Pyrene Control (ppm) |
|---|---|---|---|---|---|---|
| 0 | 42.34 | 42.34 | 38.29 | 38.29 | 27.20 | 27.20 |
| 1 | 23.87 | 41.56 | 35.94 | 37.98 | 32.18 | 26.35 |
| 2 | 22.63 | 42.36 | 33.89 | 38.02 | 22.96 | 26.39 |
| 4 | 21.06 | 40.25 | 30.17 | 37.14 | 19.08 | 27.06 |
| 6 | 17.25 | 41.35 | 25.46 | 36.55 | 15.34 | 25.99 |
| 8 | 10.65 | 42.05 | 15.36 | 37.21 | 12.98 | 25.89 |
| 10 | 5.32 | 40.01 | 7.54 | 36.55 | 6.32 | 26.54 |
| 12 | 1.32 | 40.15 | 2.01 | 38.04 | 0.55 | 27.01 |
| 15 | 0.36 | 41.56 | 0.24 | 37.18 | 0.31 | 27.32 |
| 16 | 0.00 | 40.23 | 0.00 | 36.58 | 0.00 | 26.87 |
| 18 | 0.00 | 42.35 | 0.00 | 38.19 | 0.00 | 26.33 |

Table 7.8 (a) *Biodegradation of fluorene in soil-water slurries (soil/solution ratio of 1:5)*

| Days | Fluorene (ppm) | Fluorene Control (ppm) |
|---|---|---|
| 0 | 11.32 | 10.14 |
| 1 | 10.39 | 9.82 |
| 2 | 9.13 | 9.21 |
| 6 | 4.74 | 8.82 |
| 8 | 0.00 | 8.91 |

Table 7.8 (b) *Biodegradation of phenanthrene and pyrene in soil-water slurries (soil/solution ratio of 1:5)*

| Days | Phenanthrene (ppb) | Phenanthrene Control (ppb) | Days | Pyrene (ppb) | Pyrene Control (ppb) |
|---|---|---|---|---|---|
| 0 | 1177.05 | 1230.74 | 0 | 1812.32 | 1712.45 |
| 1 | 974.97 | 1124.04 | 1 | 1564.21 | 1594.36 |
| 2 | 600.56 | 1257.07 | 2 | 1385.47 | 1648.37 |
| 3 | 434.56 | 1326.81 | 4 | 1350.48 | 1702.58 |
| 7 | 0.00 | 1382.41 | 6 | 1175.39 | 1523.84 |
| | | | 7 | 896.25 | 1511.31 |
| | | | 8 | 432.12 | 1542.36 |
| | | | 10 | 0.00 | 1568.29 |

Table 7.9 *Biodegradation of PAHs in 20% sludge with 0.3% Makon 10*

| Days | Fluorene (ppm) | Fluorene Control (ppm) | Phenanthrene (ppm) | Phenanthrene Control (ppm) | Pyrene (ppm) | Pyrene Control (ppm) |
|---|---|---|---|---|---|---|
| 0 | 3.47 | 2.06 | 8.31 | 12.62 | 9.98 | 12.90 |
| 1 | 1.51 | 2.25 | 8.01 | 13.38 | 12.25 | 13.16 |
| 3 | 1.52 | 3.88 | 4.12 | 13.78 | 7.04 | 15.14 |
| 4 | 1.44 | 3.34 | 0.72 | 12.76 | 6.54 | 10.61 |
| 5 | 0.00 | 2.18 | 0.00 | 12.44 | 4.08 | 12.00 |
| 7 | 0.00 | 3.12 | 0.00 | 13.08 | 0.00 | 11.36 |

Table 7.10 *Biodegradation of PAHs in Bioflo IIC fermenter with 20 % sludge and 0.3% Makon 10*

| Days | Fluorene (ppm) | Phenanthrene (ppm) | Pyrene (ppm) |
|---|---|---|---|
| 0 | 5.41 | 7.99 | 12.97 |
| 1 | 3.62 | 5.34 | 15.68 |
| 2 | 3.34 | 4.51 | 6.90 |
| 4 | 2.55 | 3.20 | 3.72 |
| 5 | 1.86 | 2.88 | 2.97 |
| 7 | 1.77 | 2.31 | 2.89 |
| 8 | 0.98 | 1.88 | 1.96 |
| 11 | 0.27 | 0.41 | 0.94 |
| 13 | 0.00 | 0.00 | 0.00 |
| 15 | 0.00 | 0.00 | 0.00 |

**Table 7.11** *Biodegradation of PAHs in bioslurry reactor with 20% sludge and 0.3% Makon 10*

| Days | Fluorene (ppm) | Phenanthrene(ppm) | Pyrene (ppm) |
|---|---|---|---|
| 0 | 5.41 | 7.99 | 12.97 |
| 1 | 3.62 | 5.34 | 15.68 |
| 2 | 3.34 | 4.51 | 6.90 |
| 4 | 2.55 | 3.20 | 3.72 |
| 5 | 1.86 | 2.88 | 2.97 |
| 7 | 1.77 | 2.31 | 2.89 |
| 8 | 0.98 | 1.88 | 1.96 |
| 11 | 0.27 | 0.41 | 0.94 |
| 13 | 0.00 | 0.00 | 0.00 |
| 15 | 0.00 | 0.00 | 0.00 |
| 17 **(Surfactant Addition)** | 2.54 | 0.00 | 2.89 |
| 18 | 3.96 | 0.00 | 3.21 |
| 19 | 3.71 | 0.00 | 3.14 |
| 20 | 3.65 | 0.00 | 3.12 |
| 22 **(Nutrients Addition)** | 3.68 | 0.00 | 3.16 |
| 24 | 2.42 | 0.00 | 2.12 |
| 26 | 1.02 | 0.00 | 0.65 |
| 28 | 0.51 | 0.00 | 0.45 |
| 30 | 0.00 | 0.00 | 0.00 |
| 31 | 0.00 | 0.00 | 0.00 |
| 32 **(Surfactant Addition)** | 0.00 | 0.00 | 0.00 |
| 33 | 0.00 | 0.00 | 0.00 |
| 34 | 0.00 | 0.00 | 0.00 |
| 35 | 0.00 | 0.00 | 0.00 |

**Table 7.12** *DO uptake*

| Time, t (min) | $(C_i - C)$ (mg/L) |
|---|---|
| 0.09 | 3.0 |
| 0.37 | 2.8 |
| 1.44 | 2.5 |
| 2.54 | 2.2 |
| 3.32 | 2.0 |
| 4.22 | 1.8 |
| 5.2 | 1.5 |
| 6.29 | 1.2 |
| 7.14 | 1.0 |

# APPENDIX B
# FIGURES

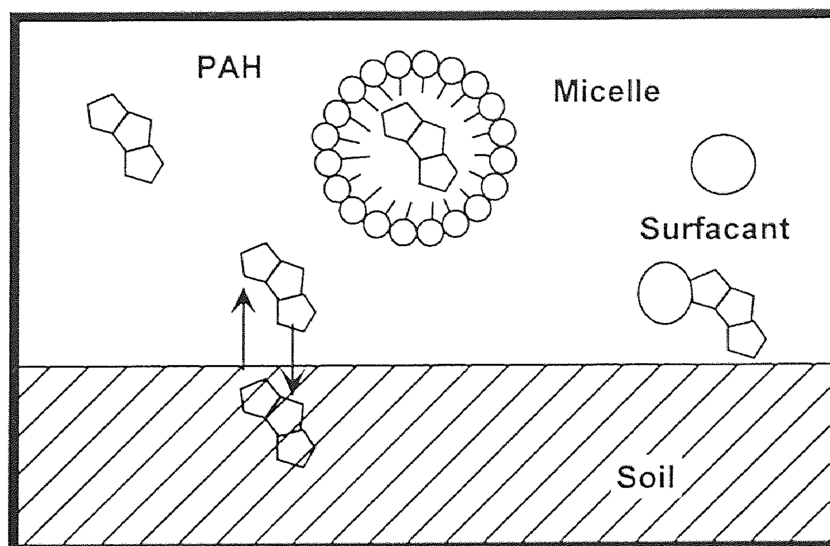


**Figure 1.1** *Distribution of PAH and surfactant in soil/aqueous system*

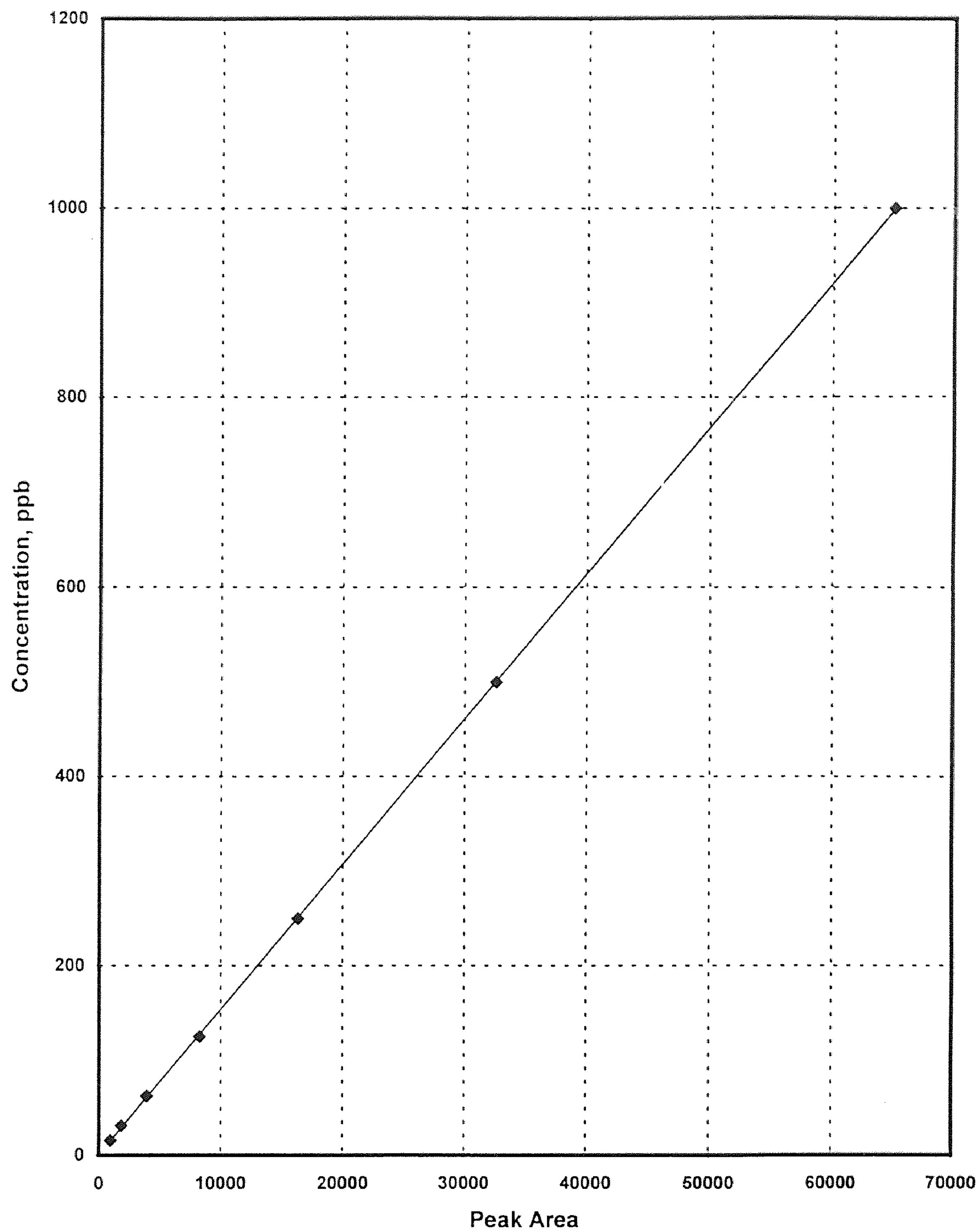


**Figure 4.1(a)** *Calibration curve for fluorene at low concentrations*

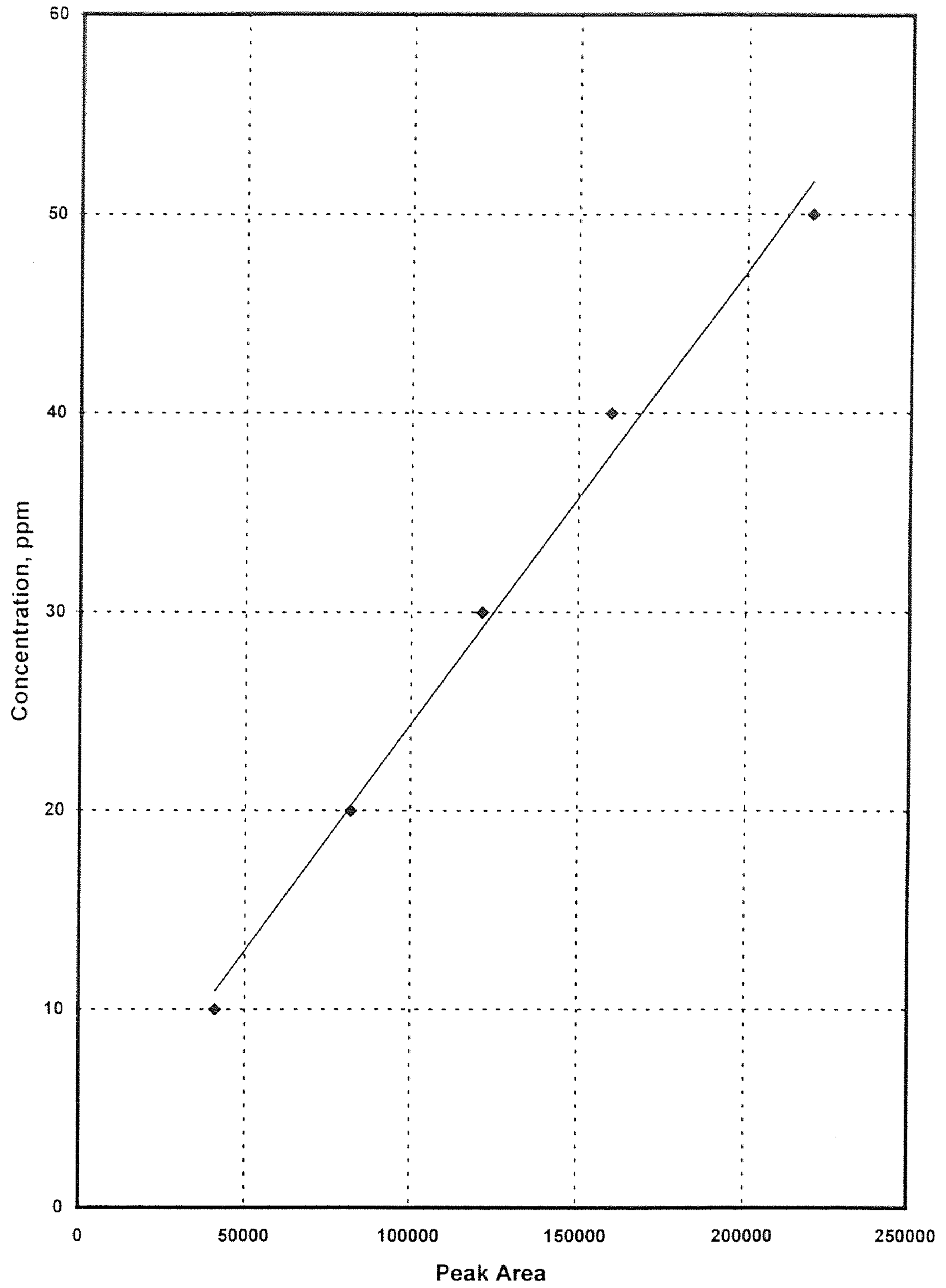


**Figure 4.1(b)** *Calibration curve for fluorene at high concentrations*

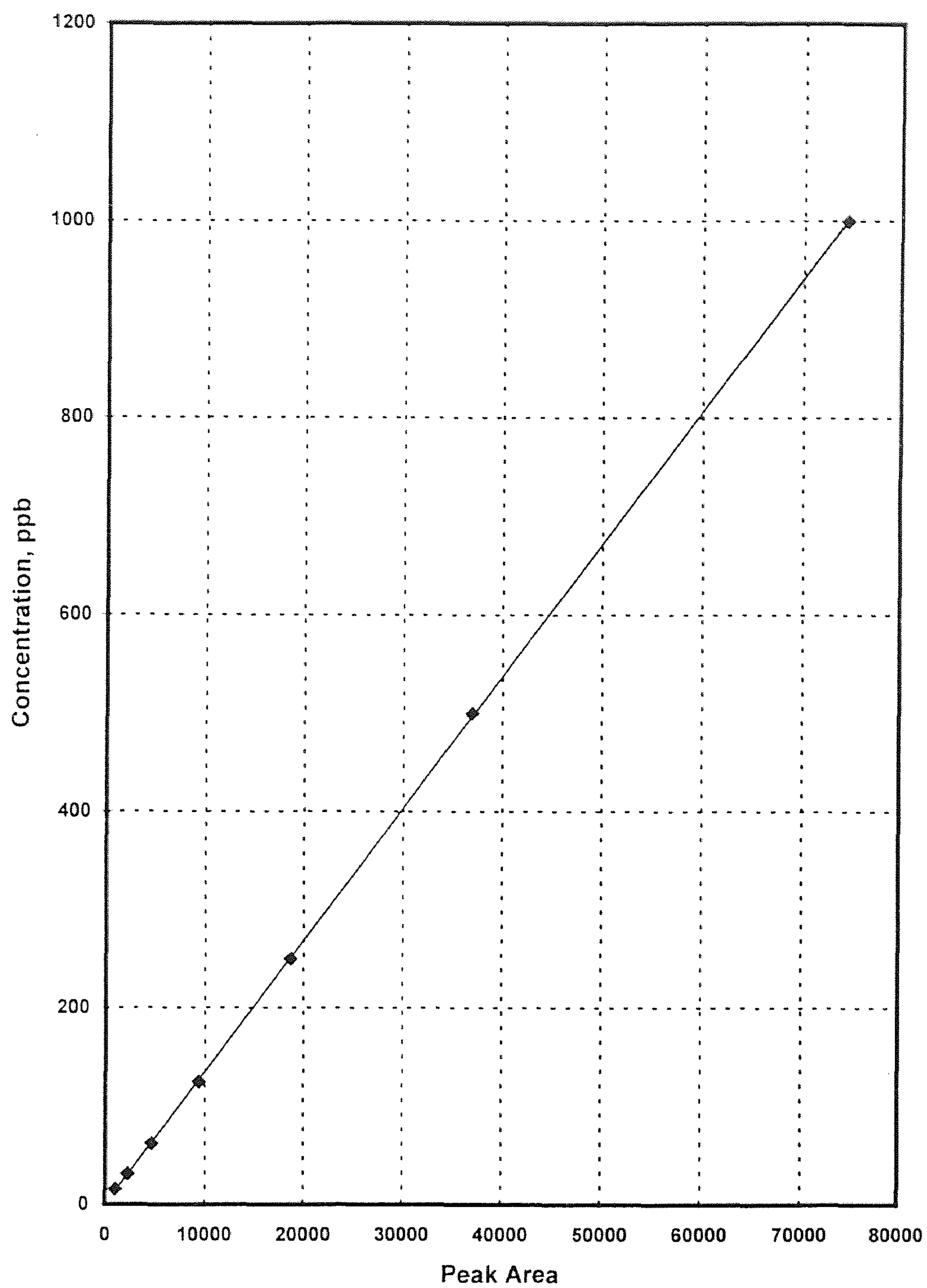


**Figure 4.2(a)** *Calibration curve for phenanthrene at low concentrations*

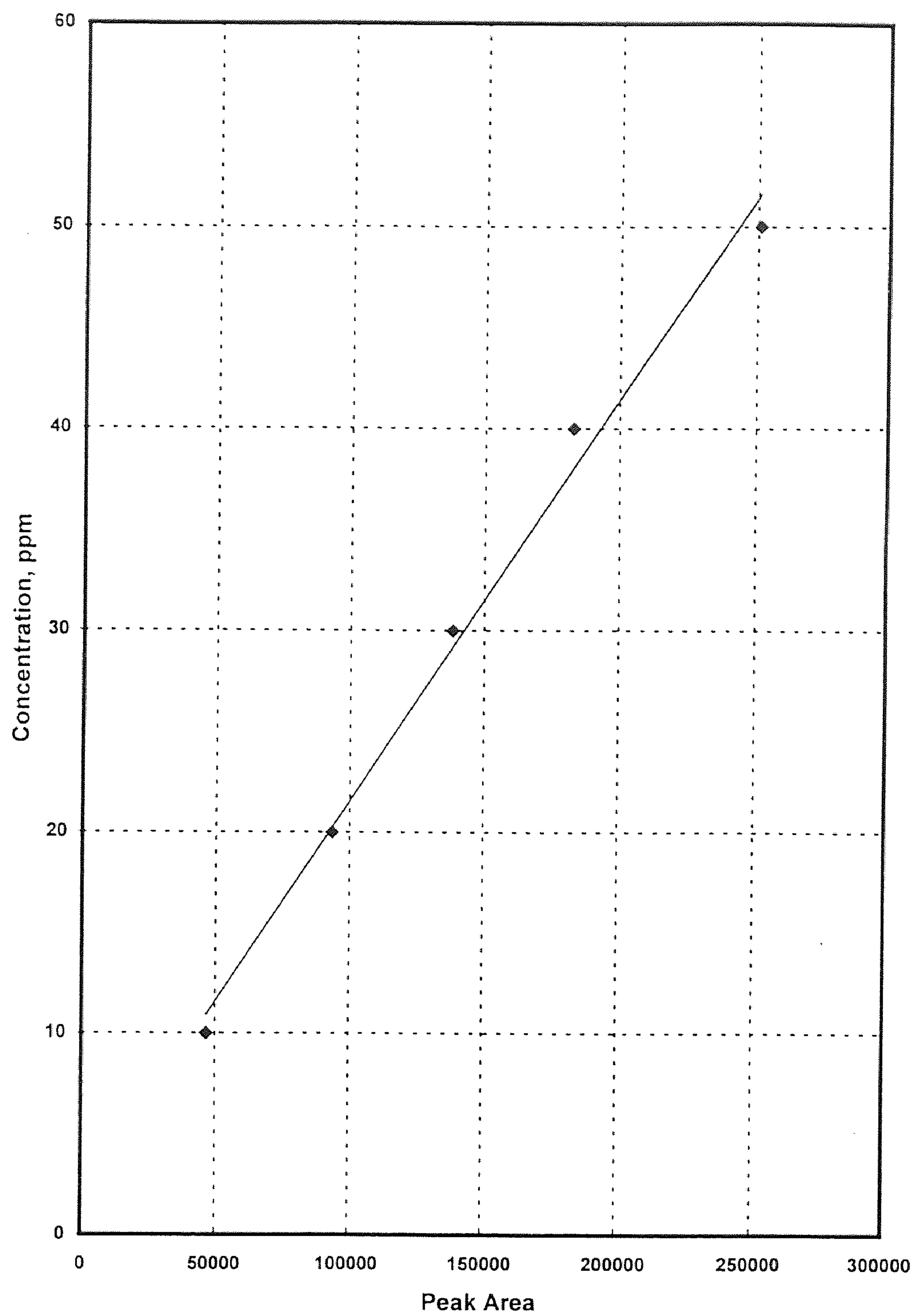


**Figure 4.2(b)** *Calibration curve for phenanthrene at high concentrations*

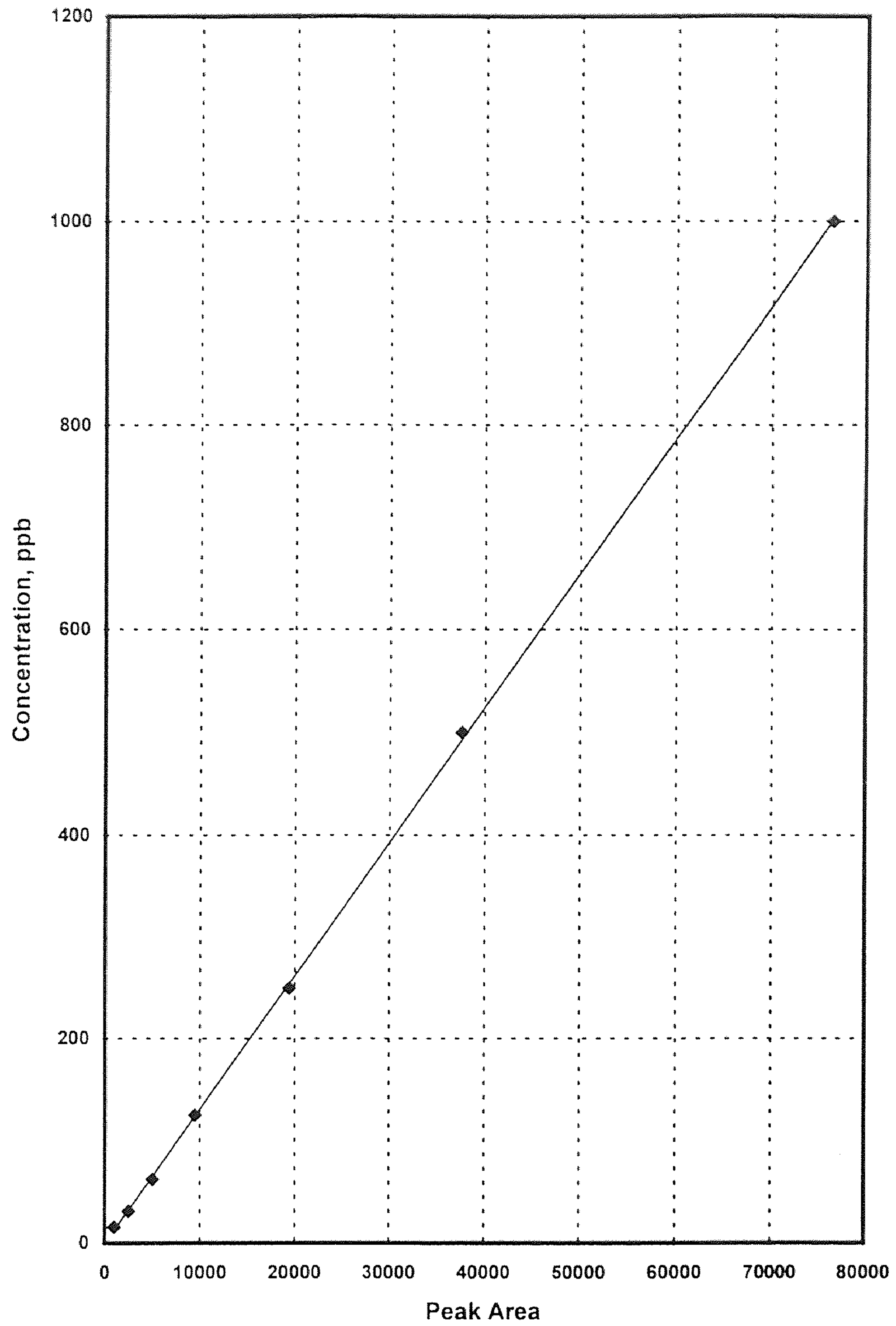


**Figure 4.3(a)** *Calibration curve for pyrene at low concentrations*

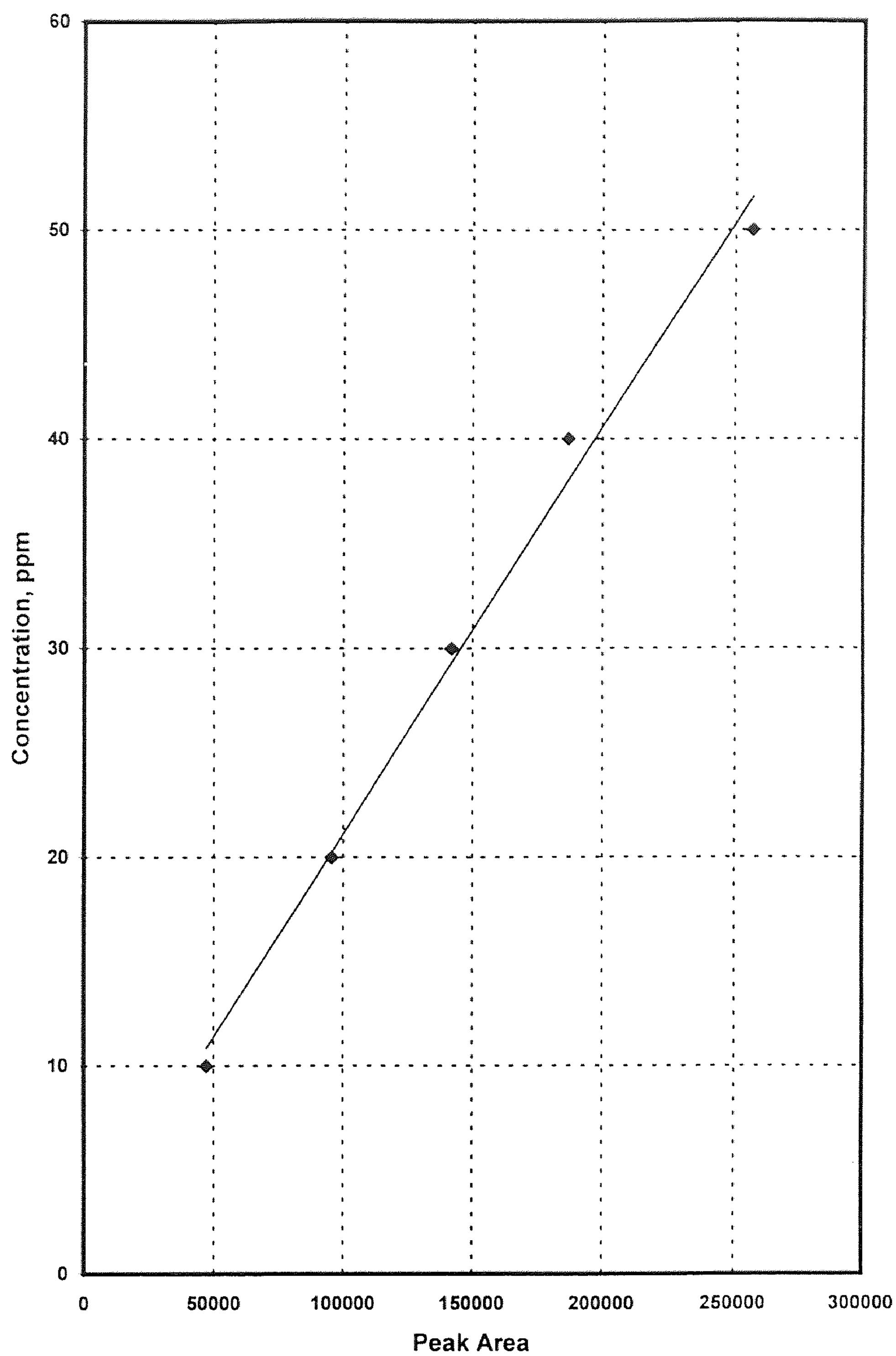


**Figure 4.3(b)** *Calibration curve for pyrene at high concentrations*

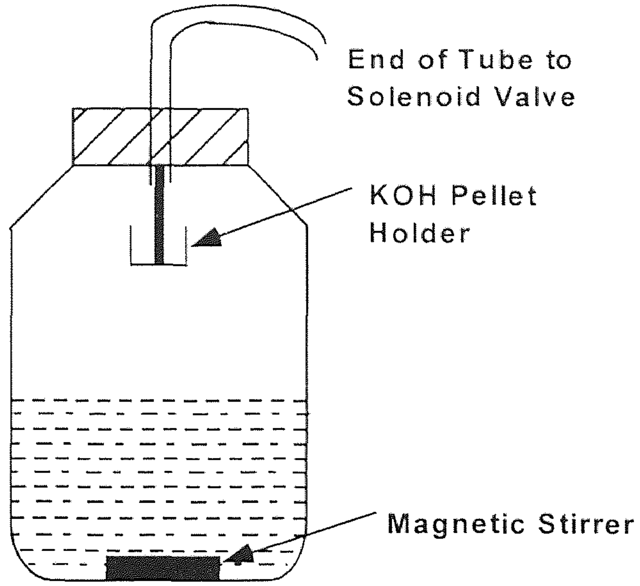


**Figure 5.1** *Respirometer Bottle*

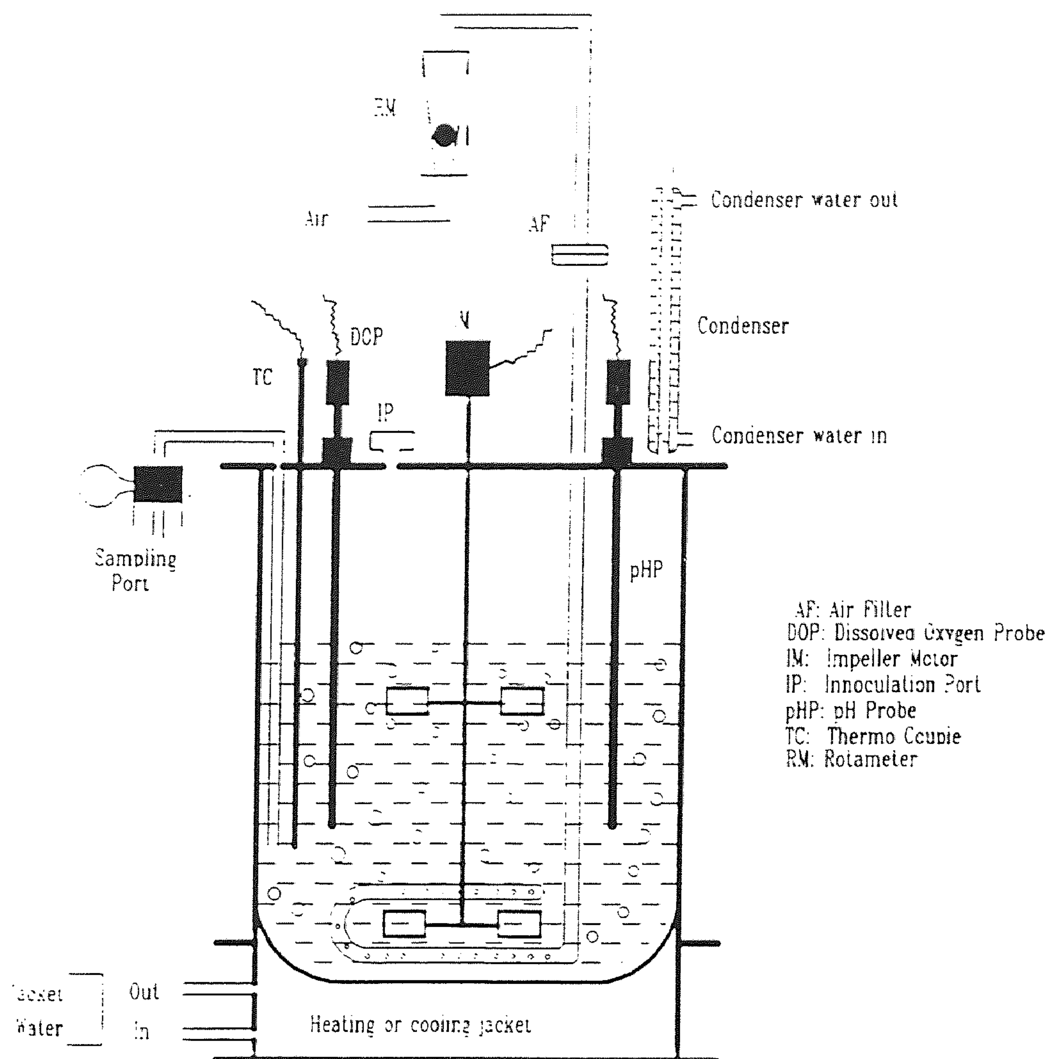


**Figure 5.2** *Batch Fermenter*

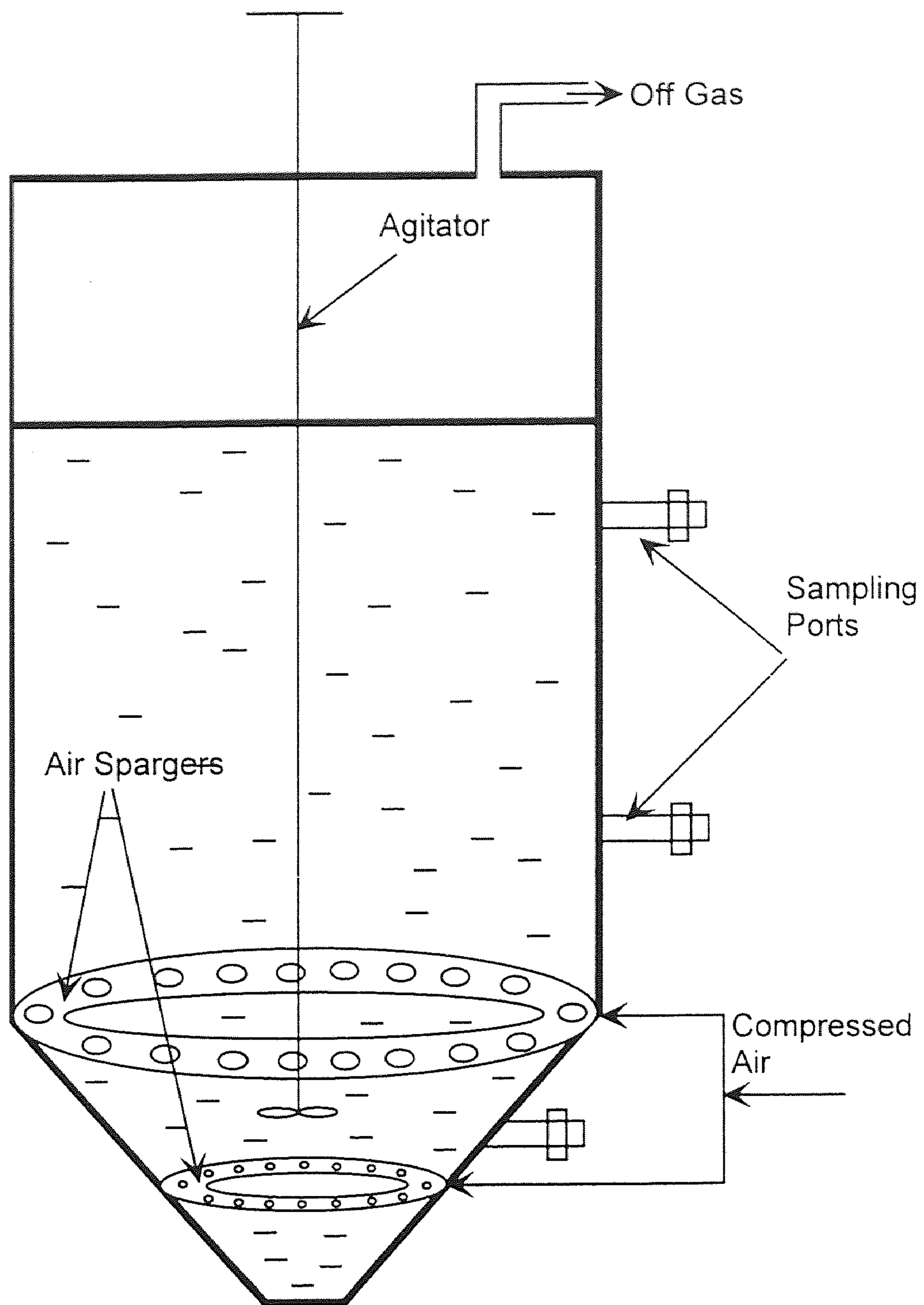


**Figure 5.3** *Bioslurry Reactor*

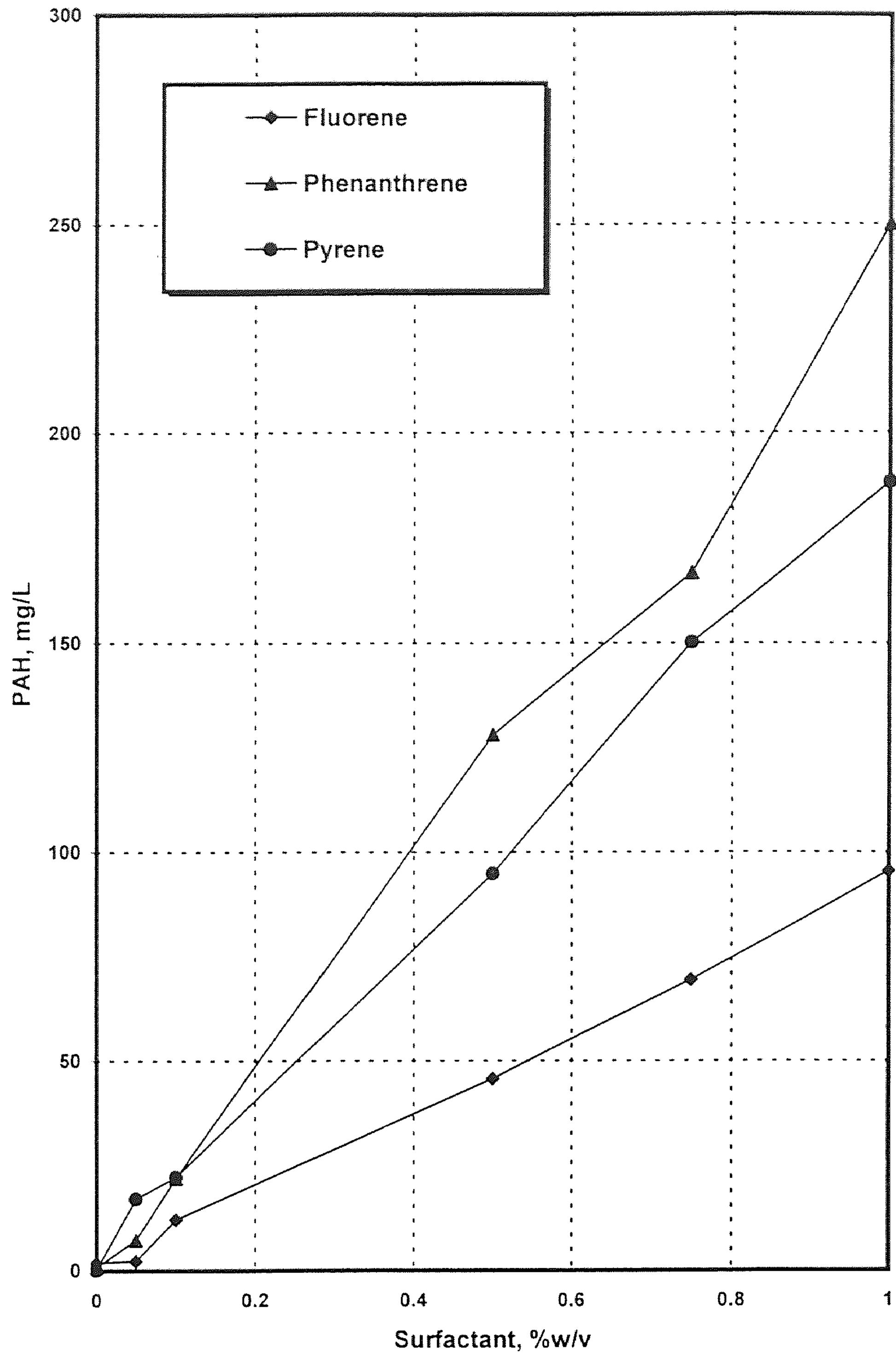


**Figure 7.1** *Solubility of model PAHs in presence of Makon 10*

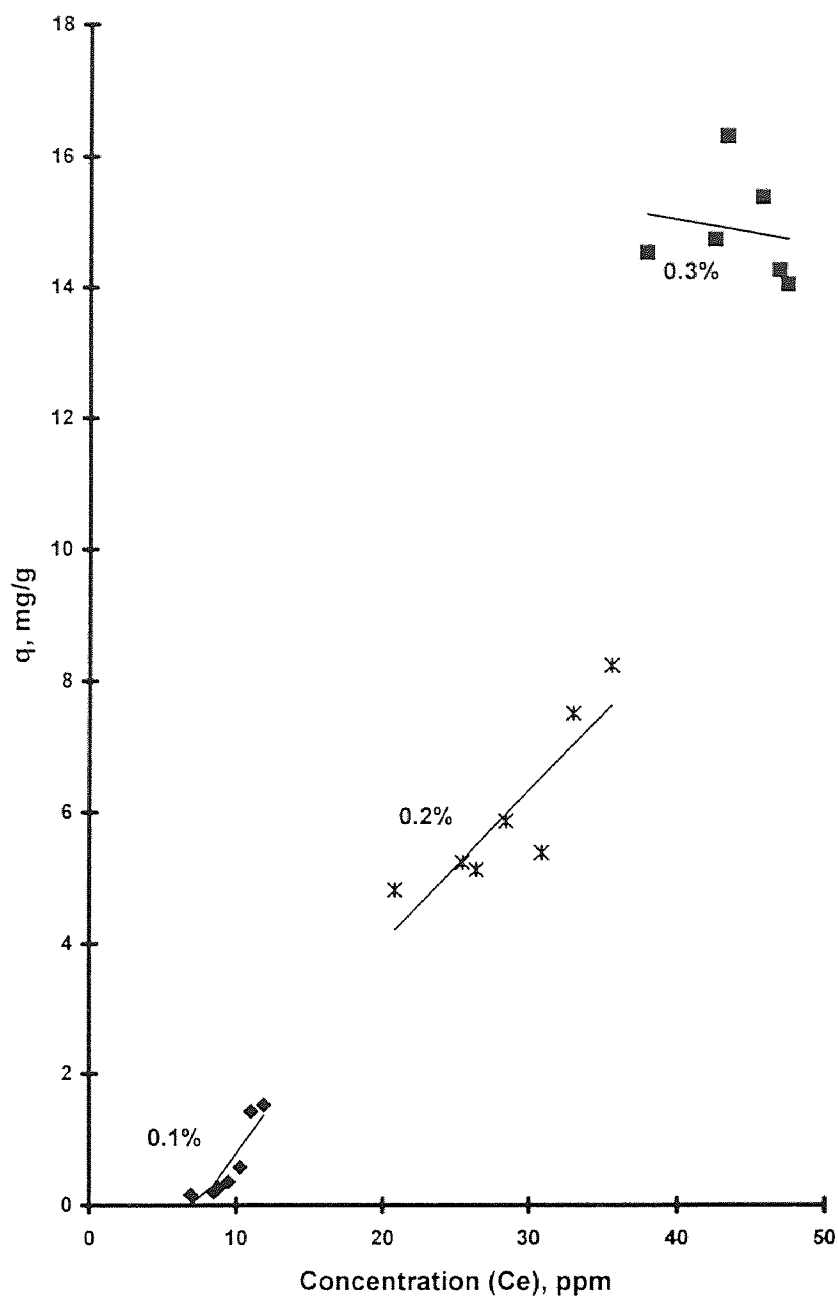


**Figure 7.2** *Adsorption isotherm of fluorene at various concentrations of Makon 10*

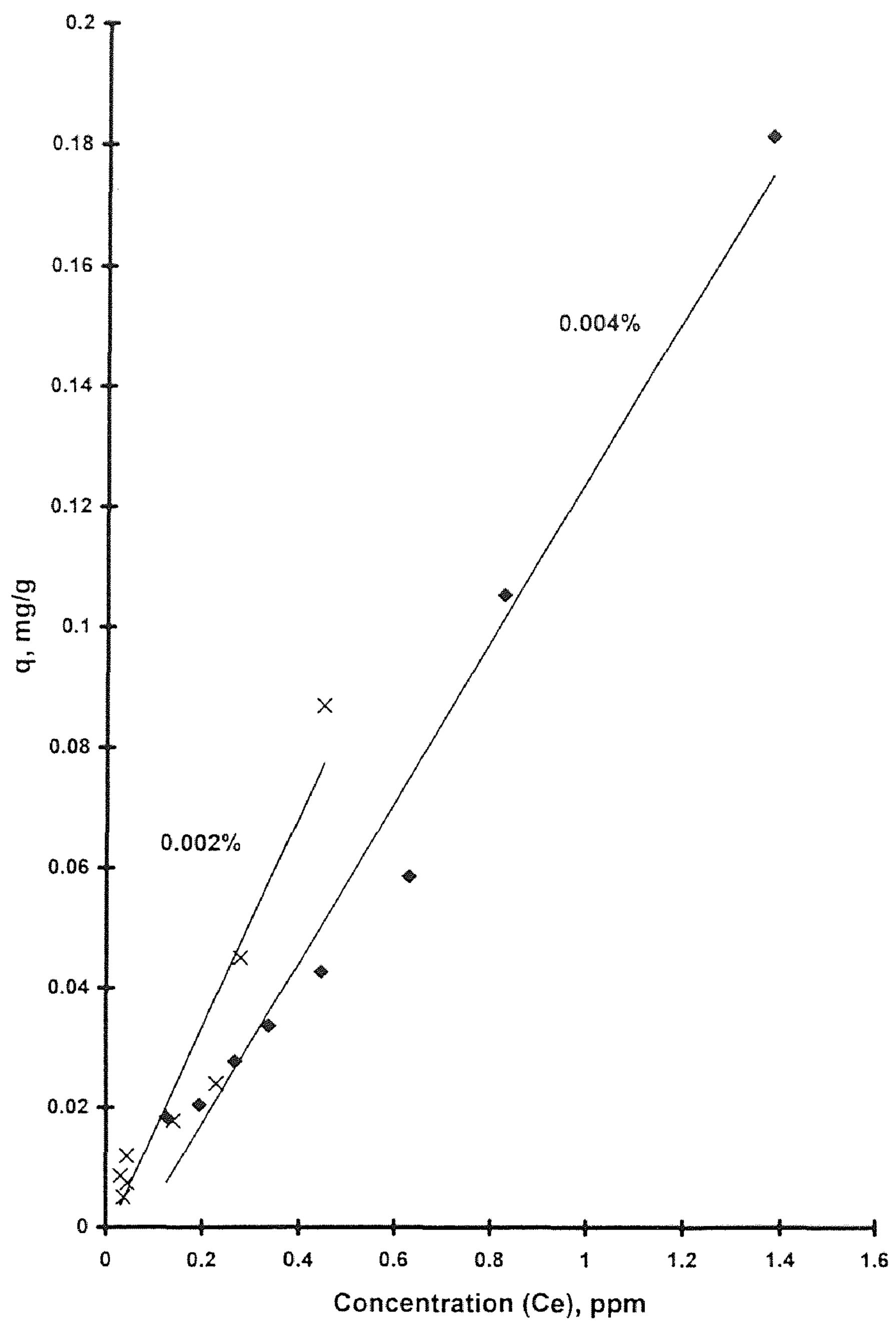


**Figure 7.3** *Adsorption isotherm of fluorene at various concentrations of Makon 10 below CMC*

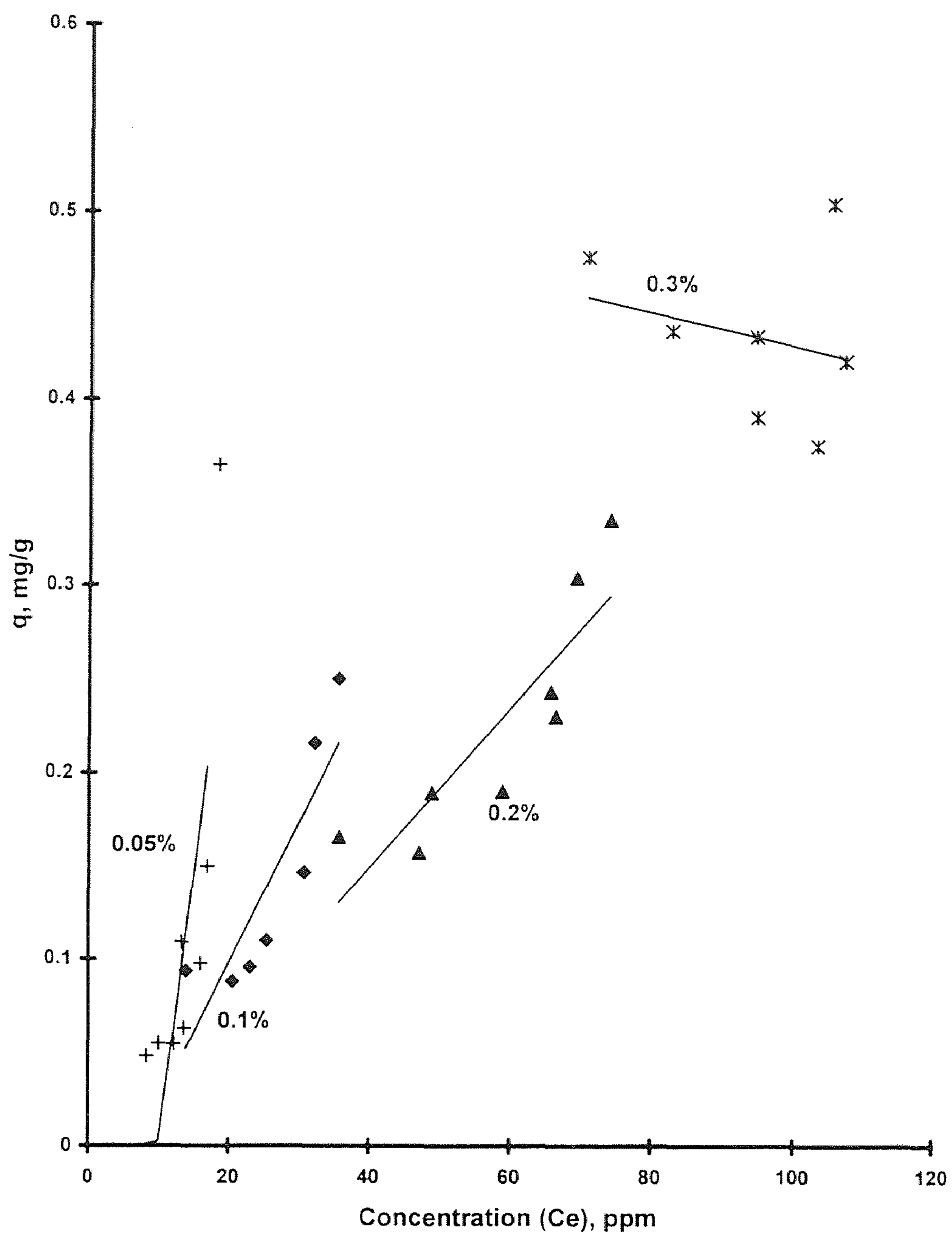


**Figure 7.4** *Adsorption isotherm of phenanthrene at various concentrations of Makon 10*

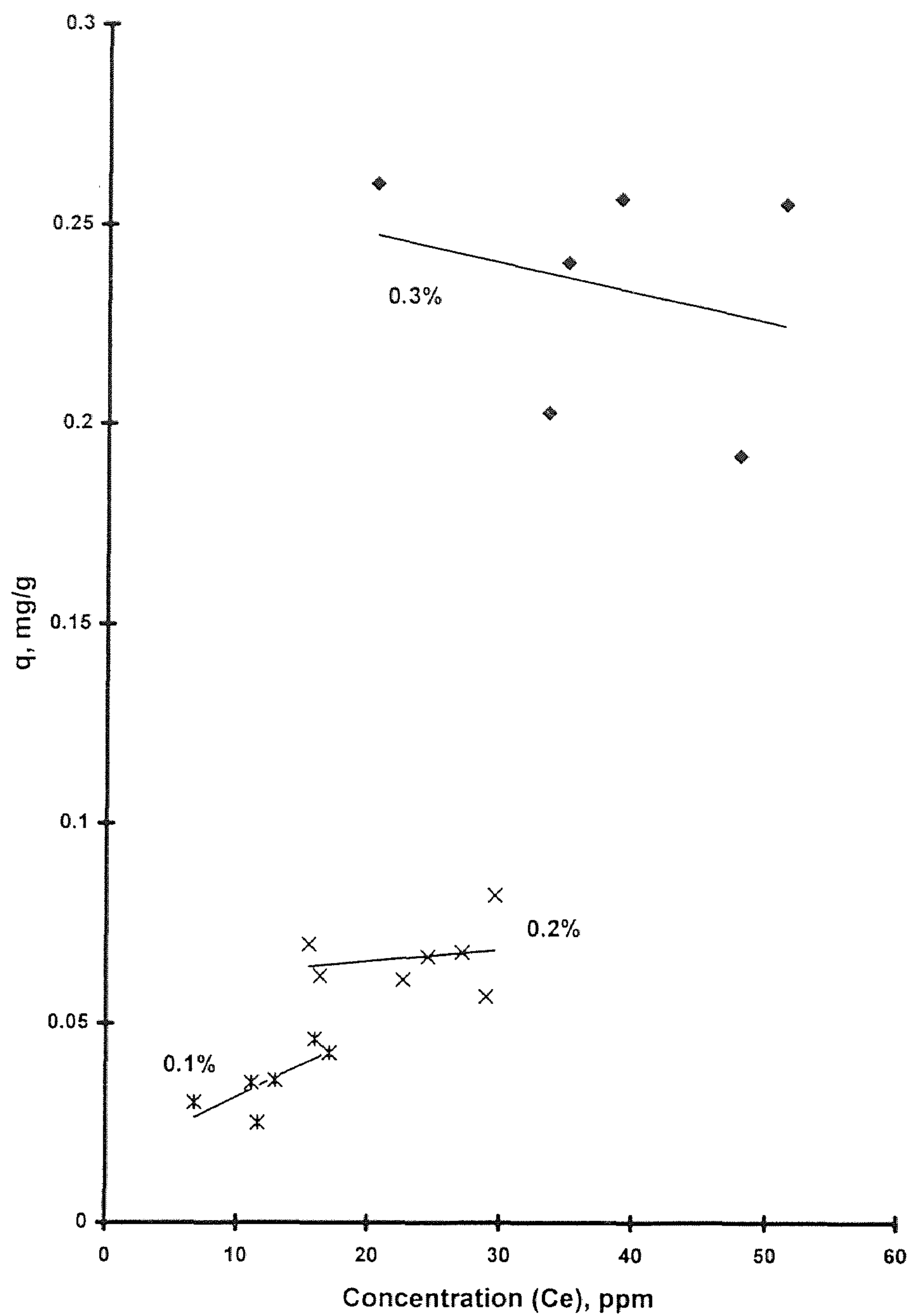


**Figure 7.5** *Adsorption isotherm of pyrene at various concentrations of Makon 10*

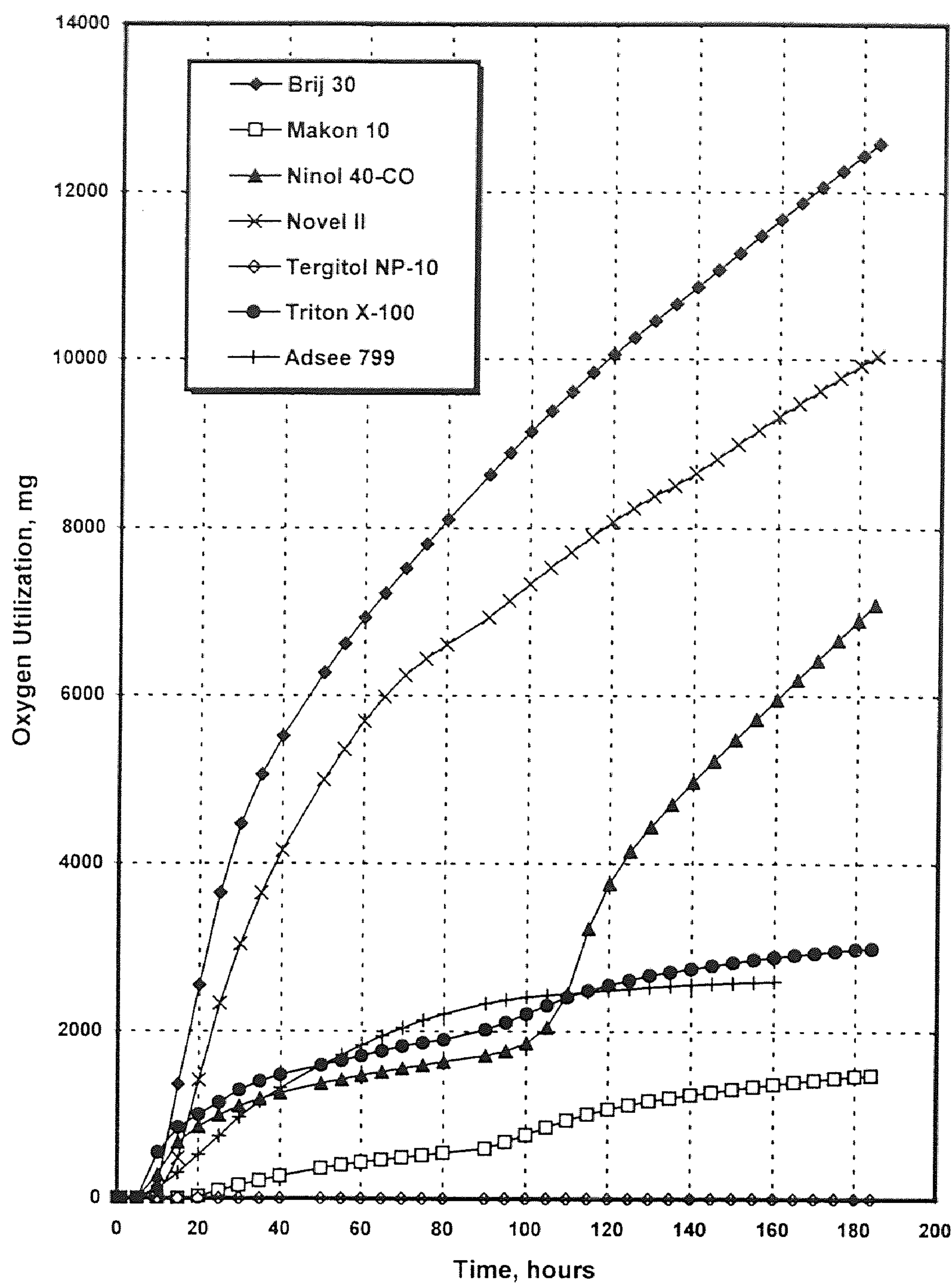


**Figure 7.6** *Cumulative oxygen utilization during surfactant degradation in respirometer*

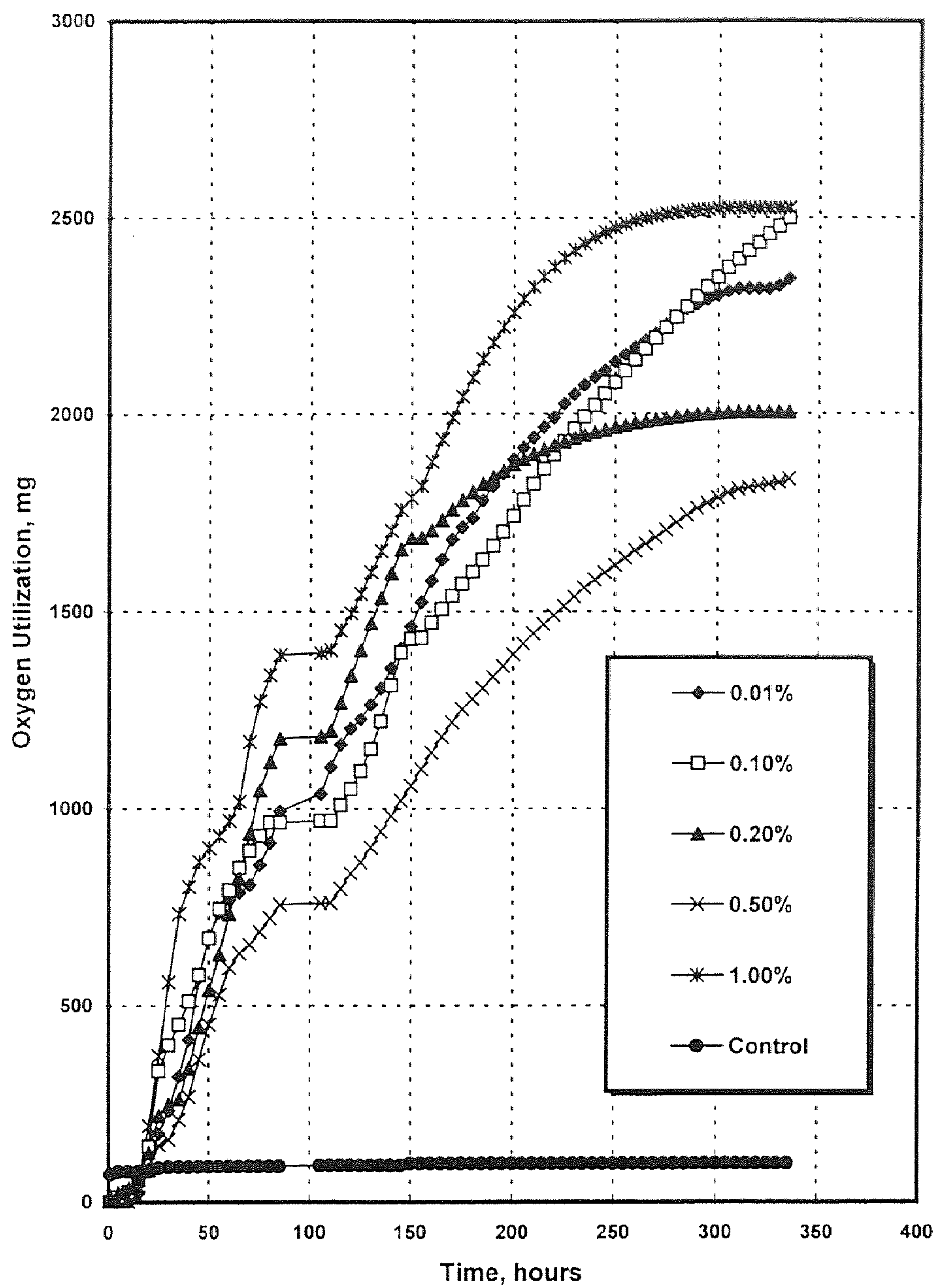


**Figure 7.7** *Inhibition of Makon 10 concentration on microbial growth*

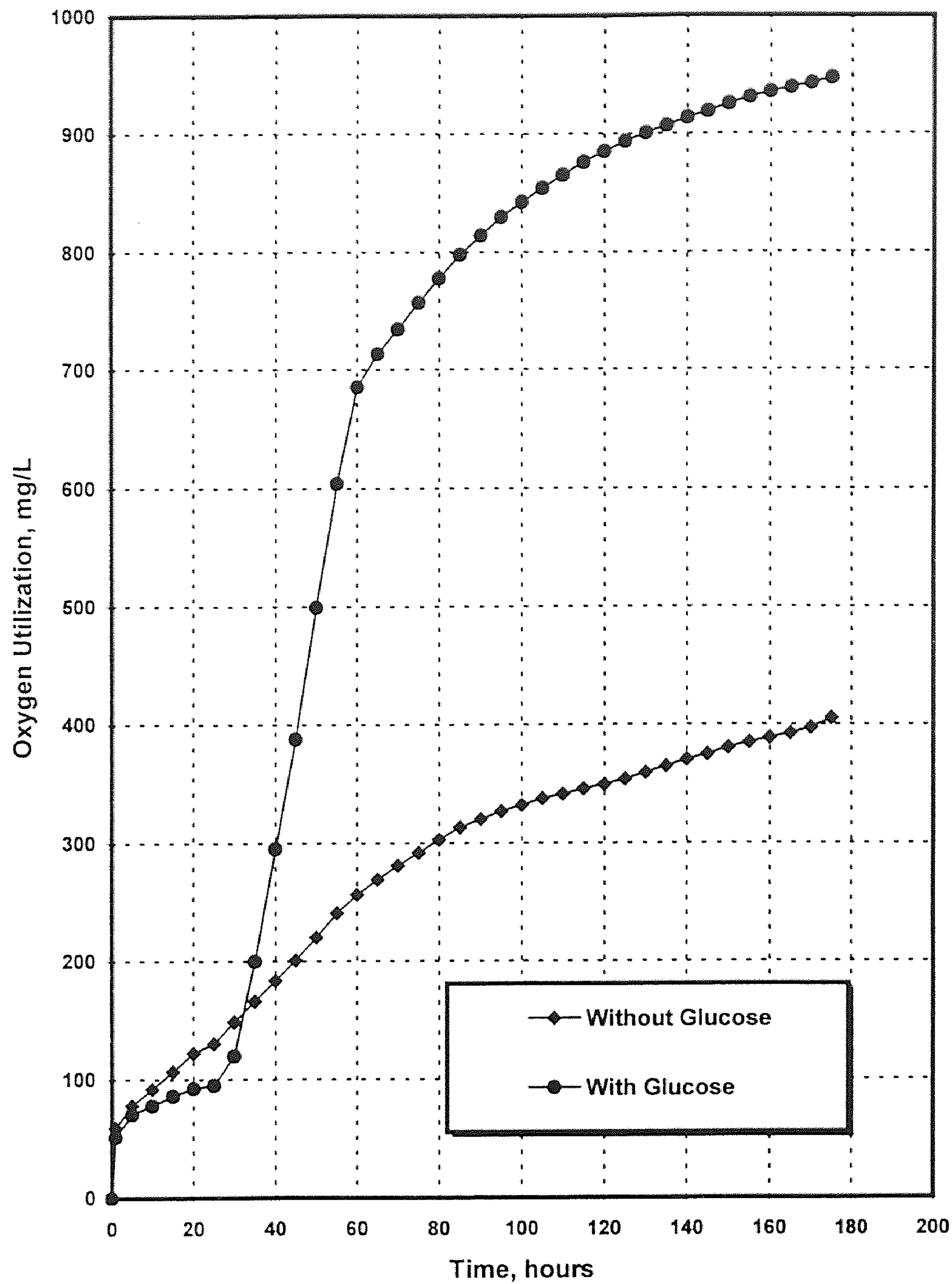


**Figure 7.8** *Cumulative oxygen utilization during biodegradation of PAHs*

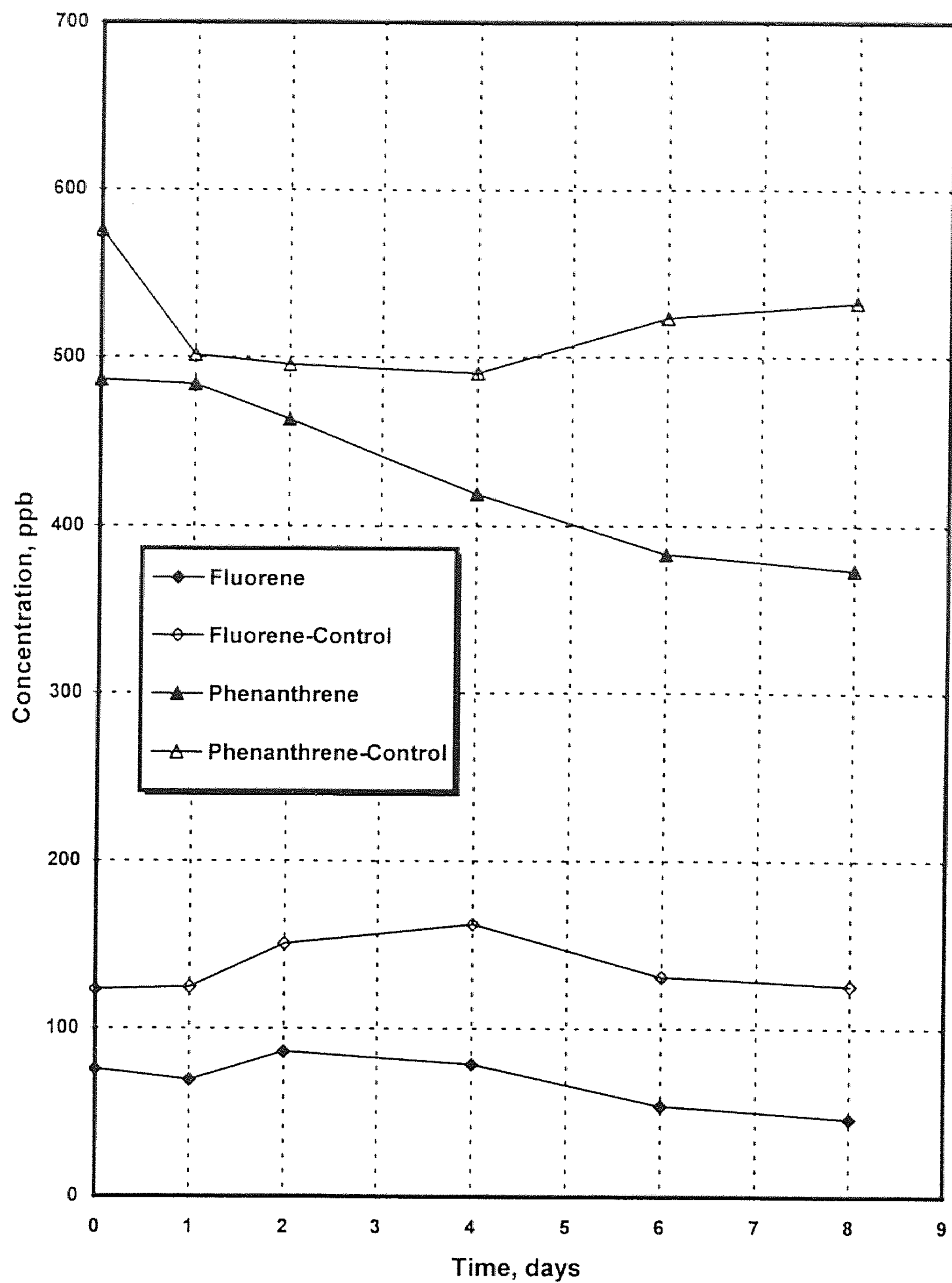


**Figure 7.9** *Biodegradation of PAHs in growth medium without surfactant*

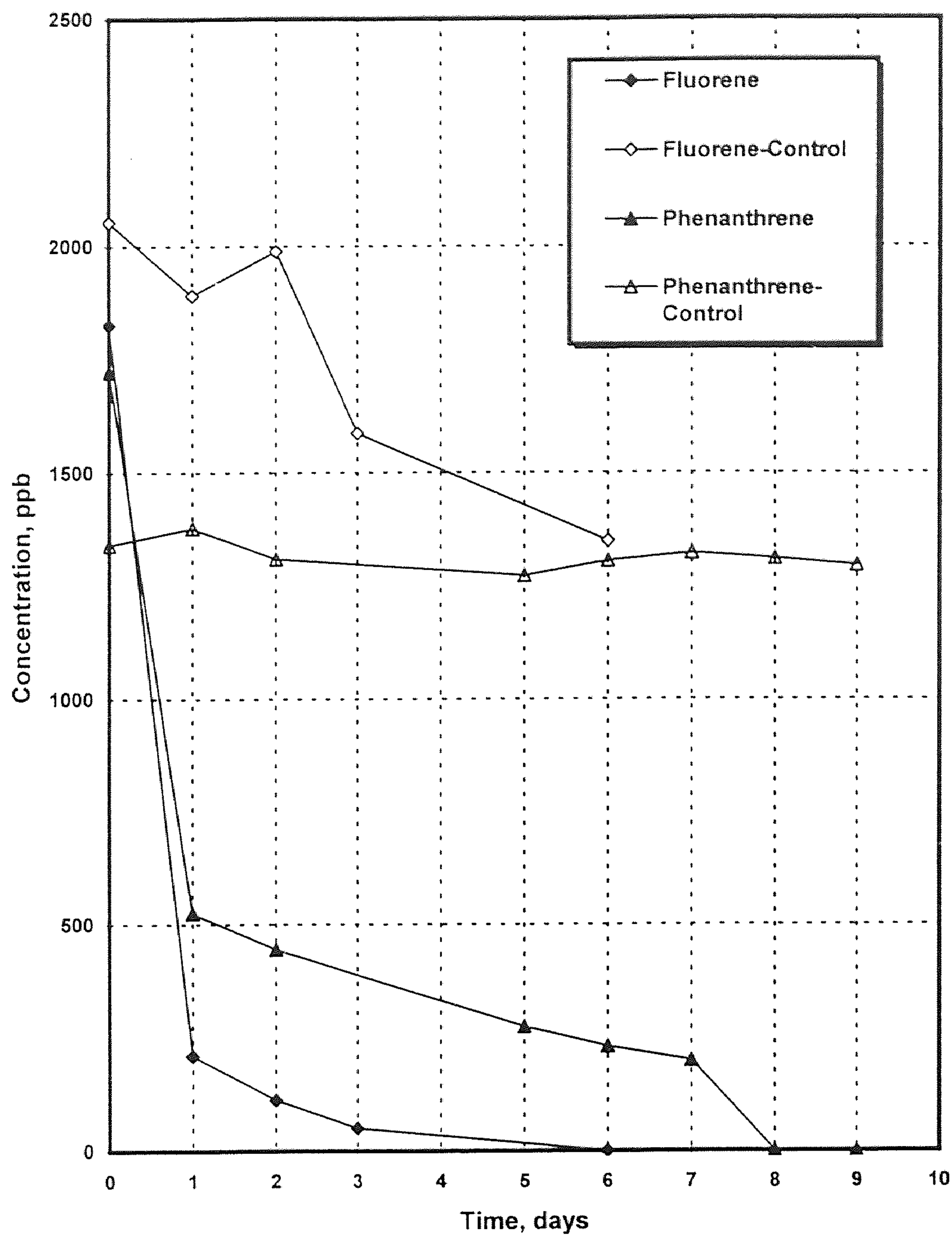


**Figure 7.10** *Biodegradation of PAHs in presence of 0.01% Makon 10*

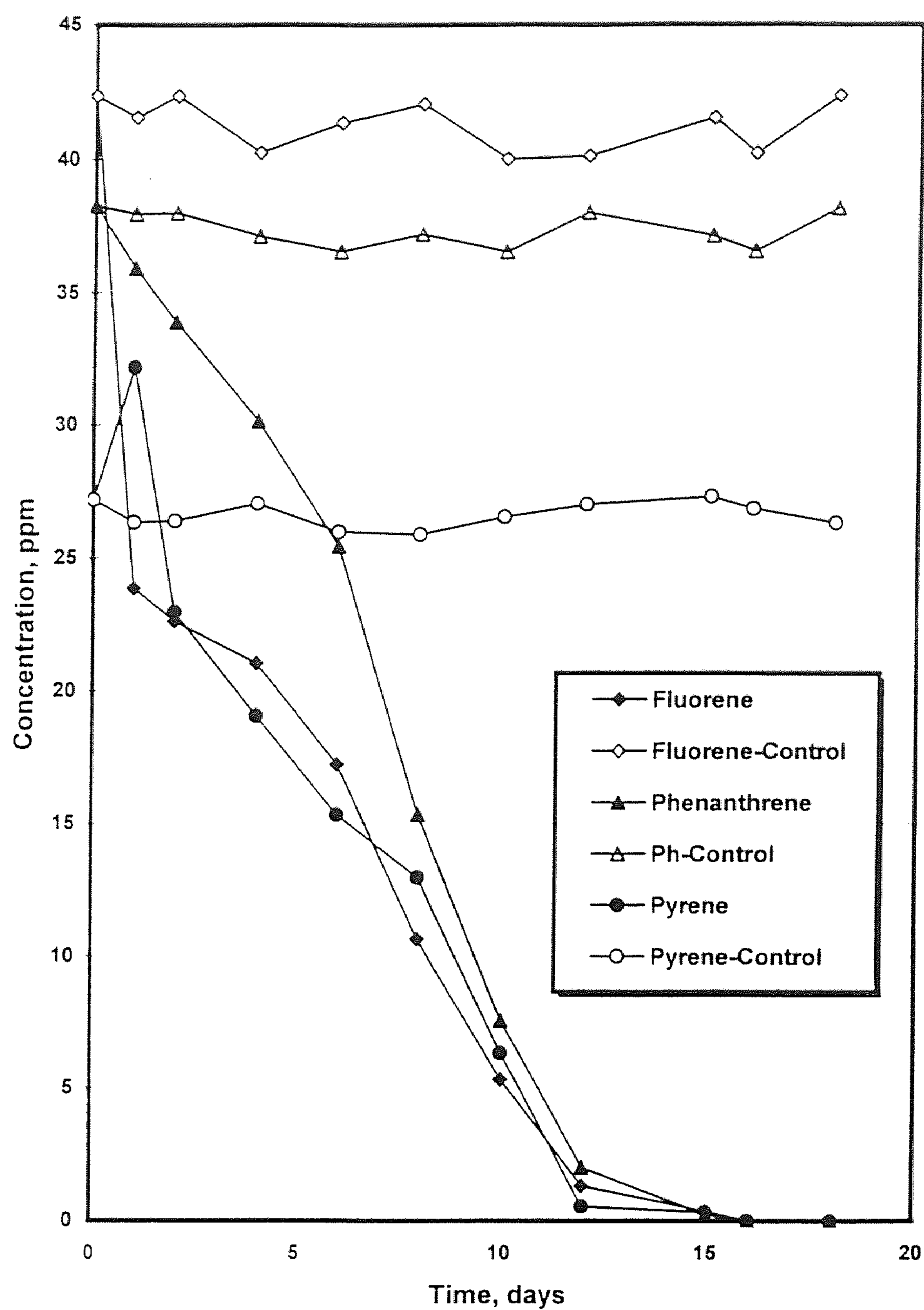


**Figure 7.11** *Biodegradation of PAHs in presence of 0.3% Makon 10*

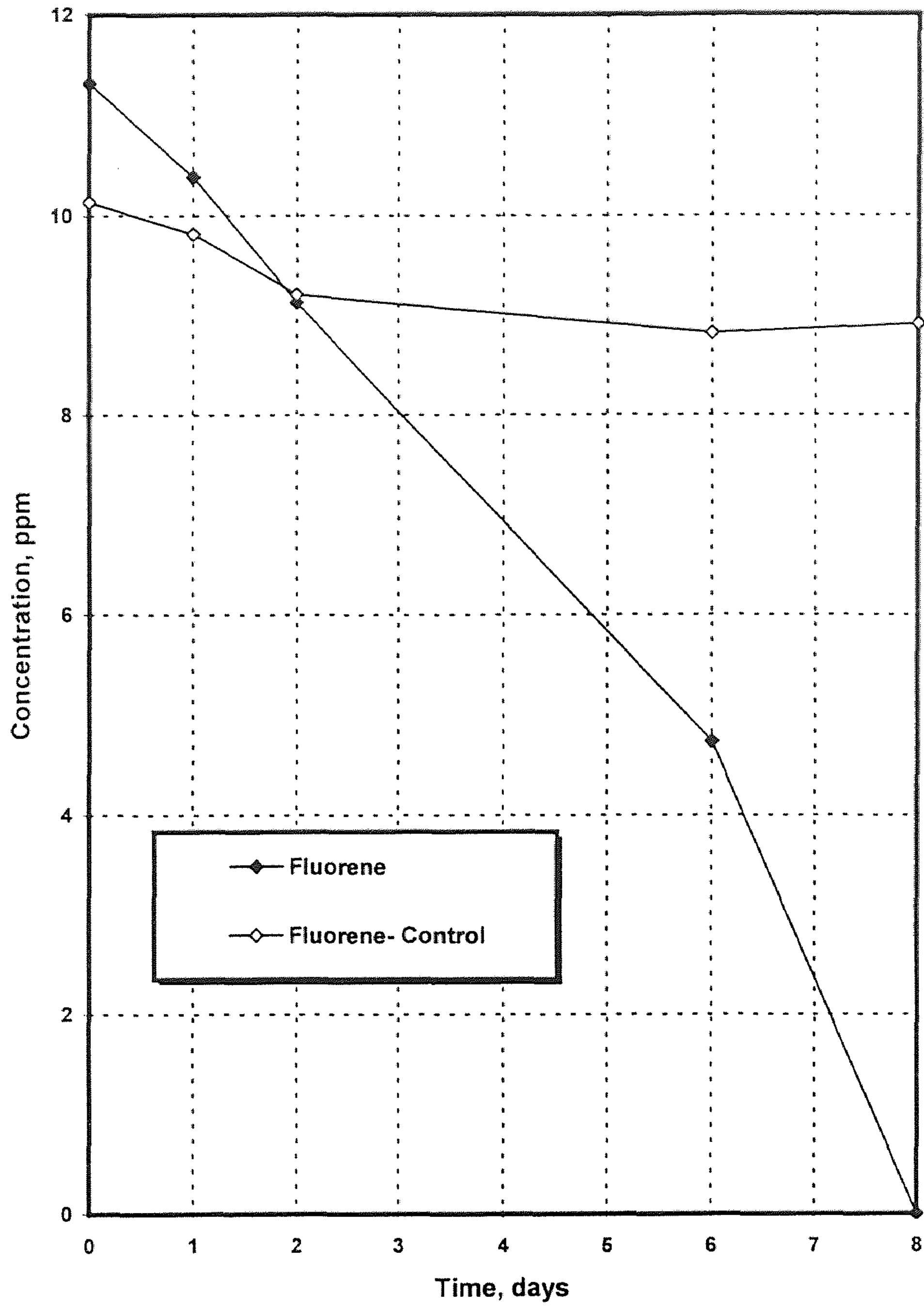


**Figure 7.12 (a)** *Degradation of fluorene in 20% soil and 0.3% Makon 10*

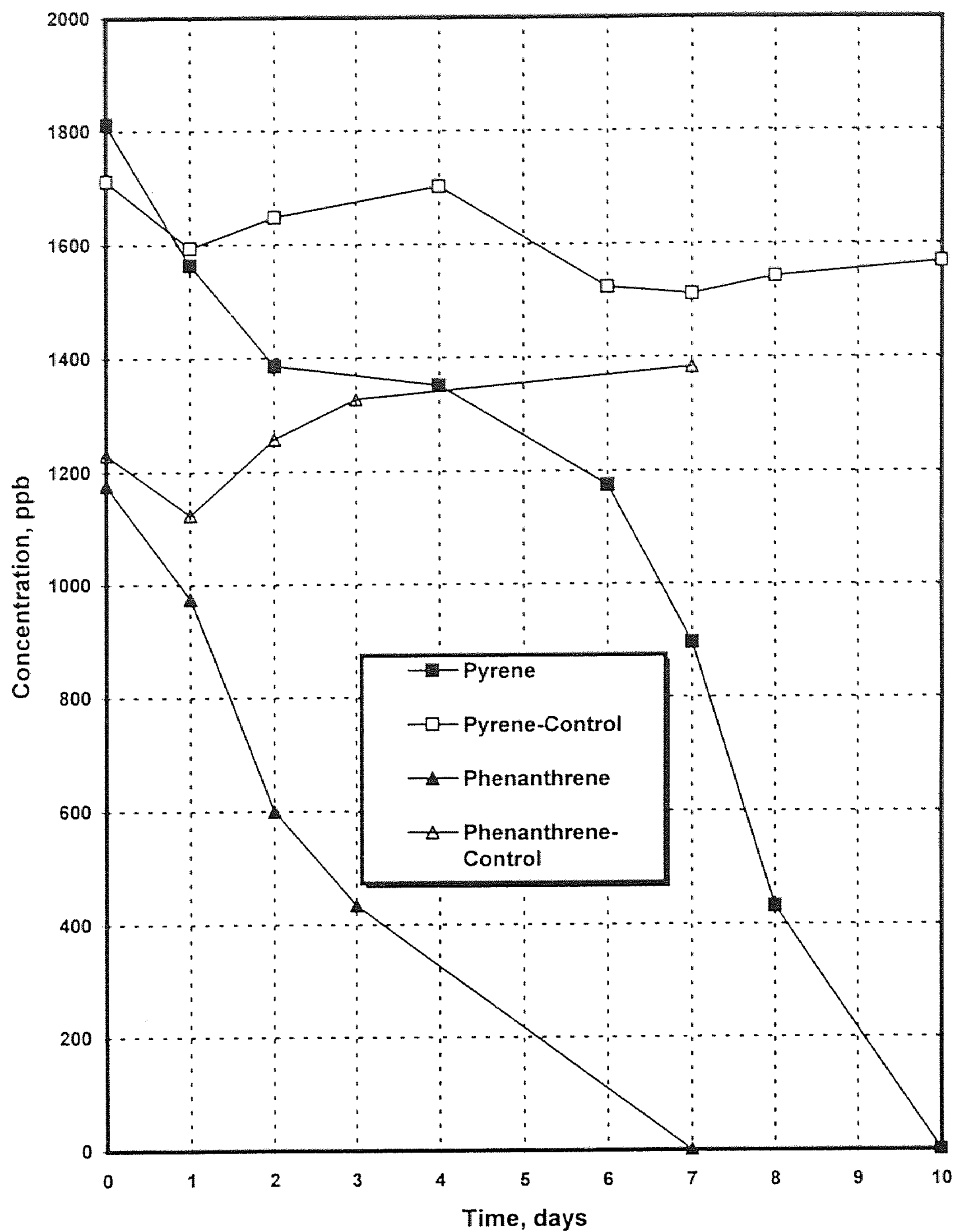


**Figure 7.12 (b)** *Degradation of phenanthrene and pyrene in 20% soil and 0.3% Makon 10*

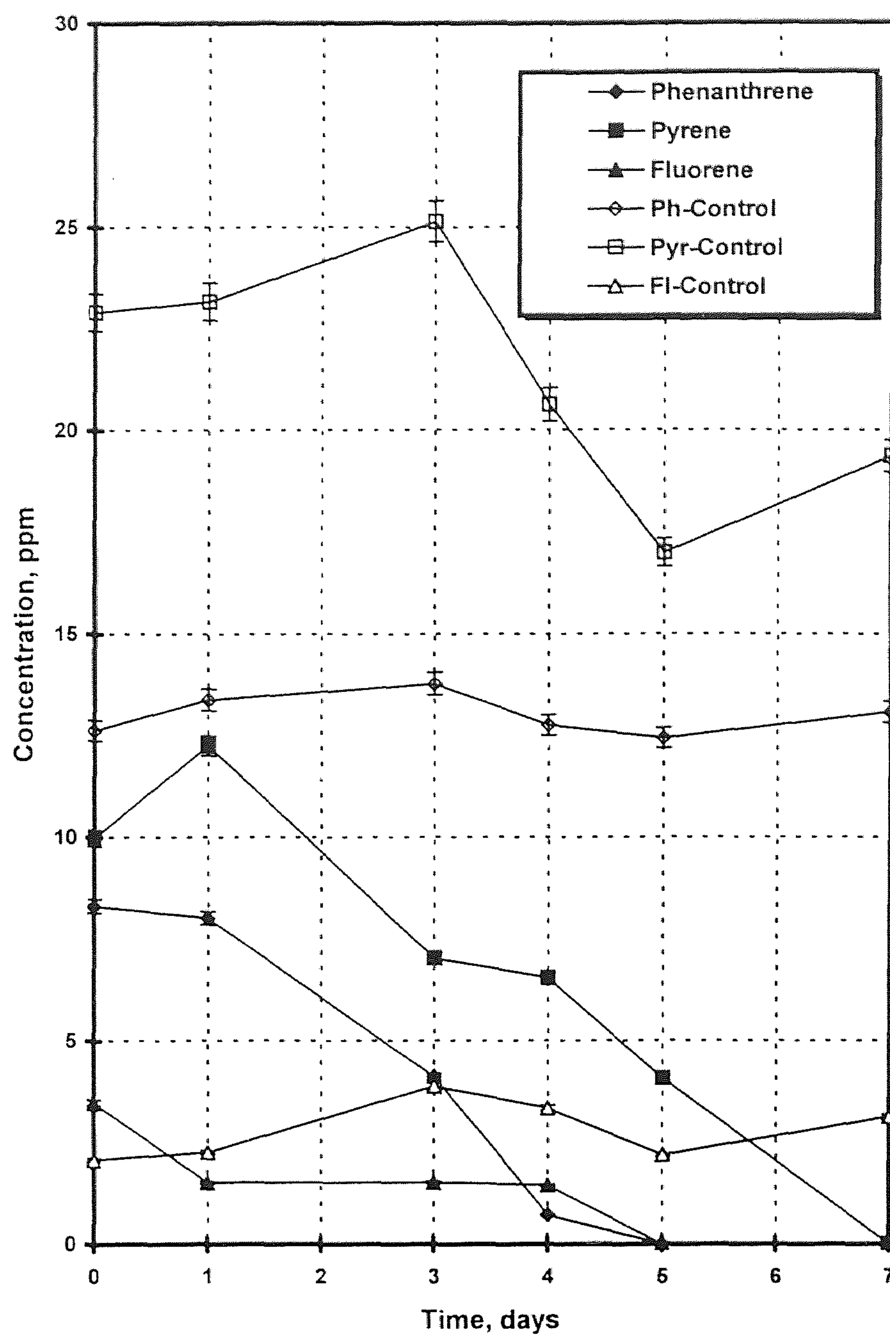


**Figure 7.13** *Biodegradation of PAHs in 20% sludge and 0.3% Makon 10*

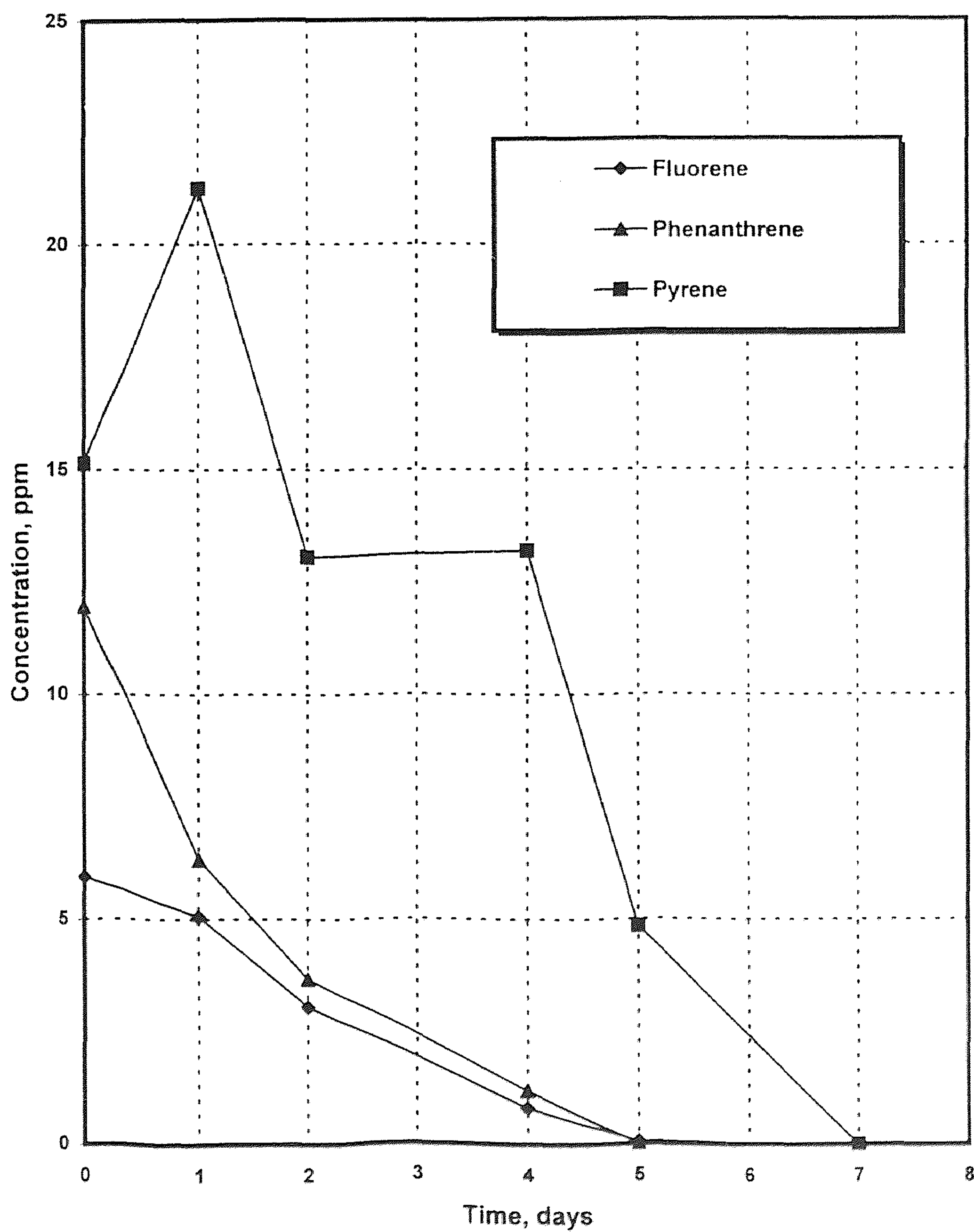


Figure 7.14 *Aqueous phase biodegradation of PAHs in 20% sludge with 0.3% Makon 10 in a batch fermenter*

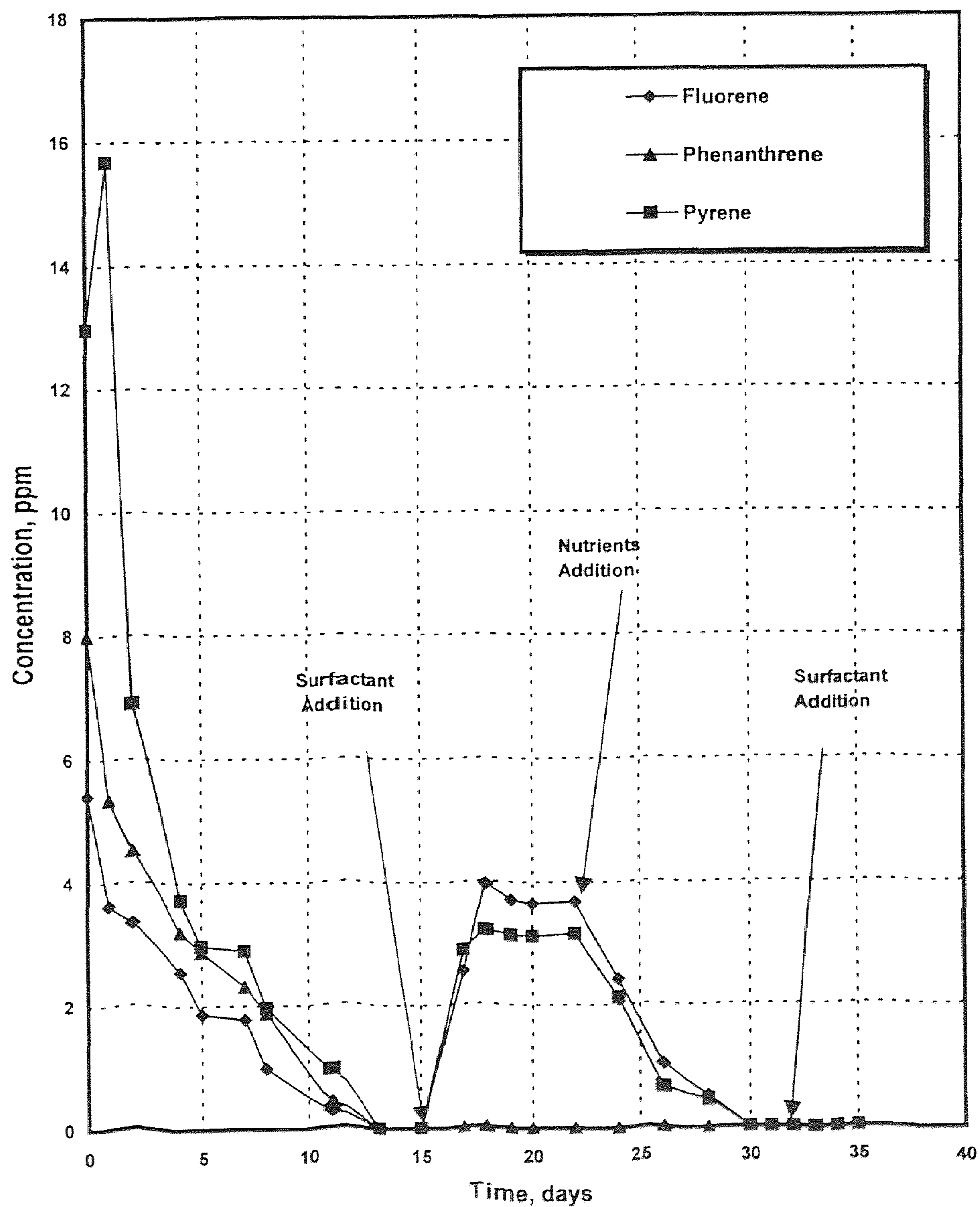


**Figure 7.15** *Biodegradation of PAHs in 20% sludge in the presence of 0.3% Makon 10 in a bioslurry reactor*

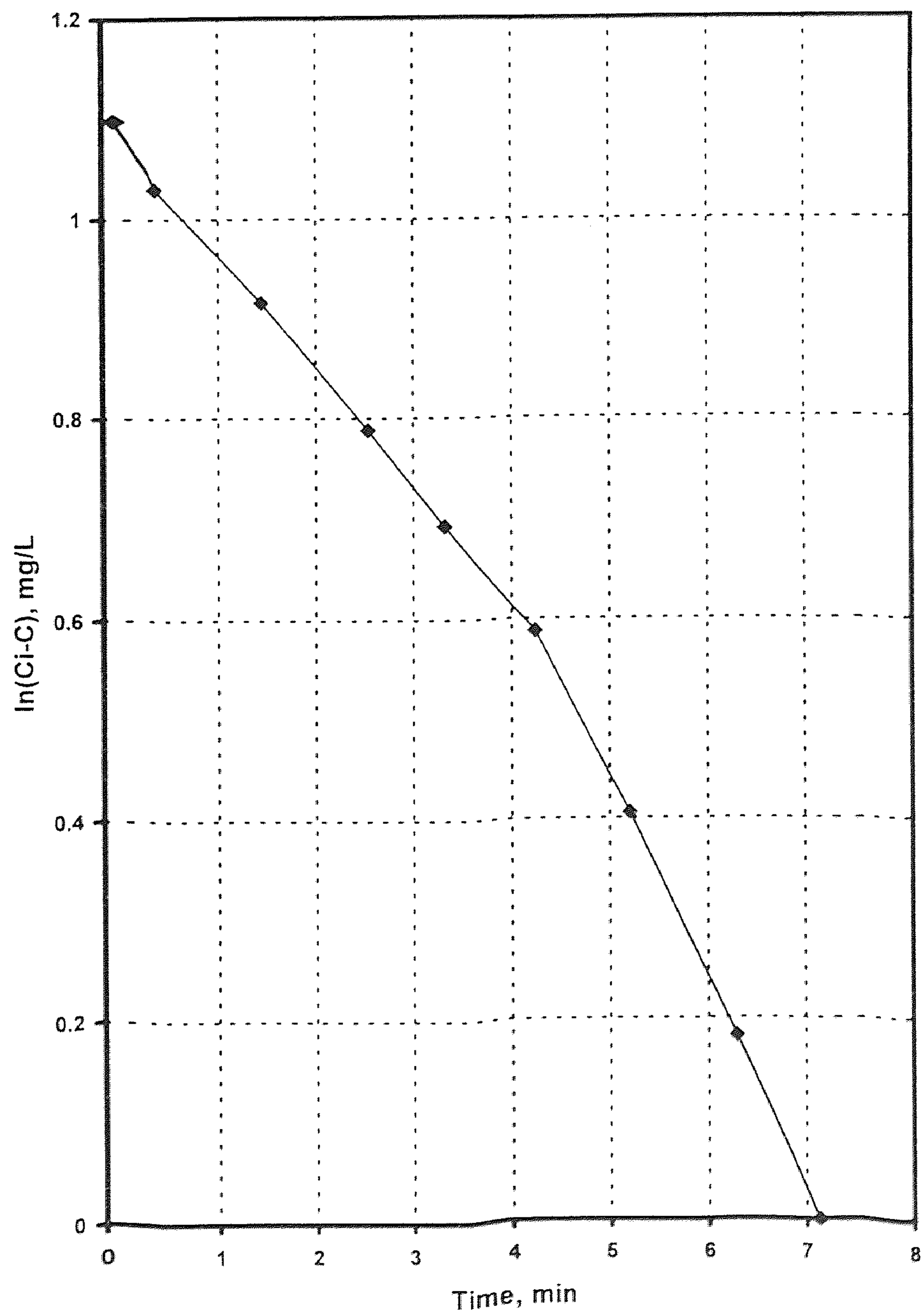


Figure 7.16 *Oxygen uptake by microorganisms*

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