PENTACHLOROPHENOL AND OXYGEN CONSUMPTION OF IMMOBILIZED MIXED BACTERIAL CULTURE.

by
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ABSTRACT

Title of Thesis: Pentachlorophenol and oxygen consumption of immobilized mixed bacterial culture.

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This study was undertaken to investigate the effects of Pentachlorophenol (PCP) concentration on oxygen consumption of immobilized microbial cells. The microbial population, from a municipal wastewater treatment plant, was immobilized in calcium alginate gel. The following studies have also been conducted in this project:

1. Comparison of the effect of PCP concentration on oxygen uptake between immobilized and free cells.
2. Comparison of the effects of PCP concentration on oxygen uptake between acclimated and an unacclimated bacterial beads.

Dissolved oxygen concentration was monitored using a

flow Clark-type oxygen probe, in a micro-assay bioreactor. It was found that PCP concentrations higher than 2000 ppm stimulated oxygen consumption rate. However, a free chloride test indicated no generation of chloride ion. For the immobilized system, instantaneous inhibition of oxygen consumption was not observed. Under identical experimental conditions, studies were made on free suspended microbial cells. Concentrations of 2000 ppm and 5000 ppm of PCP were fatal for unacclimated and acclimated free systems respectively. Using a recirculation bioreactor, PCP was not effectively degraded, under the conditions which the experiment was run, and the stimulation of respiration was without substrate consumption.

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TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
	1.1 Immobilization and its Advantages	7
	1.2 Requirements of Dissolved Oxygen	9
	1.3 Activated Sludge and Biodegradation	11
II.	OBJECTIVES	13
III.	LITERATURE REVIEW	14
IV.	MATERIALS AND EXPERIMENTAL METHODS	20
	4.1 Microorganisms	20
	4.2 Immobilization	20
	4.3 Defined Nutrient Medium	21
	4.4 Check for Viability in A Microassay Reactor	21
	4.5 Biodegradation of Pentachlorophenol in Recirculation Reactor	24
	4.5-1 Experimental Setup and Procedure	24
	4.5-2 Oxygen Measurement	26
	4.6 Control Run	26
	4.7 Free Chloride Determination	27
V.	RESULTS AND DISCUSSIONS	28
	5.1 Preliminary Studies	28
	5.2 Effect of PCP Concentration On Initial Oxygen Uptake Rate in Microassay Reactor	28
	5.2-1 Unacclimated Immobilized Beads	29
	5.2-2 Acclimated Immobilized Beads	29

5.2-3 Comparison Between Free and Immobilized Acclimated Microorganisms..31

5.2-4 Comparison Between Free and Immobilized Unacclimated Microorganisms.....34

5.2-5 Comparison Between Acclimated and Unacclimated Free Microorganisms.....34

5.3 Biodegradation of PCP in a Recirculation Reactor 34

5.4 Free Chloride Test.....37

5.5 Use of Surfactant.....37

VI. CONCLUSIONS AND SUGGESTIONS 40

6.1 Conclusions 40

6.2 Suggestions 40

APPENDIX 42

BIBLIOGRAPHY.....48

LIST OF TABLES

Table Page

I. Solubility and Partition Coefficient.....4

II. Properties of Pentachlorophenol.....6

LIST OF FIGURES

Figure Page

I. Experimental Setup of Microassay Reactor.....23

II. Experimental Setup of Recirculation Reactor 25

III. Effect of PCP Concentration on Initial Oxygen Uptake Rate in a Microassay Reactor.....30

IV. Comparison of Oxygen Uptake Rate Between Acclimated and Unacclimated Immobilized Microorganisms.....32

V. Comparison of Oxygen Uptake Rate Between Free and Immobilized Acclimated Microorganisms.....33

VI. Comparison of Oxygen Uptake Rate Between Free and Immobilized Unacclimated Microorganisms.....35

VII. Comparison of Oxygen Uptake Rate Between Acclimated and Unacclimated Free Microorganisms...36

VIII. Biodegradation of PCP by Immobilized Bacteria in a Recirculation Reactor.....38

CHAPTER I

INTRODUCTION

Halogen-containing organic chemicals have many beneficial uses as pesticides, solvents, surfactants, and plastics. However, their widespread uses have resulted in a variety of water pollution problems. The ultimate fate of chlorinated compounds in the aquatic environment has been the focus of extensive research. Biological treatment and specifically the activated sludge process is used widely for the treatment of municipal as well as industrial wastes. The large variety of microorganisms present in the activated sludge reactor and their interaction may hold the key to the effective treatment and removal of chlorinated compounds. The occurrence of chlorinated organics in wastewater and the removal by activated sludge treatment has been reported (1). The accumulation of lipid-soluble, water insoluble pesticides in living organisms is one of the most disturbing features of environmental pollution by pesticides.

PENTACHLOROPHENOL

The primary application of PCP in the U.S. is to inhibit mold and control wood-boring insects in the lumber

and construction industry. As is typical of chlorinated pesticides, PCP has proven to be somewhat resistant to biodegradation. However the ability to degrade the biocide has been demonstrated among bacterial populations and fungi, in both pure and mixed cultures (2). Most studies have concentrated on the conditions and the rate of degradation, but little information exists on the microbial degradation pathway.

The resistance of chlorophenols to microbial decomposition is said to be dependent on the number and position of chlorine atoms on the phenol ring. PCP is one of the most refractory compounds of the chlorophenol family. PCP has been cited as an example of the fallibility of microorganisms in being able to cope with unusual "man-made" chemicals (3).

USES OF PCP

PCP and its salts have probably had more varied uses than any other pesticide. It has been widely used as a pre- and post-plant herbicide in several crops, but its largest use as a herbicide has been in rice paddy fields. It also is used extensively as a wood preservative against wood rotting and staining microorganisms, and for the control of powder post beetles, wood-boring insects, and termites. Other uses have been as a biocide in cellulosic products, starches,

adhesives, proteins, leather, oils, paints, rubber, rug shampoos, textiles, food processing plants, and the control of bacteria, fungi, insects, mollusks, and other nuisance biota (4).

OCCURRENCE

Occurrence of PCP as a residue in human and animal tissues and its presence in 85% or more of the urine samples of people exposed non-occupationally to PCP suggest a rather wide distribution of this compound. Whether such ubiquitous distribution of PCP is due to the patterns of usage of PCP alone has been questioned. Other possible sources of PCP which have been suggested include degradation of hexachlorobenzene and pentachloronitrobenzene (5).

DEGRADATION OF PCP IN SOIL

Degradation in soil is affected by numerous chemical, physical and biological factors. PCP degrades more rapidly in flooded or anaerobic soil than in aerobic moist soil. The degradation of PCP in soil is primarily by reductive dehalogenation to simpler tetra-,tri-, and dichlorophenols (47) (see Appendix also).

MICROBIAL DEGRADATION OF PCP

Microbial activity affects the persistence of an organic biocide in the natural environment by means of

biodegradation and transformation. Various microorganisms such as bacteria and fungi have demonstrated their capability of degrading PCP and other chlorophenols. Very little information is available regarding PCP biodegradation in the aquatic ecosystem and a recent study has indicated that the oxidative pathway is the major mechanism for PCP degradation in a simulated environment (6).

The role of microsomal enzymes in the living organisms to cope with xenobiotics is to increase the polarity of the xenobiotics which will be discharged more effectively, eg. hydroxylation. Hexachlorobenzene is degraded to the relatively polar compound PCP. Similarly, PCP is degraded to tetrachlorohydroquinone and pentachlorophenyl acetate as their metabolites in the organisms. The following table shows these characteristics.

Table 1. Solubility and Partition Coefficient.

	Water Solubility	Partition Coefficient (octanol-water)
Hexachlorobenzene	0.006 ppm	13560
PCP	20 ppm	6400
Tetrachlorohydroquinone	21.5 ppm	3200
Pentachlorophenyl acetate	20 ppm	2350

Therefore the process of ecosystem degradation of PCP

involves formation of more water soluble and less lipophilic degradation products which are more readily eliminated by the various organisms of the ecosystem.

ENVIRONMENTAL FATE OF PCP

Hundreds of millions of pounds of PCP have been applied world wide, yet there is little information on the environmental fate and impact of this chemical. Such studies are of particular importance because of the relative environmental stability of PCP and its high toxicity to nearly all forms of life as an inhibitor of oxidative phosphorylation. More recently, PCP and dioxin impurities have been implicated in the death of farm animals chewing on PCP treated wood(Chem. Eng. News,1977). Probably the widest scale usage of PCP has been in Japan where its use as a fungicide and herbicide has resulted in contamination of almost all surface waters to 0.01-0.1 ppb (7).

CHEMICAL/PHYSICAL PROPERTIES OF PCP

PCP is a fully chlorinated phenol. Pure PCP, a white solid with needle-like crystals, is practically insoluble in water. The most widely used metallic salt of PCP is sodium pentachlorophenate. Therefore, the readily water-soluble salt is substituted in many industrial applications. PCP is soluble in most organic solvents, oils, and petroleum hydrocarbons with high aromatic and olefinic content, which

make it compatible for inclusion in many pesticide formulations.

Useful data on the properties of PCP are given in the following tabulation (Monsanto 1958, Dow 1962).

Table 2. Properties of Pentachlorophenol

Molecular weight	266.36
Melting point	190^0 C
Boiling Point	293^0 C
Density	1.85 gm/cm^3
Vapor pressure (20^0-100^0 C)	0.00011- 0.12 mmHg.
Solubility in water	14- 19ppm.

PCP is relatively stable and will not decompose when heated at temperatures up to its boiling point for extended periods of time.

Innovative Technology In Biological Treatment

In the past, biological reactors for the treatment of aqueous waste containing toxic compounds have typically utilized activated sludge in suspended form. With the complexity of the problem rising day by day, new and innovative technologies are now being investigated. The use of immobilized microorganisms has been so far limited to the selection of new immobilizing matrices and determination of

removal rates in batch reactors. In this study, performance of a packed bed recirculation reactor utilizing a calcium-alginate immobilized mixed microbial population has been investigated. The reactor was operated in a recirculation mode with an externally aerated reservoir.

1.1 IMMOBILIZATION AND ITS ADVANTAGES

The application of immobilized bacterial culture for bio-oxidation has been a subject of intensive study in recent years. The technique of immobilization has many advantages over the conventional free culture system in wastewater treatment. One of the problems an activated sludge plant encounters is washout of biomass. Moreover, the system is very sensitive to shock loads and cannot sustain frequently varying input parameters. Longer cell residence time (CRT) is required for the organisms to be acclimated and produce a population that is active in degrading the toxin. The use of the technique of immobilization solves many of these problems. This system facilitates separation and has a greater degree of operational flexibility, as continuous or semicontinuous processes become practical. Immobilized cells are much more resistant to high concentrations of toxic chemicals (8). The high density of the immobilized system results in high rate of bio-oxidation per unit volume of the bioreactor. Moreover, Immobilized cells can also be dried and stored over several months, and

can be used as reproducible biomass.

Several methods have been developed for immobilized biocatalyst preparation. There is no universal carrier nor immobilization method for all living cells, and each application should be separately tested and optimized. The support material should withstand the substrate, product and reaction conditions and it should be suitable for continuous or repeated use in the scale desired. Moreover, the method should be sufficiently gentle for the living cells. For example, fungal mycelium may simply be dried and grown in a pellet form to be used as a biocatalyst (9). Microbial cells may be flocculated or aggregated; they may be attached to a suitable carrier by adsorption or ionic bonding or they may be entrapped in a polymer matrix. Among the most used adsorption carriers are activated charcoal, porous ceramics and porous silica supports. Suitable polymers for entrapment of cells include alginate, K-carageenan, polyacrylamide and polyvinyl alcohol.

The Detox Group in this lab did an investigation on the use of calcium alginate for the entrapment of microbial cells. Cell entrapment in alginate is a rather simple and non-toxic method for immobilization, but the gel may create a diffusional barrier for both substrate and oxygen (10). It has been reported that calcium alginate gel provides little barrier to the diffusion of neutral substrates up to a molecular weight of 5,000 (11).

For microorganisms entrapped within a matrix, the mass transfer resistance around the beads may reduce the effectiveness of microbial activity per unit volume by limiting the availability of substrates, resulting in lower specific substrate utilization. It has been reported (12), that mass transfer resistance around the beads is closely related to system parameters used in the reactor, such as flow rate, bead size and bead composition, but the mass transfer within the beads is still limited by intraparticle diffusional resistance.

1.2 REQUIREMENTS OF DISSOLVED OXYGEN

For a system which utilizes aerobic microorganisms for the biodegradation of organic compounds, dissolved oxygen (DO) requirements are of great importance. Biodegradation of chlorinated organic compounds such as 2,4-dichlorophenoxy-acetic acid requires molecular oxygen as a co-substrate for metabolism (13). Aerobic microorganisms also utilize oxygen primarily as the terminal electron acceptor for aerobic respiration. Bacterial respiration is generally not affected by DO concentration above the critical value. In the present study , the demand for DO increases with increasing PCP concentration without the utilization of the toxin. The critical dissolved oxygen concentration has been defined as the concentration at which the respiration rate of cells is one half of the maximum rate. It is generally lower for

dispersed cultures than for flocculant culture (14). Relatively little is known about the influence of dissolved oxygen on the microbial degradation of organic chemicals. In one of the studies, it was found that the half life of biodegradation of nitrilotriacetic acid in natural water samples increased from 1.3 to 5.8 days as the dissolved oxygen concentration decreased from saturation level to about 0.3 mg/liter (15). In the case of microorganisms entrapped in alginate gel, diffusion can become a rate limiting factor for oxygen uptake and may further increase the half life of biodegradation. The degradative pathways involve a number of enzymes. If one of them is oxygenase, then the degradative rate will also depend on oxygen concentration.

The major disadvantage of immobilization is the increased diffusional resistance of substrates and products through immobilization matrices. However, this can be of an advantage in a system which contains highly concentrated toxin. Due to the low solubility of oxygen in water and high local cell density, oxygen transfer is often the rate limiting factor in the performance of aerobic immobilized cell systems (16). Methods that have been used to increase the availability of oxygen to immobilized cells include decreasing the particle size of the immobilization matrix and increasing the oxygen holding capacity of the medium. Increasing the oxygen holding capacity of the medium has

been attempted by the addition of hydrogen peroxide and perfluoro chemicals (16,17).

1.3 ACTIVATED SLUDGE AND BIODEGRADATION

Activated sludge is a widely used biological process for the oxidation of organic compounds in municipal and industrial wastewaters. A variety of microorganisms present in the activated sludge reactor and their interaction may hold the key to the effective treatment and removal of chlorinated compounds. The diversity of the microbial flora serves as a good environment for developing cultures that can biodegrade synthetic organics in general and chlorinated organics specifically.

PCP is among the chlorinated phenols that is most toxic, and hence, resistant to biodegradation. It has been reported that PCP shows self-inhibitory effects at high concentration. Most of the activated sludge treatment plants do not have the ability to meet state requirements for the removal of PCP from the wastewater.

For microorganisms entrapped in alginate gel, the rate constants may not be the same as those for free microbes, and factors other than microbial activity may be rate limiting. Research in the past has indicated that acclimated cultures capable of utilizing chlorinated organic substrates can be derived from activated sludge, and are effective over a wide range of substrate concentrations (18).

The main removal mechanisms in biological treatment systems are: adsorption, stripping and biodegradation. In the case of PCP, however, stripping and adsorption are of minor concern due its low vapor pressure. The stripping rate is a direct function of the thermodynamic equilibrium between the liquid and gas phases.

CHAPTER II

OBJECTIVES

The specific objective of this study has been, to investigate the effect of PCP concentration on oxygen consumption rate of an immobilized microbial population. The effect of PCP concentration on immobilized and free bacterial culture is compared. The biodegradability of PCP by immobilized bacteria in a recirculation bioreactor is also investigated.

CHAPTER III

LITERATURE REVIEW

In the study of microbial metabolism of pesticides both mixed populations and pure cultures of microorganisms have been employed. With mixed populations provided by soil, water, sewage, etc., the biodegradability of the tested compounds has been claimed to be closer to "environmental conditions" (19), however, a comprehensive study of the metabolism of a given pesticide is usually approached with experiments employing pure cultures under rigidly controlled laboratory conditions.

The biodegradation of several chlorinated hydrocarbons was studied. Under aerobic condition with raw sewage as the source of microorganisms, Leigh (20) found that Lindane could not be degraded while heptachlor was bio-oxidized to 1-hydroxy chlordene and an unidentified substance. Simizine was shown to metabolized by Aspergillus fumigatus in experiments performed by Kaufman et al. (21), since the product was ammelide, no ring cleavage seemed to occur. Diazinon labeled in position 2 of the pyrimidine ring was incubated with soils and the release of radioactive carbondioxide found by Sethuanathan and McRae (22) gave evidence for the biodegradation of this pesticide.

Wright and Cain (23) reported that an Achromobacter sp. isolated from soil utilizes 4-carboxy-1-methylpyridinium chloride as sole carbon source for growth. Since this compound is a photolytic decomposition product of paraquat it follows that paraquat can be degraded by a combination of photochemical and biochemical reactions.

A mixed population of microorganisms derived from activated sludge was shown by Schwartz (24) to metabolize isopropyl metachlorocarbanilate (CIPC). Kearny and Kaufman (25,26,27,28,) have found that a species of Pseudomonas striata could utilize CIPC as a sole carbon source for growth.

Microbial metabolism of a derivative of chlorophenol, 2,4-dichlorophenoxyacetic acid (2,4-D), has been one of the most intensively studied topics in the biodegradation of pesticides. Steenson and Walker (29) showed that both 2,4-D and 4-chloro-2-methylphenoxyacetic acid could be metabolized by an Acromobacter sp. in the presence of peptone and when the peptide concentration was at or below 0.3 mM . When concentrations of these two compunds were increased to 0.6 mM, inhibitory effects of the substrates were noted.

The disappearance of PCP from moist organic soil was reported by Young and Carroll (30) and biodecomposition was assumed to be involved. Nevertheless, many researchers have reported that PCP was resistant to microbial attack in soils

or in waters. Heidman et al. (31) treated a synthetic wastewater containing PCP and glucose as carbon sources and have found that PCP was not treatable by the activated sludge.

Kirsch and Etzel (32) using a prolonged batch culture enrichment technique have obtained a mixed culture which removed PCP from the culture medium and liberated radioactive carbon dioxide.

PCP inhibits oxidative phosphorylation. Bostrom and Johansson (33) found that, in vivo, PCP inhibited pyruvate kinase and lactate dehydrogenase activities in the livers of eels.

In a microbial system using Micrococcus denitrificants, Imai et al. (34) studied oxidative phosphorylation in a cell-free preparation of membrane fragments. They have shown that PCP at 5 x E-4M reduced ATP formation by 59%. From these data it appears that PCP has been classified as an inhibitor in energy transfer.

The ultimate fate of halogen containing organic chemicals in the ecosystem, and in particular in the aquatic environment, has been a focus of extensive research. There has been a growing interest in obtaining precise descriptions of biodegradation rates of specific chemicals in wastewater treatment systems to characterize decay rates in the environment. Most of the literature to date on biodegradation of chlorinated organic chemicals deals with

the use of free microorganisms.

Shamat and Maier (35) reported that activated sludge biomass could be used to develop microbial population capable of completely metabolizing chlorinated organic compounds. They also studied the kinetic parameters of these microorganisms in order to assess the feasibility of removing chlorinated organic wastes by the activated sludge process.

Westmeier and Rehm (36) studied the biodegradation of 4-chlorophenol by calcium alginate entrapped Alcaligenes sp.A7-2. When they compared the degradation rates of free and immobilized cells, they found that calcium alginate protects the cells against high concentrations of 4-chlorophenol and allows rapid degradation. No degradation products could be determined by HPLC detection after complete mineralization. They observed that high frequency feeding of small amounts of 4-chlorophenol was more favorable than low frequency feeding of large amounts. They also found that repeated use of immobilized microorganisms increased the degradation rates, but starvation for about three days caused a rapid decrease in degradation.

Tanaka et al. (37) studied diffusion characteristics of several substrates of varying molecular sizes into and from calcium alginate gel beads. It was found that the diffusion of high molecular weight substrates was limited more strongly by the increase of calcium alginate concentration

in the gel beads than by the increase in calcium chloride concentration used in curing the beads. Substrates having molecular weight less than 20,000 were easily taken into the gel.

In another study Gosmann and Rehm (38) investigated oxygen uptake of three different microorganisms, Pseudomonas putida, Saccharomyces cerevisiae and Aspergillus niger, all immobilized in calcium alginate gel. The oxygen uptake was compared with the respiration of free cells. It was shown that, the specific oxygen uptake of microorganisms decreased at lower cell concentrations. On the other hand, by increasing cell concentration in the gel, the cells had to compete for oxygen, and diffusion became the limiting factor for oxygen uptake.

In another study conducted in this laboratory (39), it was found that a concentration of 750 ppm 2-chlorophenol was fatal for the microorganisms in free form, while in immobilized form the microorganisms could withstand up to 5000 ppm of 2-chlorophenol. The rate of oxygen uptake was independent of the dissolved oxygen concentration in the bulk. Maximum activity was observed at a temperature of $37^{o}C$.

The ability of immobilized Flavobacterium cells to degrade the biocide has been reported (40). The ability of immobilized Flavobacterium cells to degrade nine initial PCP concentrations between 25 and 500 ppm was investigated. The

experiments were conducted in 125 ml flasks. Another set of experiment was conducted in a continuous column reactor, packed with 200 gms of beads. In the batch reactors, the bacteria degraded PCP up to 150 ppm. Although the degradation rate initially increased with increasing PCP concentration, inhibition was noted at higher concentrations. No degradation was detected at high concentrations. For the column reactor , the reactor could degrade about 100 mg PCP/hr per liter of reactor volume.

CHAPTER IV

MATERIALS AND EXPERIMENTAL METHODS

4.1 MICROORGANISMS

A mixed liquor from the Livingston (N.J.) wastewater treatment plant was used in this study. The microorganisms were acclimated at room temperature with PCP for a period of 15 days with a total concentration of 150 ppm. However the microorganisms used for the biodegradation runs were not acclimated to PCP. They were acclimated to phenol (100 ppm) and 2-chlorophenol (10 ppm) as the only carbon source, successively over a period of ten days (approximately 10 spikes) with continuous aeration. The culture was then centrifuged (International portable refrigerated centrifuge, Model PR-2) at 3000 rpm and 15^{o}C to obtain concentrated pellets.

4.2 IMMOBILIZATION

Immobilization of the microorganisms in calcium alginate gel was conducted as follows. Distilled water and concentrated pellets (6% dry biomass, w/w) were taken in a ratio of 5:2 by weight in a blender. Sodium alginate (0.75% w/w) was then added slowly to the mixture, with continuous

stirring to obtain a homogeneous cell suspension. With the help of a syringe pump (Sage Instruments, Model 351), the homogeneous cell suspension was then extruded as discrete droplets in a slowly stirred solution of 0.2 M calcium chloride. On contact with calcium chloride, the droplets hardened to form beads about 3-3.5 mm in diameter. Here, calcium chloride acted as a cross-linking agent. The beads were then cured in calcium chloride for 24 hours at 4^{o}C before use.

4.3 DEFINED NUTRIENT MEDIUM

The composition of buffered defined medium used in this work as follows (41):

Magnesium chloride	100 mg
Manganese sulfate	10 mg
Ferric chloride	0.5 mg
Potassium phosphate (pH 7.2)	2.0 g
Water	100 ml

The above solution was then diluted to 1000 ml by adding distilled water.

Fixed nitrogen was excluded from the defined medium in order to prevent biological growth within the beads.

4.4 CHECK FOR VIABILITY IN A MICROASSAY REACTOR

To study the viability of free and entrapped

microorganisms, a microassay bioreactor was used. The experimental set up is shown in Figure 1. Viability is defined here as the ability of microorganisms to consume dissolved oxygen from the medium for endogenous respiration (39). The microassay reactor consists of a 1.9 ml water jacketed reaction vessel with a small magnetic stirring bar. The concentration of dissolved oxygen was monitored using a Clark-type dissolved oxygen probe. Water at the optimum temperature (37^{o}) was circulated in the jacket through a water bath (Haake, TYP F-4391). The reactor was mounted on a stirrer plate, and the magnetic bar maintained uniform oxygen concentration. The oxygen probe was connected through an amplifier to a chart recorder assembly (Schlumberger, Model EU-205-11) which recorded changes in dissolved oxygen concentration (42).

The microassay bioreactor was sterilized in an autoclave at $120^{o}C$ and then washed successively with methanol and distilled water, before starting a new run. It was then rinsed several times with sterile defined medium. Sterile defined medium (1.8 ml) was then added to the reactor and saturated with oxygen by bubbling air through it. The saturation concentration of oxygen from air in water at $37^{o}C$ was estimated to be 200 nmole/ml. After saturation with oxygen, 5 beads weighing a total of 0.05 g ($\pm$ 3.0 percent) were shocked at $42^{o}C$ for 2-3 minutes in distilled water and then put into the reactor.

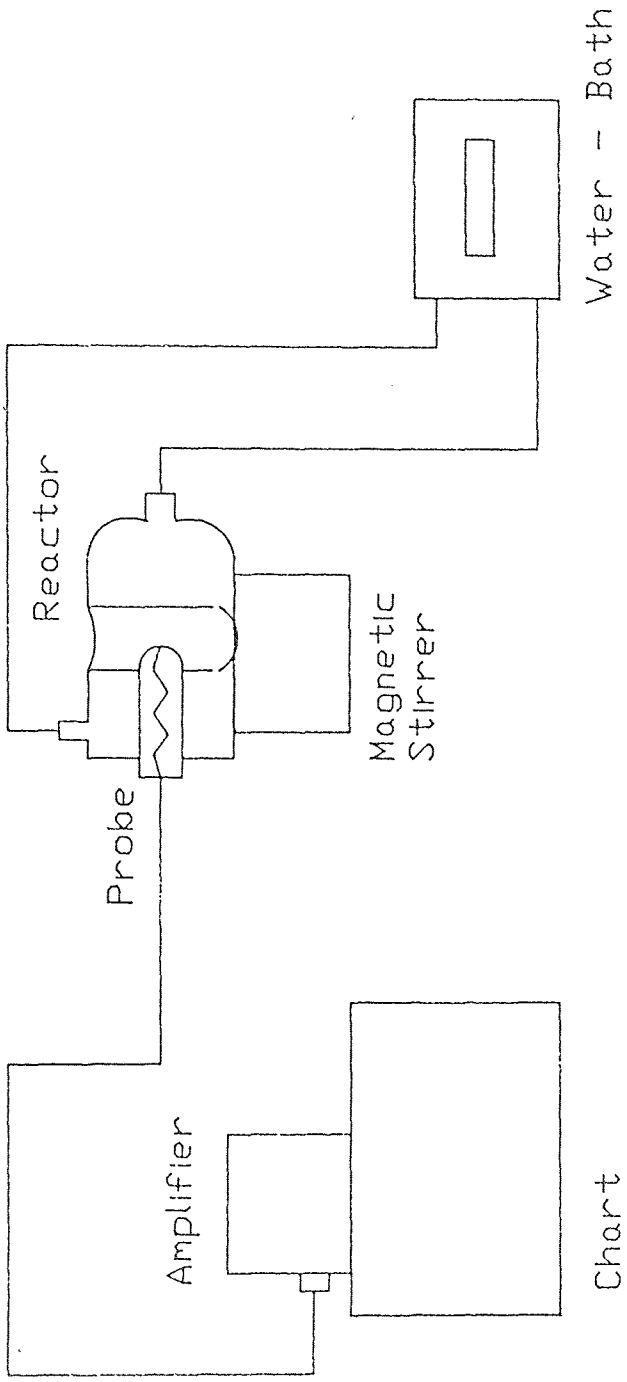


Fig. 1. Experimental Setup of Micro-Assay Reactor

The shock treatment was carried out to revive the microorganisms from their dormant state (39).The Reactor was then sealed from the top, and the concentration of dissolved oxygen monitored on the strip chart recorder. Sufficient mixing was allowed to overcome any mass transfer resistance.

4.5 BIODEGRADATION OF PCP IN A RECIRCULATION REACTOR.

4.5-1 Experimental setup and procedure

The experimental setup of the reactor is shown in Figure 2. The reactor was 2.5" in diameter and 10" in length. The reservoir was 4.5" in diameter and 9" in length. The total reaction volume was 2 liters. The reaction medium was circulated between substrate reservoir and packed reactor using a peristaltic pump (Horizon Ecology company).

The DO concentration was monitored using an impingement flow Clark-type dissolved oxygen probe. Filtered air was sparged into the reservoir at the rate of 1.0 liter/min. The reactor contained a thermometer and a pH probe (Orion Cat no: 91-04). An online flow meter regulated the flow rate of the recycle stream. A peristaltic pump (Cole Parmer Model 7016) was used to take samples from the reservoir. Samples were taken periodically and analyzed with a HPLC (Waters 501) for concentration of PCP. The activity of the beads was also tested periodically by stopping the air supply to the reservoir. A starting concentration of 20 ppm PCP was fed to

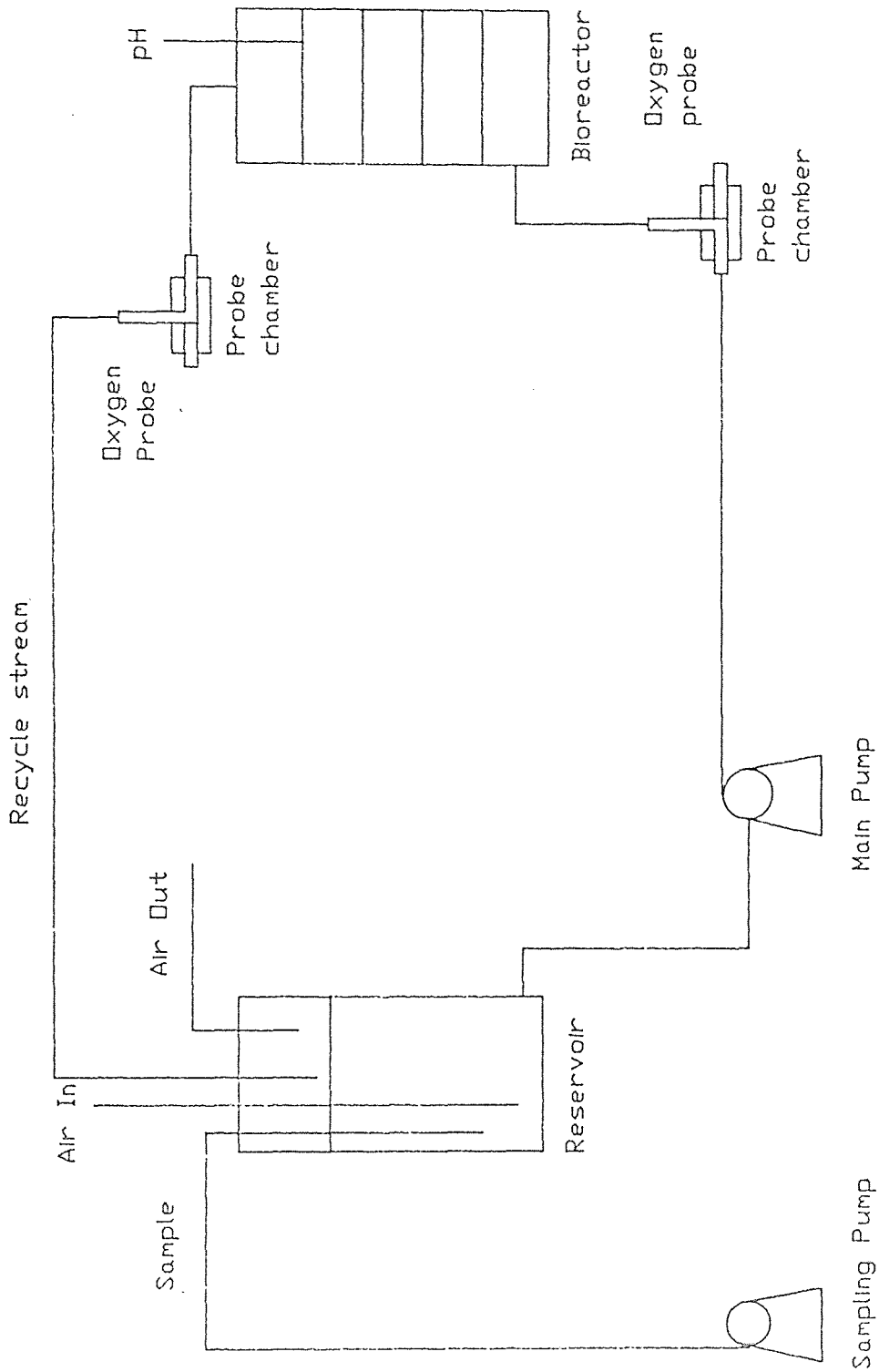


Fig. 2. Experimental Setup of Recirculation Reactor.

the system.

4.5-2 Oxygen measurement

The oxygen measurement unit consisted of an impingement chamber, oxygen sensor, signal conditioner and recorder (Omega Rd 2000). The oxygen sensor was used for the detection of dissolved oxygen producing an output in the millivolt range which was very sensitive to external vibrations and other factors such as the presence of stray magnetic field (10). Therefore, the output signal needed to be conditioned by a conditioner and then amplified to a certain level so that it could be used as input to the recorder. Since the signal from a Clark-type oxygen probe increased with the flow until a plateau was reached, the first step in oxygen measurement was to determine the minimum flow rate through the chamber for correct measurement. This was found to be 100 ml/min. The second step was the oxygen consumption measurement. In order to arrive at this point, the medium in the reservoir was saturated with air up to the maximum level (estimated to be 237 nmole/ml) and then aeration was stopped (43). The fall in the oxygen concentration in the system was continuously monitored using a strip chart recorder.

4.6 CONTROL RUN

An air sparged reactor was used at temperatures and

flow rates identical to the recirculation reactor without a biomass, to account for the removal of PCP by physical processes, stripping being the predominant mechanism. PCP, if exposed to direct sunlight, can undergo photodegradation. To minimize this effect, the reactor system and all samples were protected from direct sunlight.

4.7 FREE CHLORIDE DETERMINATION

To determine free chloride concentration silver nitrate test was used (44), before and after runs.

CHAPTER V

RESULTS AND DISCUSSION

5.1 PRELIMINARY STUDIES

Previous studies conducted in this lab (39) have shown that the method of immobilizing microorganisms in calcium alginate is very easy, practical and gives well defined integral beads.

Studies have also been made on effect of calcium chloride concentration on oxygen uptake rate.It was found that 0.1M calcium chloride was the optimum value. However, 0.2M has been used for better bead stability. Using the microreactor, parameters such as concentration of sodium alginate, biomass concentration and temperature were optimized with respect to oxygen uptake rate. The loading biomass in a recirculation reactor has been investigated in this lab (10). The optimum value was found to be 50 gms. of wet beads in a 2000 lit. recirculation reactor.

In the present study the parameters shown above were adopted, during the course of investigation.

5.2 EFFECT OF PCP CONCENTRATION ON INITIAL OXYGEN UPTAKE RATE IN MICROASSAY REACTOR

The effect of PCP concentration on the initial oxygen

uptake rates for free and immobilized microbial systems was studied. Comparisons on oxygen uptake between acclimated and unacclimated systems were also made.

5.2-1 UNACCLIMATED IMMOBILIZED BEADS

Microbial pellets which were not acclimated to PCP were immobilized and were cured in 0.2M calcium chloride solution for 24 hrs. Five beads of 6% dry biomass were used in the microassay reactor at 37 C. The results of this experiment is shown in Figure 3. Up to concentration of 2000 ppm, the oxygen uptake rate decreased with increasing PCP concentration. There was a sharp increase of the oxygen uptake rate as PCP concentration increased to about 5000 ppm. As the PCP concentration increased uptake rate of oxygen increased at rates lower than the initial rates. The uncoupling of oxidative phosphrylation (45) by PCP, i.e, the stimulation of respiration without utilization of substrate is responsible for the data that were generated.

5.2-2 ACCLIMATED IMMOBILIZED BEADS

Microbial pellets which were acclimated to PCP over a period of 15 days were immobilized and cured for 24 hrs before use in the microassay reactor. For sake of comparison, same dry biomass concentration of the unacclimated beads were used. Comparison of the two systems

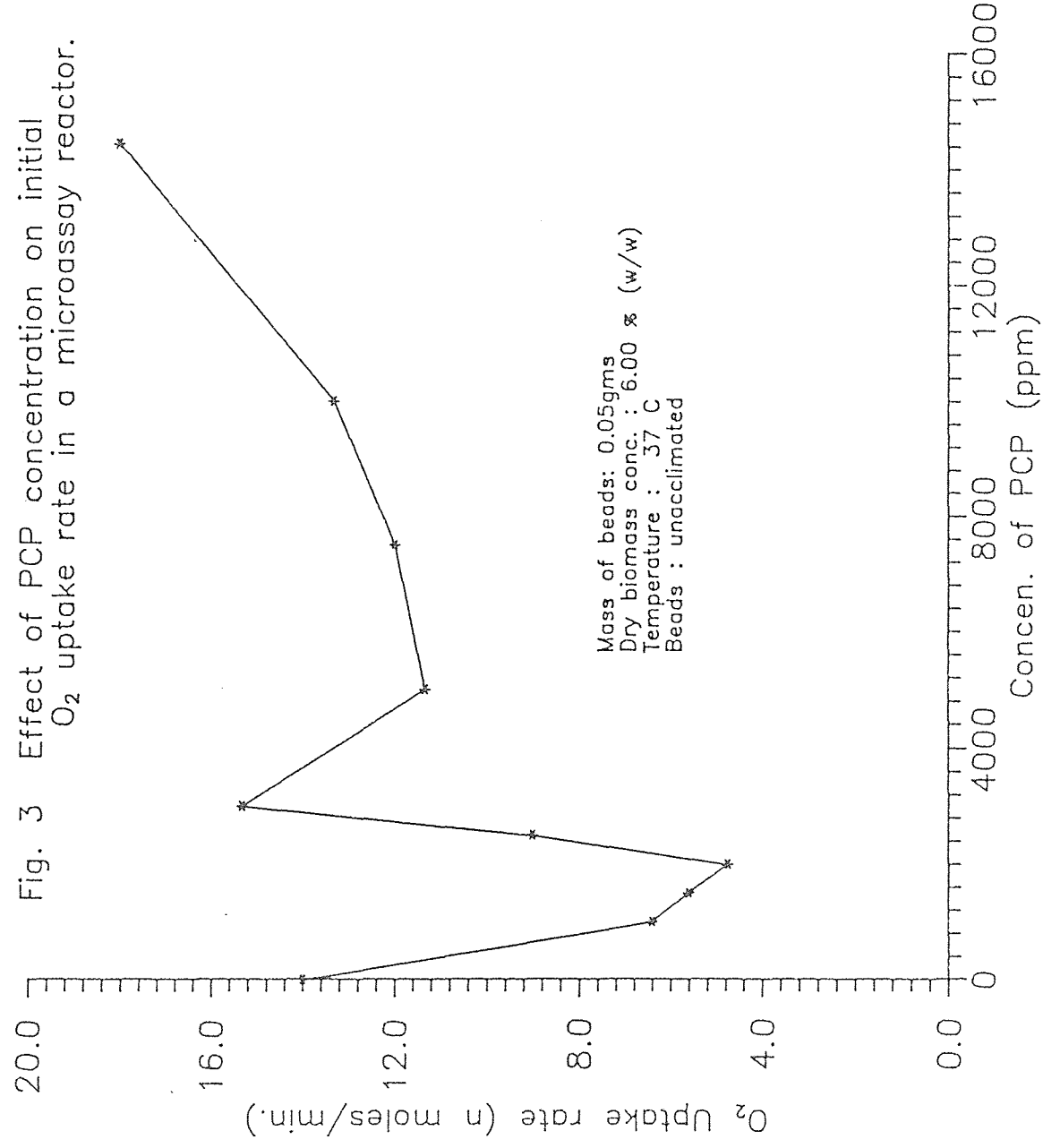


Fig. 3 Effect of PCP concentration on initial O_2 uptake rate in a microassay reactor.

is shown in Figure 4. Obviously the oxygen uptake rate for the acclimated is lower than that of the unacclimated beads. The uptake rate shown for the acclimated beads is that of colony of microorganisms that survived the toxic effects of PCP during acclimation period. The acclimated system has less active biomass involved in respiration. However, both systems withstood very high concentration of PCP, up to about 15,000 ppm.

5.2-3 COMPARISON BETWEEN FREE AND IMMOBILIZED ACCLIMATED MICROORGANISMS.

Using a microassay reactor, oxygen uptake rates of free acclimated pellets were measured, at same conditions as the previous systems. The uptake rates obtained were compared to that of acclimated immobilized beads. This is shown in Figure 5. For the free system the instantaneous inhibition occurred at a PCP concentration of only 5000 ppm, whereas the immobilized system withstood concentration higher than 15,000 ppm. This manifests one of the advantages of the technique of immobilization. At lower concentration the free system is shown to have higher uptake rates, due to diffusional resistance into the beads.

Fig. 4 Comparison of O_2 uptake rate between acclimated and unacclimated immobilized microorganisms.

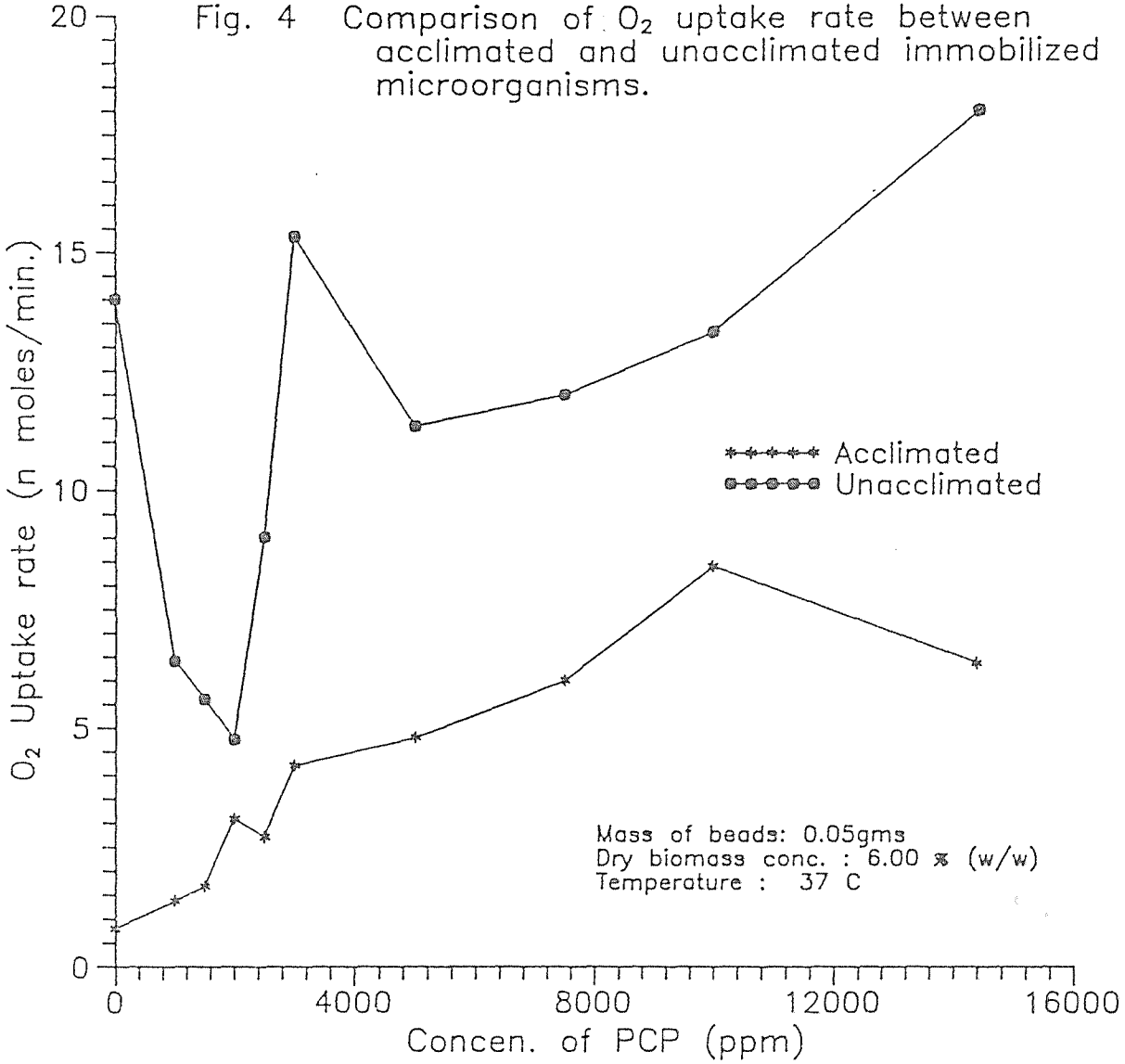
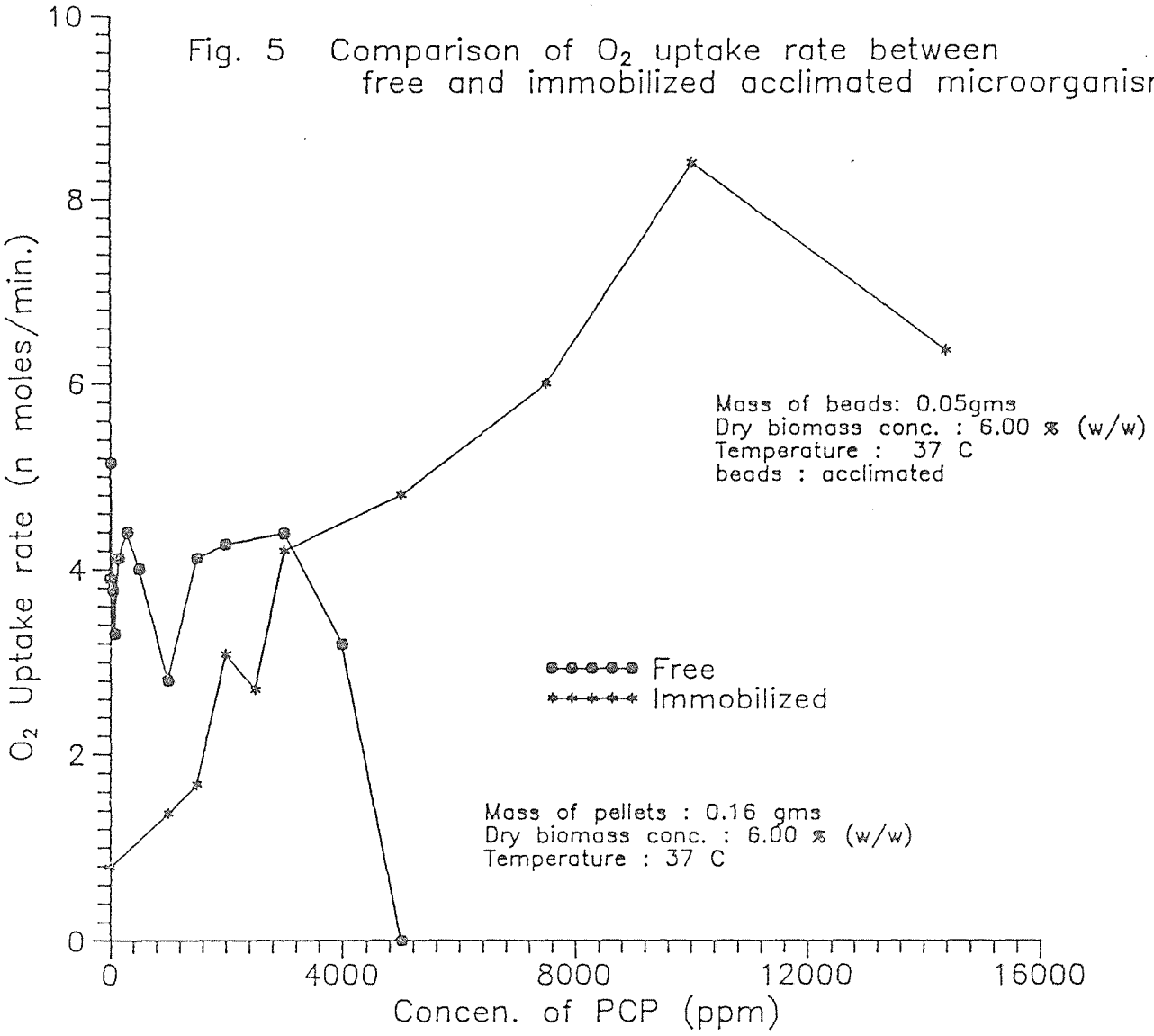


Fig. 5 Comparison of O_2 uptake rate between free and immobilized acclimated microorganisms.



5.2-4 COMPARISON BETWEEN FREE AND IMMOBILIZED UNACCLIMATED MICROORGANISMS.

The oxygen uptake of unacclimated free microbial cells was measured using the microassay reactor. The runs were made under identical experimental conditions as that of the immobilized beads. The uptake rates have been compared, and the result is shown in Figure 6. At 2000 ppm the uptake of oxygen is totally inhibited for the free system. Though the rates for the immobilized system are lower than that of the free system, the immobilized cells were able to sustain higher concentration of PCP.

5.2-5 COMPARISON BETWEEN ACCLIMATED AND UNACCLIMATED FREE MICROORGANISMS.

In Figure. 7 comparison of oxygen uptake rate for free acclimated and unacclimated systems is shown. Acclimation produces a colony that withstands high concentrations of PCP. As shown in the figure, the instantaneous inhibition occurred at 2000 ppm and 5000 ppm for unacclimated and acclimated free cells respectively.

5.3 BIODEGRADATION OF PCP IN A RECIRCULATION REACTOR.

To investigate the ability of the entrapped microorganisms to degrade PCP, a recirculation reactor was used.The reactor volume was 2 l, and the loading of biomass was 50 gms (wet weight) of beads. The feed flow rate was

Fig. 6 Comparison of O_2 uptake rate between free and immobilized unacclimated microorganisms.

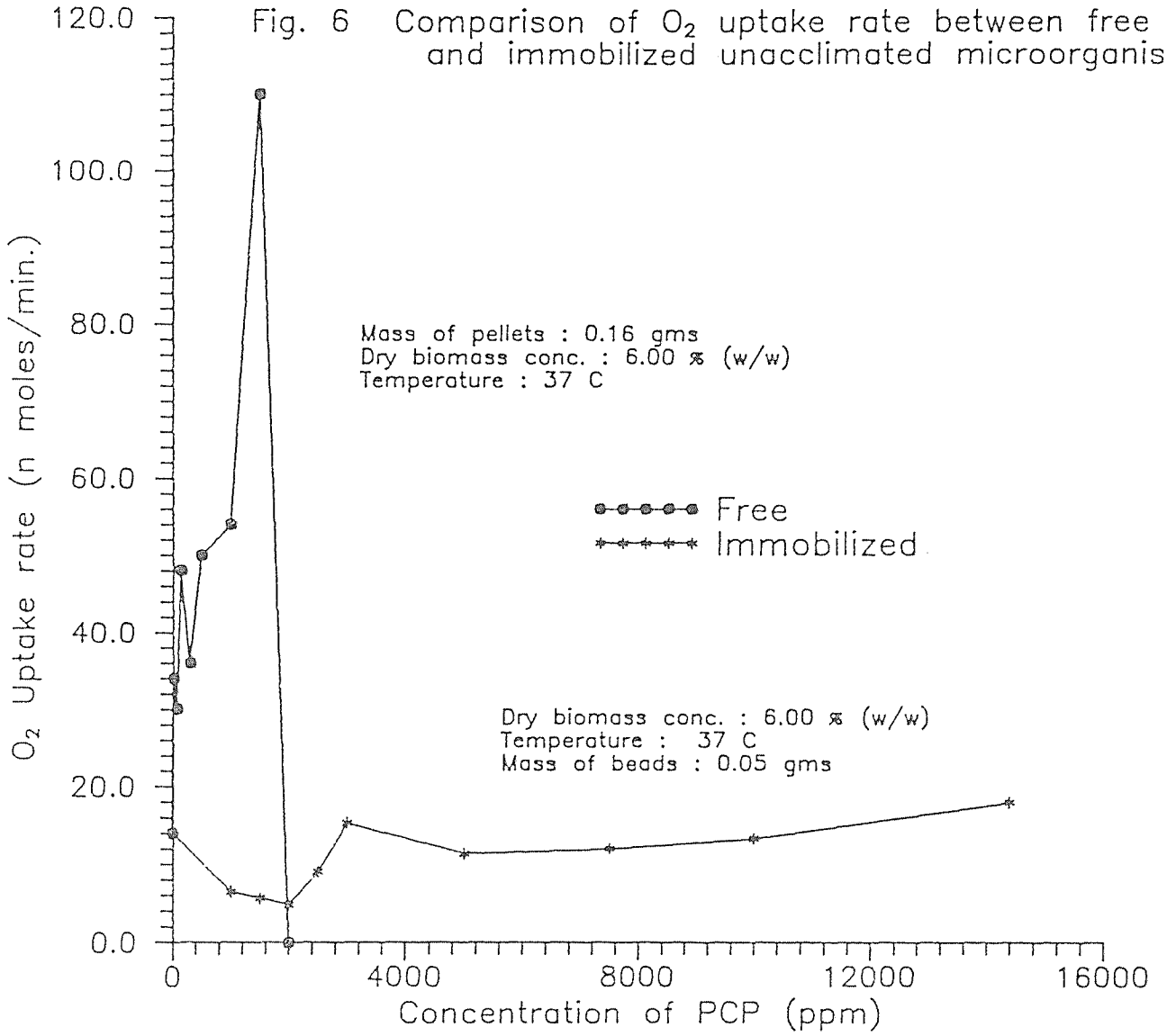
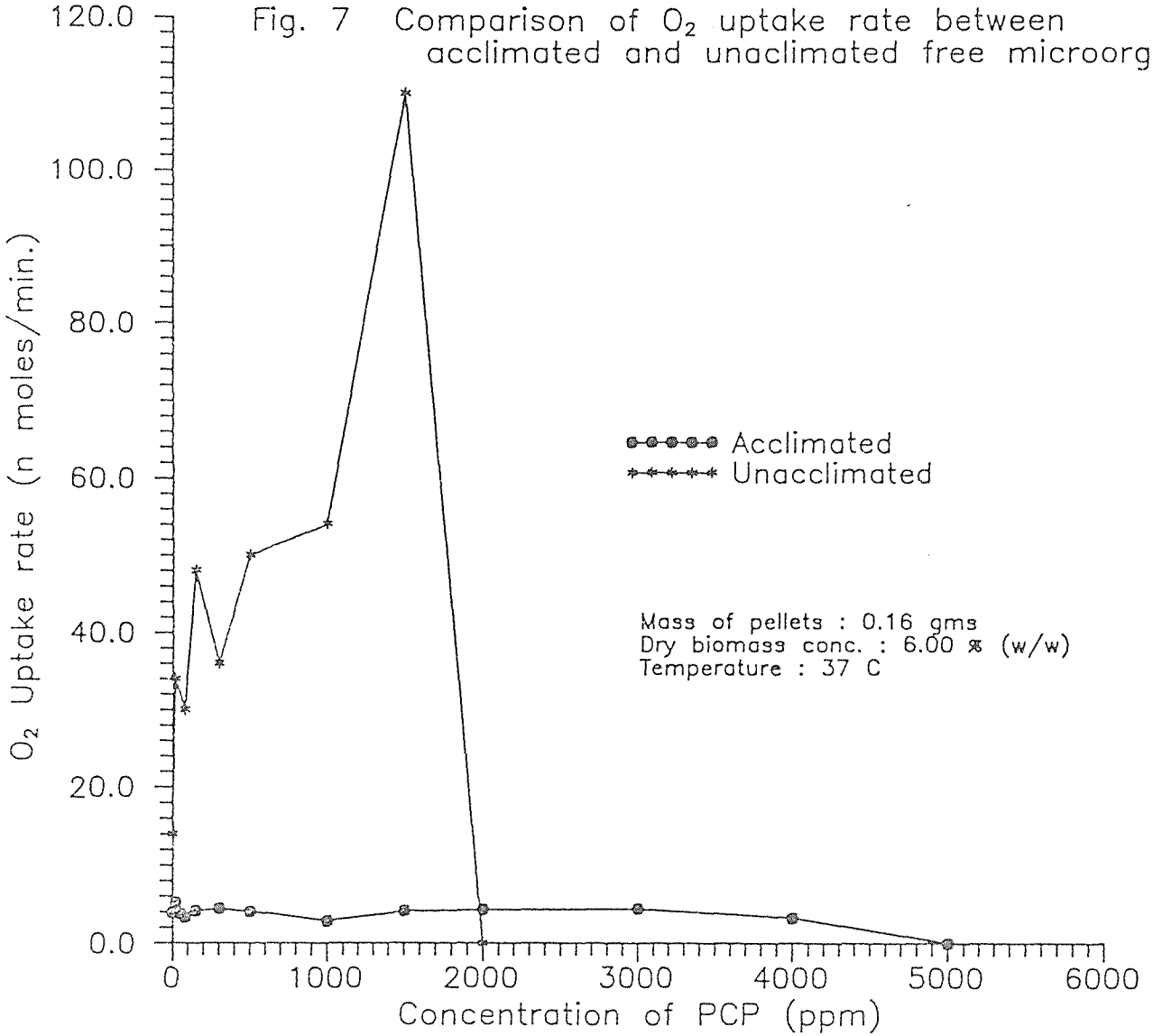


Fig. 7 Comparison of O_2 uptake rate between acclimated and unaclimated free microorganisms.



maintained at 220 ml/min. The experiment was conducted at room temperature. Initially 20 ppm of PCP was spiked into the reservoir. Daily samples were taken and the PCP concentration was monitored with HPLC. After 5 days the PCP concentration has dropped less than 10 % of the original concentration. This is accounted partly by stripping, adsorption and possibly photodegradation. This is shown in Figure 8. Essentially no biodegradation of PCP took place.

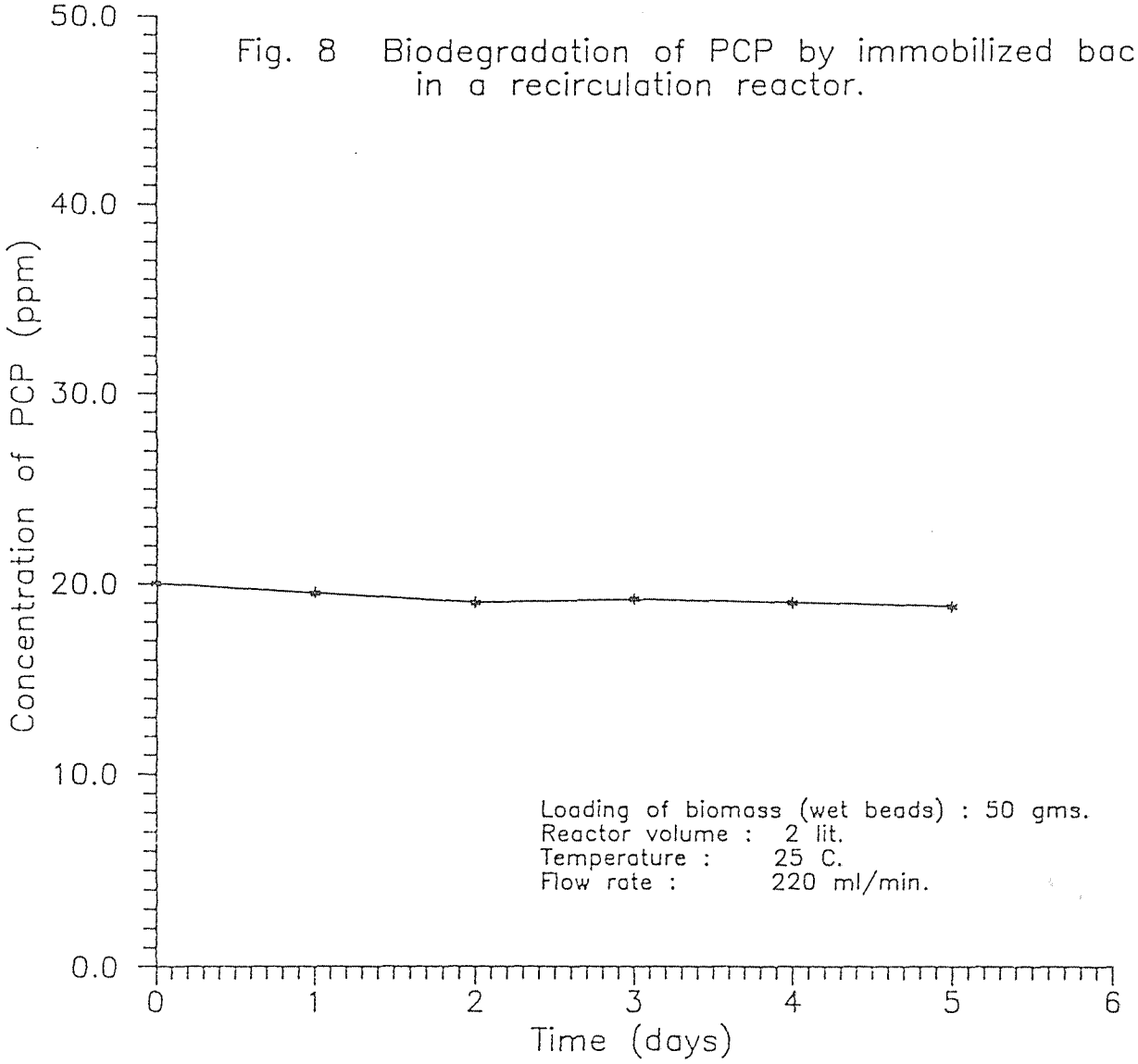
5.4 FREE CHLORIDE TEST

For the biodegradation runs in the recirculation reactor, free chloride test was performed before and after the runs. The results indicate that there was no free chloride generated during the five day run. The chloride was again repeated in a small scale in a microassay reactor. Five beads (6% dry biomass concentration) were used in this run. The experiment was run for a period of 5 hrs. A chloride test using silver nitrate solution indicated no new traces of free chloride.The solution was constantly being resaturated.

5.5 USE OF SURFACTANT

An attempt was made to use a nonionic surface-active agent to enhance the solubility of PCP in aqueous medium. This surfactant was provided by Rohm and Haas company. It belongs to a nonylphenol series, with an average of five

Fig. 8 Biodegradation of PCP by immobilized bacteria in a recirculation reactor.



polyoxyethylene chains. Its trade name is Triton-57 (46). It has a HLB (Hydrophile-Lipophile Balance) of 10, and is 90 % biodegradable as determined by loss of foam. A test was made to check its toxic effect on the microorganisms. There was no apparent adverse effect as it did not inhibit respiration. However, its use was abandoned due to the problems in analysis of PCP on the HPLC.

CHAPTER VI

CONCLUSIONS AND SUGGESTIONS

6.1 CONCLUSIONS

At high concentration of PCP, the rate of oxygen consumption increased for the immobilized system. There was no instantaneous inhibition of oxygen consumption. The free system was instantly inhibited at concentrations in the range between 2000-5000 ppm. A mixed microbial population entrapped in calcium alginate and shown to degrade chlorophenol, can not degrade PCP in a recirculation reactor which was running in a batch mode. Microbial population acclimated to PCP did not produce an enzyme system that degrades PCP. It is also found that high concentrations of PCP stimulated respiration. A chloride test using silver nitrate titration indicated no generation of free chloride.

6.2 SUGGESTIONS

In the present work, the feasibility of using an immobilized bacteria to degrade PCP was shown to be unsuccessful, under the conditions the experiment was conducted. In order to obtain better results the following recommendations are suggested.

1. Preliminary studies be done on the effects of PCP on microorganisms, especially the uncoupling of oxidative phosphorylation of PCP.

2. Very long acclimation period be allowed, so that a colony which can degrade PCP will be evolved.

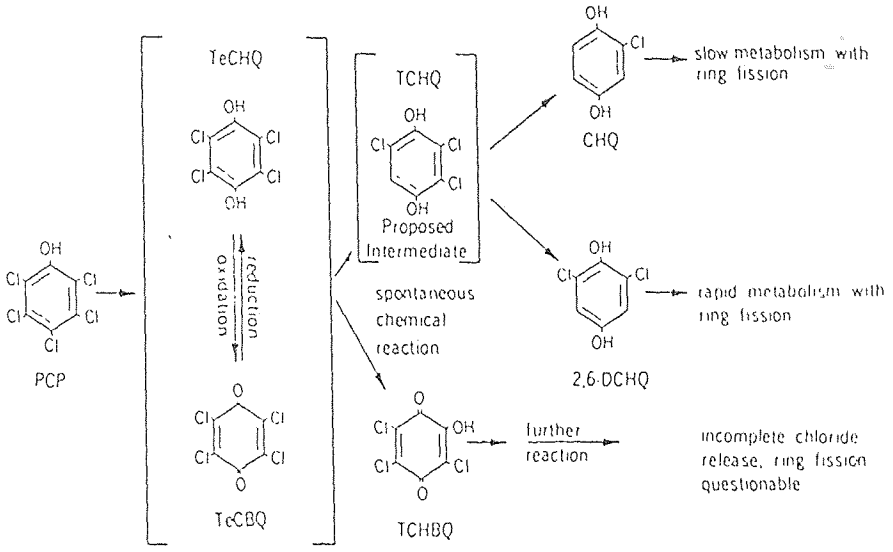
3. Use a microbial population from wood treatment plants which use PCP or Flavobacterium.

4. To degrade PCP effectively, use of high biomass concentration is recommended.

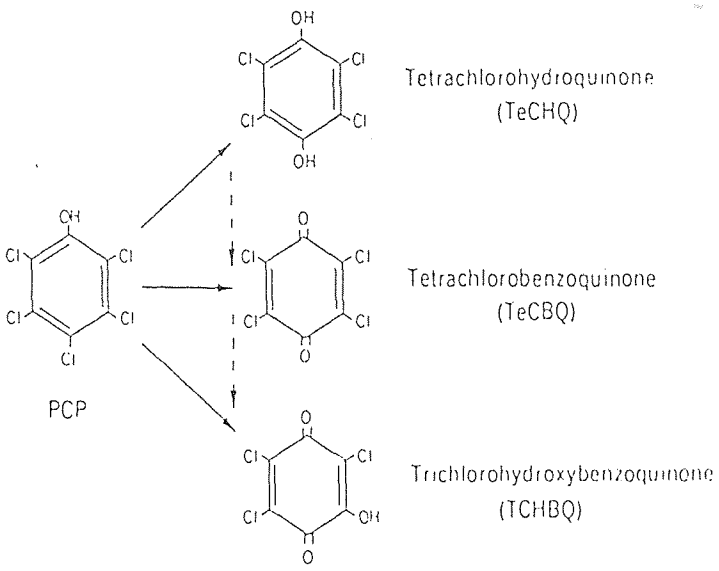
5. PCP be fed with other co-substrates which might induce enzymes that degrade PCP, eg. cresols.

APPENDIX

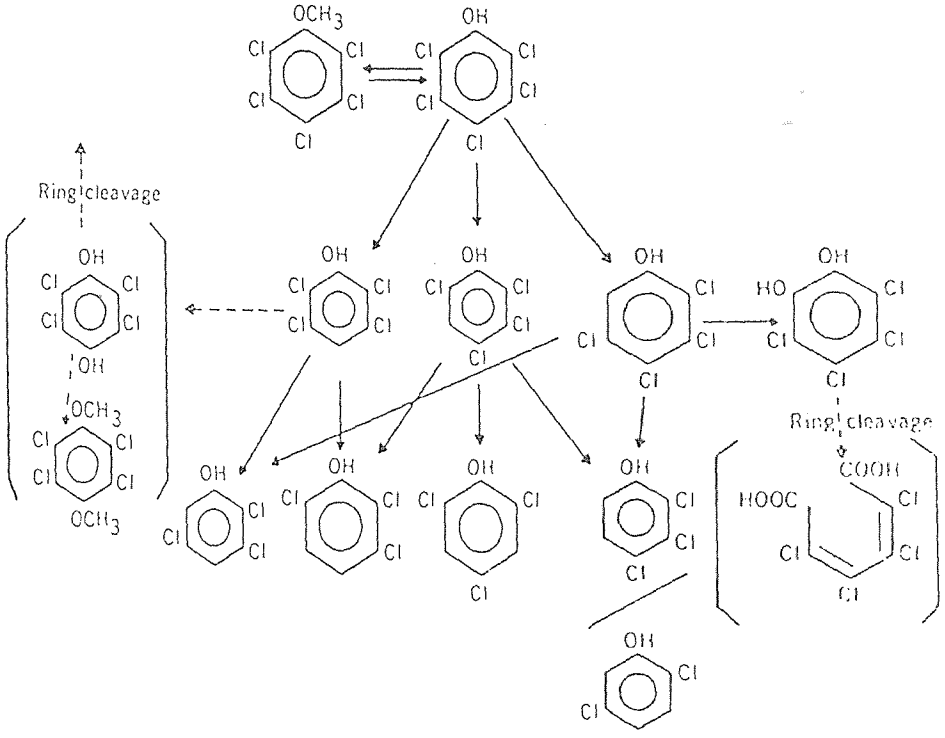
One of the objectives of this research has been, to lay foundations and provide basic information for further investigation. The pathway by which a complete mineralization of PCP occurs is not yet identified. However, there are some sources which suggest proposed pathways. Some of these are given in this part of the report.



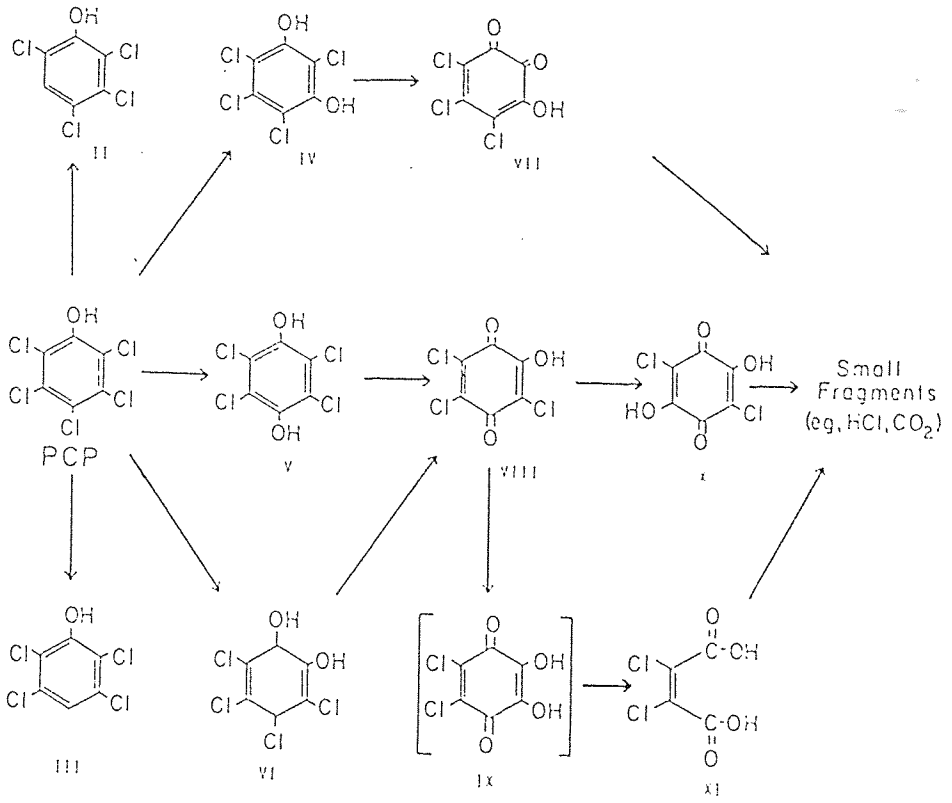
Hypothetical pathway for the biodegradation of pentachlorophenol by the bacterial culture, KC-3. (47).



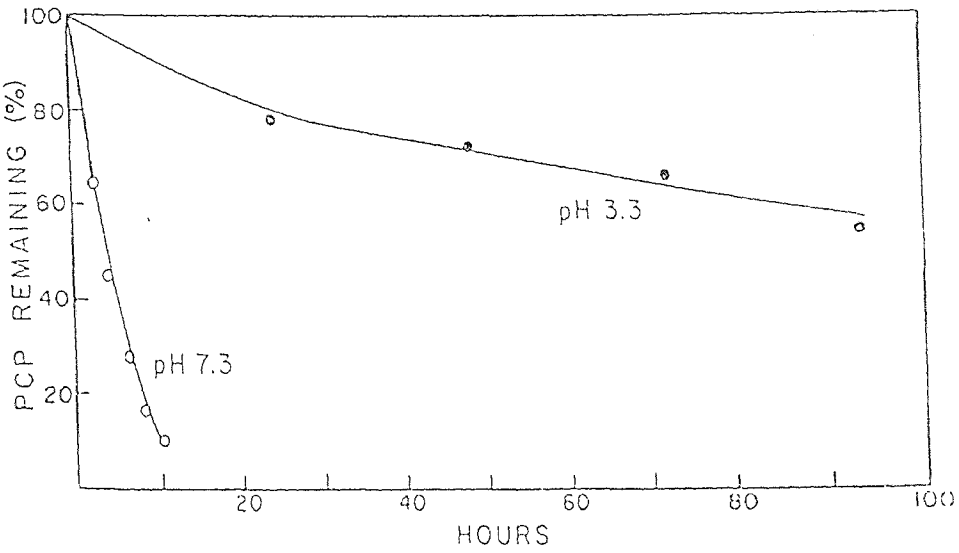
Products of PCP metabolism in cultures of the mutant ER-7.(47)



Proposed pathway of PCP degradation in soil. (47)



Proposed photolysis pathway for PCP. (47)



Photodecomposition rate of PCP at pH 3.3 and 7.3. (47)

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