USE OF RECIRCULATION REACTOR TO STUDY BIODEGRADATION OF AROCLOR 1242, A POLYCHLORINATED BIPHENYL

by
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APPROVAL SHEET

Title of Thesis: Use of recirculation reactor to study biodegradation of Aroclor 1242, a Polychlorinated Biphenyl

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ABSTRACT

Title of Thesis: Use of Recirculation Reactor to Study Biodegradation of Aroclor 1242, a Polychlorinated Biphenyl.

Babu S. Sanji, Master of Science in Environmental Science and Toxicology, 1988

Thesis directed by: Sam S. Sofer, Professor
Sponsored Chair in Biotechnology

The performance of immobilized microorganisms in the detoxification of hazardous waste was investigated using a recirculation flow reactor, run in a batch mode. A mixed microbial population from a wastewater treatment plant was immobilized in calcium alginate gel. The bio-oxidation ability of these microbes towards a model toxin Aroclor 1242 (R) (PCB 1242) was studied.

A non-ionic surfactant (Triton X-405 (R)) was used as a medium in the enhancement of the solubility of Aroclor 1242 in aqueous phase.

Dissolved oxygen concentration was monitored using a flow Clark-type dissolved oxygen probe. Oxygen consumption

was compared to the biodegradation of Aroclor 1242. Physical removal of Aroclor 1242 by adhering to the system without microorganisms was also determined and was compared with the biodegradation value obtained. It was found that biodegradation was the primary removal mechanism. Total removal of Aroclor 1242 was 96, 98.6 and 98% for three consecutive spikes of which physical removal accounted for up to 25%.

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CHAPTER I

INTRODUCTION

For the past 50 years polylchorinated biphenyls (PCBs) have been widely used in a number of industrial applications and hence are widely distributed in the environment. Although PCBs can be partially degraded by biological, chemical and photochemical means, concern has risen over their persistence in the environment.

Polychlorinated biphenyls are mixtures of approximately 100 individual compounds and upon total or partial chlorination of biphenyl they are termed as polychlorinated biphenyls. These chlorinated aromatics were commercially produced in a series of complex mixtures called Aroclors.

The Aroclors with four or more chlorines are of greatest environmental concern. Various Aroclors identified are Aroclor 1221, 1232, 1242, 1248, 1254 and 1260, the last two digits representing the wt% of chlorine present in the Aroclor.

Some bacterial species capable of degrading PCBs have been discovered. Biodegradation of less chlorinated biphenyls is possible using microorganisms present in the

activated sludge [1].

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

In the present work, performance of a packed bed recirculation reactor utilizing calcium alginate immobilized mixed microbial population has been investigated. The reactor was operated in a recirculation mode with an externally aerated reservoir.

Commercial mixture of Aroclor 1242 (PCB 1242) was used as a model toxin for the biodegradation study. It is also known that lowly chlorinated Aroclors are more easily biodegradable when compared to highly chlorinated Aroclors [2].

1.1 IMMOBILIZATION AND ITS ADVANTAGES

The application of immobilized bacterial cells for biodegradation has been a subject of intensive study in recent years. The technique of immobilization has many advantages over the conventional free cell system in treatment of aqueous waste. Washout of biomass is one of the

most common problems encountered in present day activated sludge processes. Moreover, the system is very sensitive to varying input conditions. Long residence times are required for the organisms to be acclimated and evolve a population that is compatible with the feed. Many of these conditions can be significantly improved using immobilized microorganisms. 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 [3]. In addition, the cell density of an immobilized system can be much higher than that of the free cell system, resulting in higher rates of biodegradation per unit volume of the reactor. Immobilized cells can also be dried and stored as a convenient source of reproducible biomass.

Numerous methods have been developed for immobilized biocatalyst preparation [3]. 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 desired scale. 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 [4]. 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.

Attachment on the surface of a solid support offers an advantage because the support is stronger than the gel matrix. Diffusion of oxygen and substrate is no longer a major problem, as growth is on the surface. The disadvantages are that the microorganisms cannot take shock loadings of toxic compounds as they are not protected, and the problem of washout still remains when working under severe operating conditions of flow.

In the present study, calcium alginate was investigated and accepted for the entrapment of cells.

1.2 REQUIREMENTS OF DISSOLVED OXYGEN

In a system utilizing aerobic microorganisms for biodegradation of organic compounds, dissolved oxygen 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 [5]. Aerobic microorganisms also utilize oxygen primarily as the terminal electron acceptor for aerobic

respiration. In general, bacterial respiration does not appear to be affected above a critical dissolved oxygen concentration. 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 [6]. 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 [7]. 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.

A disadvantage of immobilization is the increased diffusional resistance of substrates and products through immobilization matrices. But this can be of advantage in case of system exposure to high concentration of toxic compound. 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 [8]. 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. Aldercruetz et al. and Larson et al. [8,9] attempted the addition of H_2O_2 , with a view to increase the oxygen holding capacity of the medium.

1.3 ACTIVATED SLUDGE AND BIODEGRADATION

Biological treatment, and the activated sludge process specifically, are used widely for treatment of municipal and 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 diversity of the microbial flora serves as a good environment for developing cultures that can biodegrade synthetic organics in general and chlorinated organics specifically.

Chlorinated aromatics are among the organic chemical pollutants that are most resistant to biodegradation and hence toxic. 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 [11].

In general, biological treatment systems involve two major competing removal mechanisms: physical removal and biodegradation [12]. The properties of chlorinated aromatics have shown that they are both chemical and thermally stable [13]. Thus it is necessary to investigate the physical removal mechanism.

1.4 USE OF SURFACTANTS

Nonionic surfactants are surface active agents used widely in industrial, household detergent applications and in making of emulsifying agents. The surfactants are also used as solubilizing agents in preparation of aqueous solutions for the compounds having very low water solubility. Some are also known as nonionic alkylphenyl polyether alcohols. Triton surfactants are basically divided into two major categories, octylphenol and nonylphenol surfactants. The hydrophile - lipophile balance (HLB) value on a scale of 0 to 20 separates the surfactants as lipophilic (oil-loving) and hydrophilic (water-loving) [14].

The surfactants when added with the compound which is very low soluble in water shears the surface of the micelles of the compound and hence makes the compound soluble in water. Several studies have been reported on the

use of surfactants in the biodegradation studies on Aroclors [2]. Nonionic surfactants Triton DF-16, Triton N-57, and Triton X-405 were tried and Triton X-405 was used for this study as it increased the solubility of Aroclor 1242 in water to a desirable extent (Triton is the trademark of Rohm & Haas Company).

In the present study, calcium alginate immobilized mixed microbial population was used to treat a model toxin Aroclor 1242 (PCB 1242).

Previous studies using calcium alginate beads [12], showed that a flow rate of 200 to 300 ml/min was necessary to eliminate external mass transfer resistance, hence a similar flow rate within the above range was adopted for this study.

1.5 DESIGN CONSIDERATIONS

The recirculation reactor has been described by Chambers et al.[15]. It is a tool designed to determine a regime of operation with respect to the flow in order to minimize mass transfer resistance. This helps to determine an accurate operating window while optimizing with respect to the reaction rate. In a low flow regime, the substrate concentration at the surface of the immobilized bacterial beads is lower than the bulk concentration because of external resistance to mass transfer. As the flow rate is increased, the reaction rate increases until a plateau is

reached, indicating that the resistance to external mass transfer has been overcome and the concentration of substrate at the surface is essentially equal to the bulk substrate concentration. Based on this principle, the performance of a reactor to study biodegradation of Aroclor 1242 has been investigated.

Oxygen is a co-substrate in aerobic biodegradation. The configuration of the recirculation flow reactor enables continuous measurement of the rate of dissolved oxygen consumption.

CHAPTER II

OBJECTIVES

The primary objective of this research was to determine the feasibility of treating aqueous wastes containing Aroclors using immobilized microorganisms.

The first step was to establish a method to increase the solubility of Aroclor 1242 in aqueous phase. This was accomplished using nonionic surfactant (Triton X-405).

In the next step the method of analysis for Aroclor 1242 was set up. EPA method 608 was modified for this purpose.

The feasibility of biodegradation of Aroclor 1242 was explored using a recirculation flow reactor system employing immobilized microorganisms.

CHAPTER III

LITERATURE SURVEY

The ultimate fate of chlorinated aromatic chemicals in the ecosystem, and in particular in the aquatic environment, has been a focus of extensive study.

There has been a growing interest in obtaining precise descriptions of biodegradation of specific chemicals in the wastewater treatment systems to characterize the decay rates in the environment. Most of the literature to date on biodegradation of chlorinated aromatic chemical deals with the use of specific strains of free microorganisms.

Liu [2] reported that various mixtures of commercial PCBs (Aroclors) could be rapidly biodegraded by Pseudomonas Sp. 7509. The rate of biodegradation could be enhanced using sodium ligninsulfonate. He also studied the kinetic parameters of these microorganisms and found that lower chlorinated Aroclor 1221 degraded faster than higher chlorinated Aroclor 1254.

Lewandowski et al. [16] studied the microbial response of mixed liquors from two different treatment plants to industrial organic chemicals. Although the mixed liquors came from very different systems, their responses to

the industrial chemicals as well as the initial and final populations, were very similar. It was also found that the primary removal mechanism for the compounds tested (phenol, 2-chlorophenol) was biodegradation and removal by stripping adsorption were negligible under the conditions of operation.

Bedard et al. [1] designed a rapid assay that assessed the PCB degradative competence and identified strains of aerobic microorganisms capable of degrading highly chlorinated biphenyls. These specific strains were capable of degrading specific congeners of the PCB mixtures, thereby highlighting the differences in congener specificity and predicted degradative competence on commercial PCBs.

Biodegradation of PCBs by microorganisms and the degradative pathway of PCBs were investigated by Osami Yagi and Ryuichi Sudo [17]. Ten strains of diphenylmethane assimilating bacteria were isolated from 300 types of soil by an enrichment culture method and one strain BM-2 later characterized as Alcaligenes, from the morphological and physiological studies, was able to utilize PCB mixtures as a sole source of carbon and this strain was used throughout the investigation.

From an initial concentration of 100 mg/l of PCB (tri-chloro) the concentration decreased to approximately 20 mg/l within 6 days of the start of experiment. The analysis

was done using gas chromatography and spectrometry.

Tucker et al. [18] investigated the susceptibility of commercial PCB mixtures to primary degradation by activated sludge microorganisms.

The activated sludge culture used for this study was obtained from a local municipal sewage treatment plant and was acclimated on synthetic sewage.

The following PCB mixtures manufactured by Monsanto Company were studied. They were Aroclor 1254, 1242, 1016 and Aroclor 1221. Due to the low water solubility of PCB mixture it was fed with ethanol. They reported that only less chlorinated Aroclors, Aroclors 1221 and 1016 was appreciably biodegraded and Aroclors 1242 and 1254 were comparatively less biodegraded. The periodic sample analysis was carried out using a ECD gas chromatography and UV spectrophotometry.

The presence of commercial mixtures of chlorinated biphenyls in both marine and estuarine environments is well documented. Sayler et al. [19] investigated that estuarine microorganisms were capable of degrading mixtures of PCBs. A strain of Pseudomonas Sp. 1008 was isolated and was enriched by direct plating. An enrichment broth was formulated and then supplemented with Aroclor 1254. Degradation studies were performed using flasks of broth and the culture grown on it. After 3 to 8 weeks of incubation, the flasks were extracted and analyzed. It was concluded

that Pseudomonas strain 1008 was capable of degrading Aroclor 1254 and also not inhibited by its presence.

Effect of activated sludge treatment process on the transformation and removal of PCBs in synthetic wastewater was investigated by Aly, et al. [20].

They concluded that over 90% of the PCBs were removed from the wastewater effluent while most of these compounds were adsorbed on the activated sludge. This study involved two phases, the effect of the presence of PCBs on the efficiency of activated sludge process and the role of activated sludge on removal of PCBs. It was observed that the removal values of PCBs decreased with increase in degree of chlorine. A 24 hour study indicated about 80-90% removal of Aroclors 1221, 1232 & 1242 and between 75-85% removal of Aroclors 1248, 1254 and 1260. The biodegradation study was conducted using a batch-type, feed and draw lab scale activated sludge columns.

Furukawa et al. [21] investigated the biodegradabiltiy of commercial PCB mixtures and their metabolic products by using Acinetobacter Sp. P6.

Dichlorobiphenyl, tri-, and tetrachlorobiphenyls (Aroclors 1221, 1232, 1242 & 1248) were primarily susceptible to biodegradation using Acinetobacter Sp. P6, previously grown in the culture medium and incubated for 1 to 60 hours. GC mass spectrometry was employed for the analysis of PCBs and in obtaining the metabolite data of the

PCBs.

An investigative study concerning the effects of two microorganisms Nocardia Sp. (NCIB 10603) and Pseudomonas Sp. (NCIB 10643), on the degradation of poychlorinated biphenyls was performed by Baxter et al. [22]

Aroclor 1242 and Aroclor 1016 were exposed to these cultures. Typical chromatograms of both Aroclor 1242 and 1016 were obtained from the samples analyzed after 0, 10, 20, 30 and 60 days of exposure. The percent degradation of Aroclor 1242 was about 88 and that of Aroclor 1016 was 96 at the end of 52 days. This again signifies that less chlorinated PCBs are more easily biodegradable when compared to highly chlorinated PCBs..

Bedard et al. [23] isolated and characterized a strain of Alcaligenes eutrophus H 850 and investigated the biodegradability of Aroclors and environmentally transformed PCBs using H 850 microorganisms.

Gas-chromatographic analysis showed an overall reduction of 81% of Aroclor 1242 and 35% of Aroclor 1254 in 8 days.

Griffin et al. [24] investigated the feasibility of degrading PCBs using three enriched mixed cultures which were isolated from soil samples and river sediment. Predominant microorganism was found to be Alcaligenes Odorans. They concluded that, PCB isomers that were more water soluble and had lower chlorination were not only

degraded at a faster rate, but were also completely utilized by the mixed cultures.

CHAPTER IV

MATERIALS AND EXPERIMENTAL METHODS

4.1 MICROORGANISMS

Activated sludge (mixed microbial population) from the Livingston (N.J.) wastewater treatment plant was used in this study. The microorganisms were acclimated at room temperature with phenol (100 ppm) and 2-chlorophenol (10 ppm) as the only carbon source, successively over a period of ten days (approximately 10 spikes, 5 spikes each) 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 DEFINED NUTRIENT MEDIUM

The composition of buffered defined medium used in this work as follows [25]:

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.

4.3 IMMOBILIZATION

The entrapment of microorganism in calcium alginate gel was conducted as follows. Distilled water and concentrated pellets (45 mg dry biomass/gm of pellet) 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 0.1 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.

4.4 CHECK FOR VIABILITY IN A MICROASSAY REACTOR

Viability studies of free and entrapped microorganisms were done in a microassay bioreactor. Viability is defined here as the ability of microorganisms to consume dissolved oxygen from the medium for endogenous

respiration. The experimental setup is shown in Figure 1. 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 at room temperature. 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 [26].

Before the start of each run, the reactor was sterilized in an autoclave at 120^{o}C and then washed successively with methanol and distilled water. It was then rinsed several times with sterile defined medium. Sterile defined medium (1.6 ml) was then added to the reactor and saturated with oxygen by bubbling air through it. The saturation concentration of dissolved oxygen in water under ambient conditions is 210 nmole/ml [27]. 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.

The shock treatment was carried out to revive the microorganisms from their dormant state. 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.

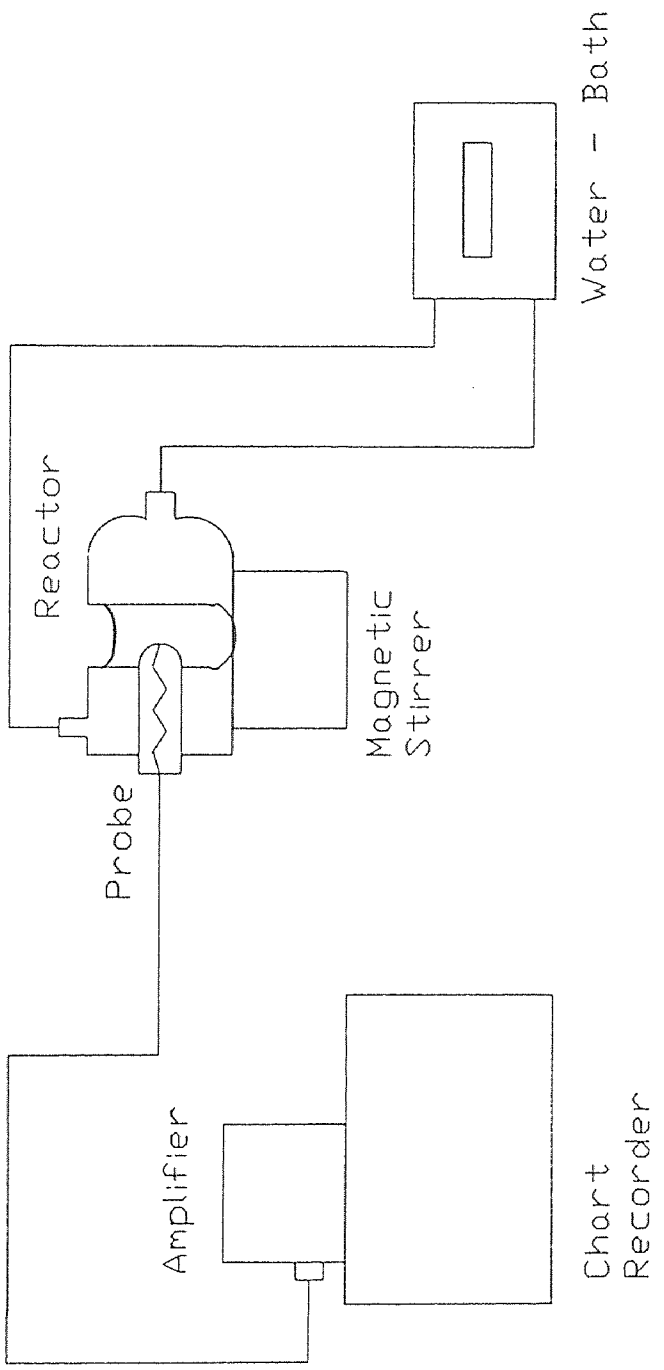

Fig. 1. Experimental Setup of Micro-Assay Reactor

4.5 PREPARATION OF WORKING SOLUTION

0.05 G of Aroclor 1242 was dissolved in 20 ml of spectrograde Acetone. An equimolar quantity of Triton X-405 (nonionic surfactant) was added and the solution was made up to 100 ml with distilled water and stirred for 30 minutes. Following this, acetone was stripped out using a rotary evaporator (Buchi RE 111, Rotavapor).

The solution containing Aroclor 1242 in aqueous medium was used for conducting the experiments.

4.6 BIODEGRADATION OF AROCLOR 1242 IN RECIRCULATION REACTOR

4.6.1 Experimental setup and procedure

The scale-up from microassay reactor to recirculation reactor was 1000 times (0.05 g wet beads in microassay reactor to 50g wet beads in recirculation reactor). The experimental setup of the reactor is shown in Figure 2. The reactor is 2.5" in diameter and 8" in length. The reservoir is 4.5" in diameter and 10" in length. The total reaction volume was 2 liters. The reaction medium was circulated between substrate reservoir and packed reactor using a centrifugal pump (Eastern Centrichem Pump).

An impingement flow Clark-type dissolved oxygen probe monitored the concentration of dissolved oxygen. Filtered air was sparged into the reservoir at the rate of 1.5 liter/min. The reactor contained a thermometer and a pH probe (Orion Cat no: 91-04). An online flow meter regulated

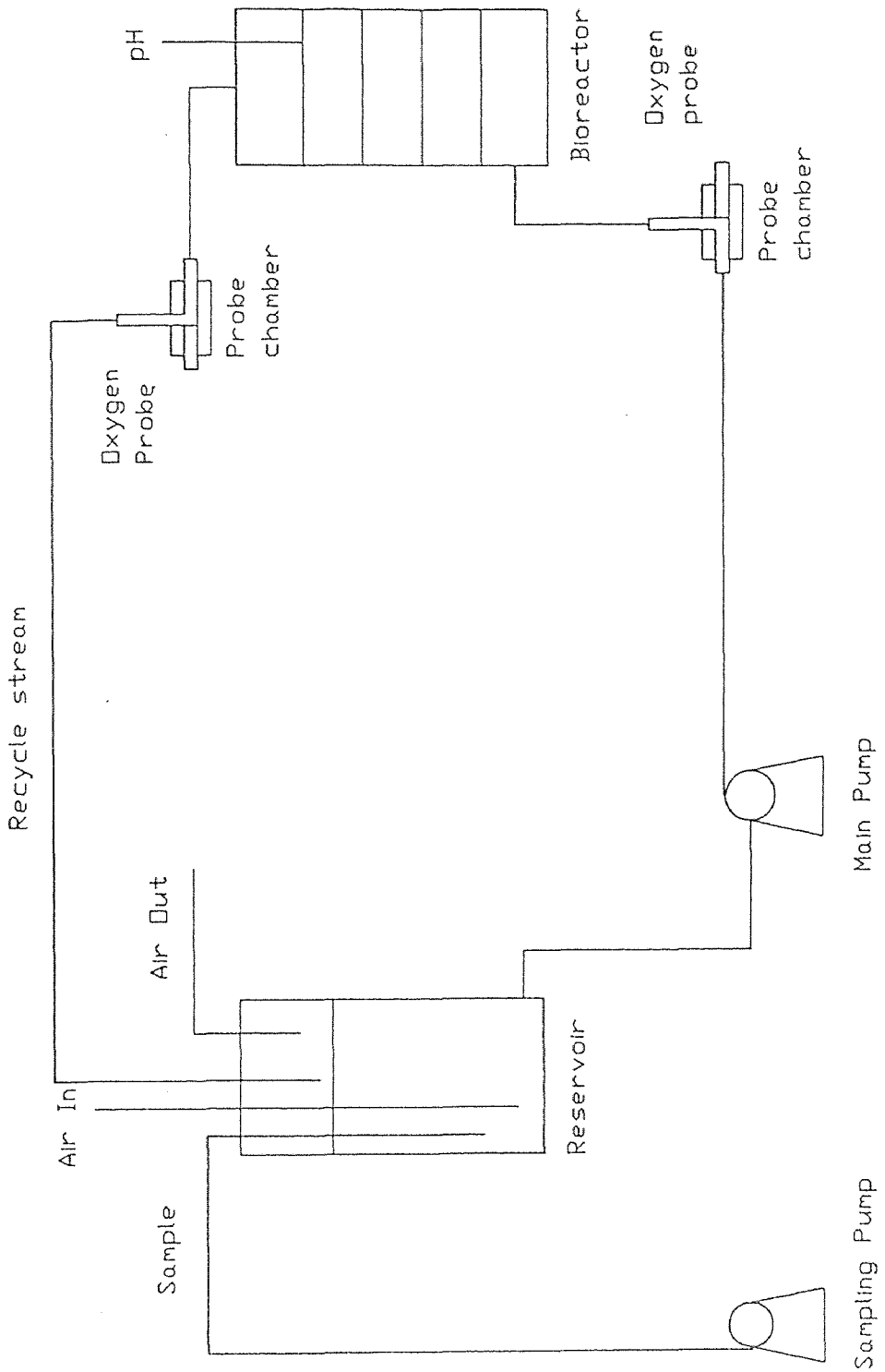

Fig. 2. Experimental Setup of Recirculation Reactor.

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 chromatograph (Varian GC 6000 vista series) for concentration of Aroclor 1242. The activity of the beads was also tested periodically by stopping the air supply to the reservoir. A starting concentration of 850 ppb Aroclor 1242 was used. Repeated spikings of 2800 and 2810 ppb were given to the system after the concentrations came down to 40 ppb.

4.6.2 Oxygen measurement

The oxygen measurement unit consisted of an impingement chamber, oxygen sensor, signal conditioner and recorder (Omega Rd 2000). 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 measurement of oxygen consumption. Periodically, air supply to the medium in the reservoir was stopped and the fall in oxygen concentration in the system was monitored using a strip chart recorder.

4.6.3 METHOD OF ANALYSIS

A. Preparation of Sample:

5 ml of the sample containing Aroclor 1242 was

dissolved in 15 ml of spectrograde iso-octane and the mixture was stirred vigoursly 60 minutes to ensure complete extraction.

The two phases were separated in a separating funnel and the organic phase was collected to perform the analysis.

B. Analysis on Gas Chromatograph

Analysis was performed on a Varian Gas Chromatograph (6000 vista series), containing 6 ft mixed phase column and operated at 200^{o}C. Sample detection was done by an electron capture detector (Ni^{63}). A sample chromatogram is shown in Fig 3. A sample calculation for determining the concentration of Aroclor 1242 is presented in appendix A.

4.7 CONTROL RUN

Control runs were done under identical conditions of temperature and air flow but without biomass, to account for the removal of Aroclor 1242 by physical processes such as adsorption or evaporation. Adhesion to the surface of the system was the predominant mechanism.

4.8 FREE CHLORIDE DETERMINATION

Free chloride was determined by titration using standard silver nitrate solution [28].

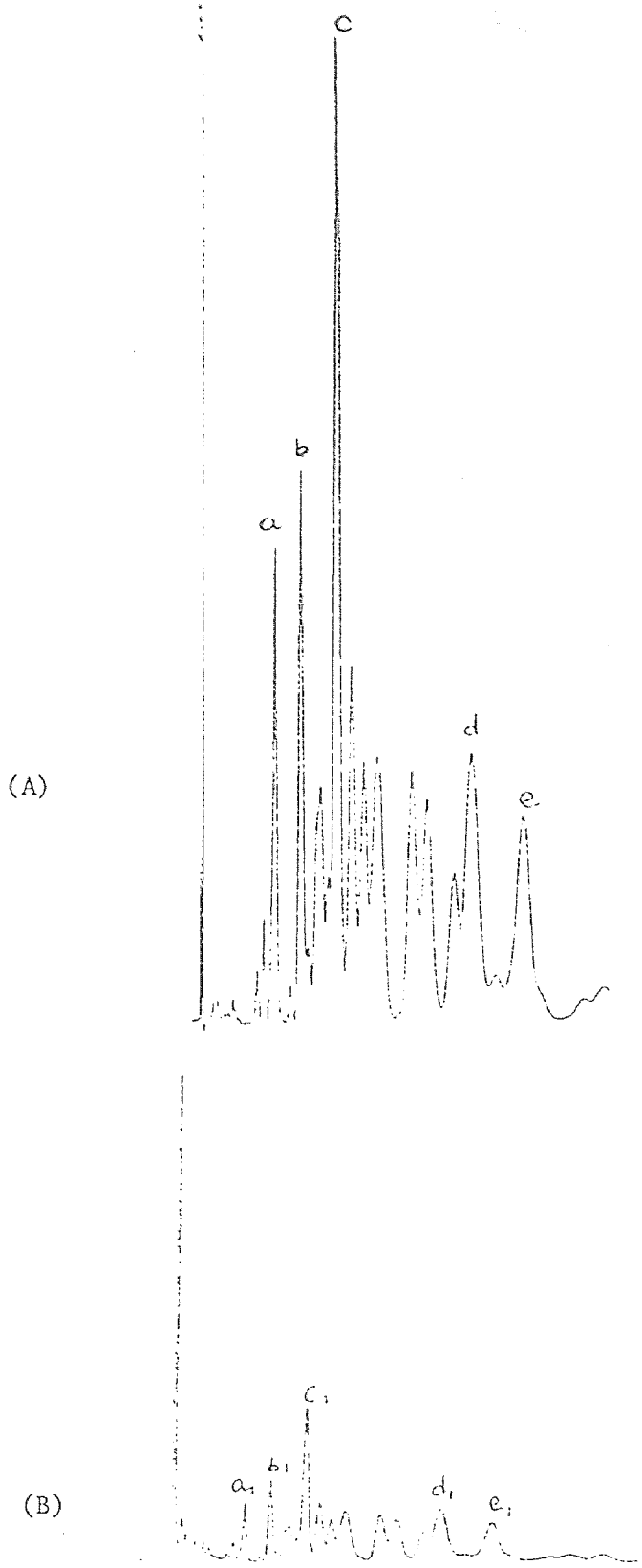

Fig. 3. A Sample Chromatogram of Aroclor 1242 (Second Spike).
(A) Initial Conc. of Aroclor 1242 (2800 ppb).
(B) Conc. at the end of Second Spike (60 ppb).

CHAPTER V

RESULTS AND DISCUSSION

5.1 INTRODUCTION

Previous studies on bead stability and viability [29] were performed and the above were optimized with respect to oxygen uptake.

The effect of calcium chloride concentration on oxygen uptake was studied with beads cured in 0.1, 0.2, 0.3, 0.5 and 1.0 M calcium chloride solution. A concentration of 0.2 M was reported to be the best and the same was adopted for this experimental study.

Study on optimum biomass concentration and temperature with respect to oxygen uptake was also performed and a concentration of 25 mg biomass/gm of mixture was found to be optimal and this value was adopted for this study.

5.2 PHYSICAL REMOVAL OF AROCLOR 1242 IN RECIRCULATION REACTOR

Control runs performed on the physical removal of Aroclor 1242 showed that 95% of Aroclor 1242 was removed by means of adhesion to the surface of the system in the first spike and only 25% physical removal was observed after the second spike (Fig 4). This showed that the system was

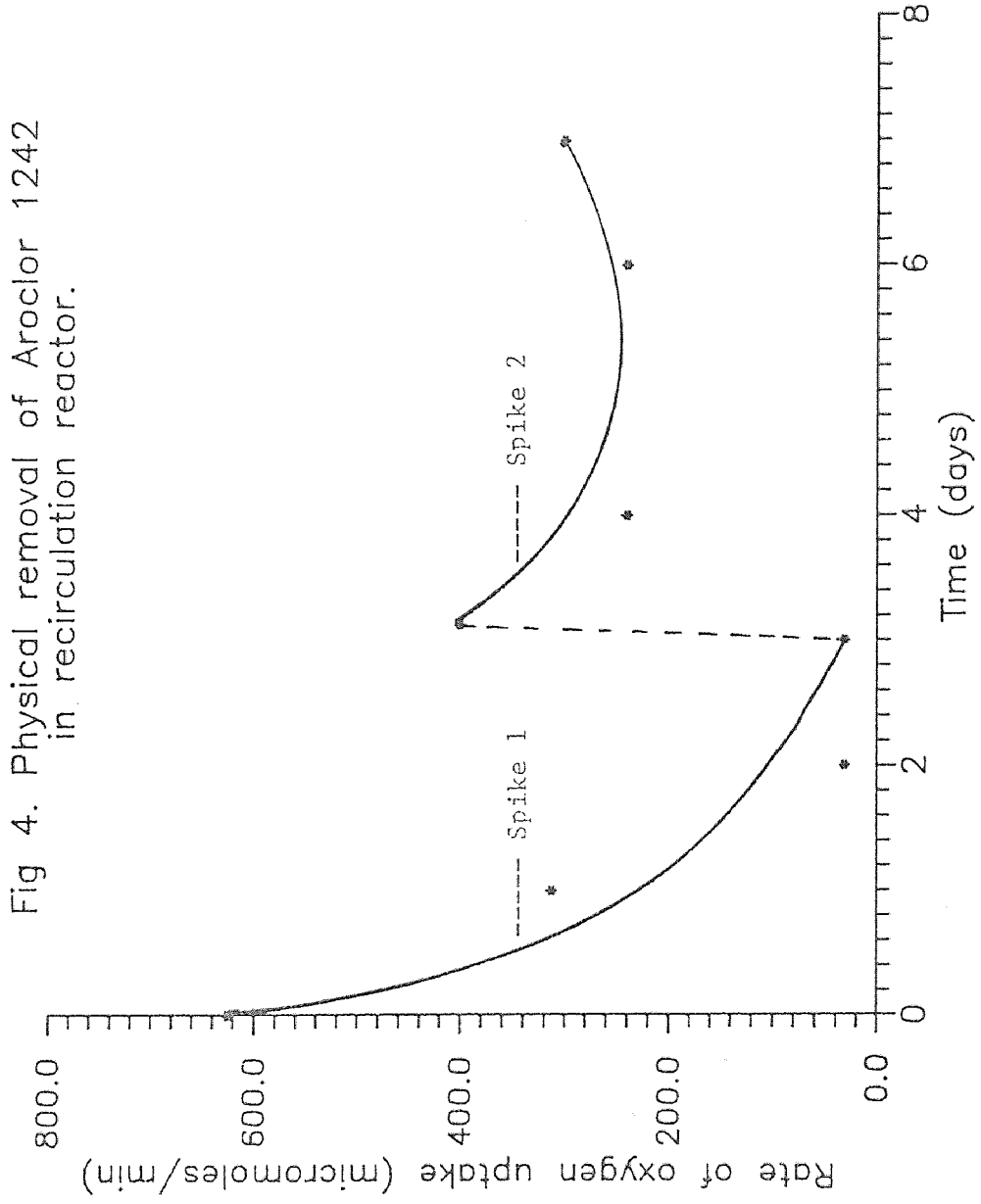

Fig 4. Physical removal of Aroclor 1242 in recirculation reactor.

stabilized at the end of second spike.

5.3 BIODEGRADATION OF AROCLOR 1242 IN RECIRCULATION REACTOR:

5.3.1 VARIATION IN CONCENTRATION OF AROCLOR 1242 WITH TIME:

Three spikes of Aroclor 1242 were studied over a period of 8 days, at the end of which the system broke down due to excessive fungal growth.

As seen in Fig 5., initial spiking concentration of 850 ppb was reduced to 30 ppb over a period of 4 days. In the second and third spikes each, approximately 96% of Aroclor 1242 was consumed in 2 1/2 days in each spike from a starting concentration of 2800 ppb.

Upon comparison the slow rate of removal of Aroclor 1242 in the first spike may be attributed as the stabilization of the system.

5.3.2 VARIATION OF pH WITH TIME

Fig. 6. shows the constant decrease of pH during the course of the experiment, which indicated that Aroclor 1242 was degraded to HCl by the microorganisms.The decrease in pH can also stimulate fungal growth as several studies have indicated the dominance of the fungus over bacteria in lower pH range.

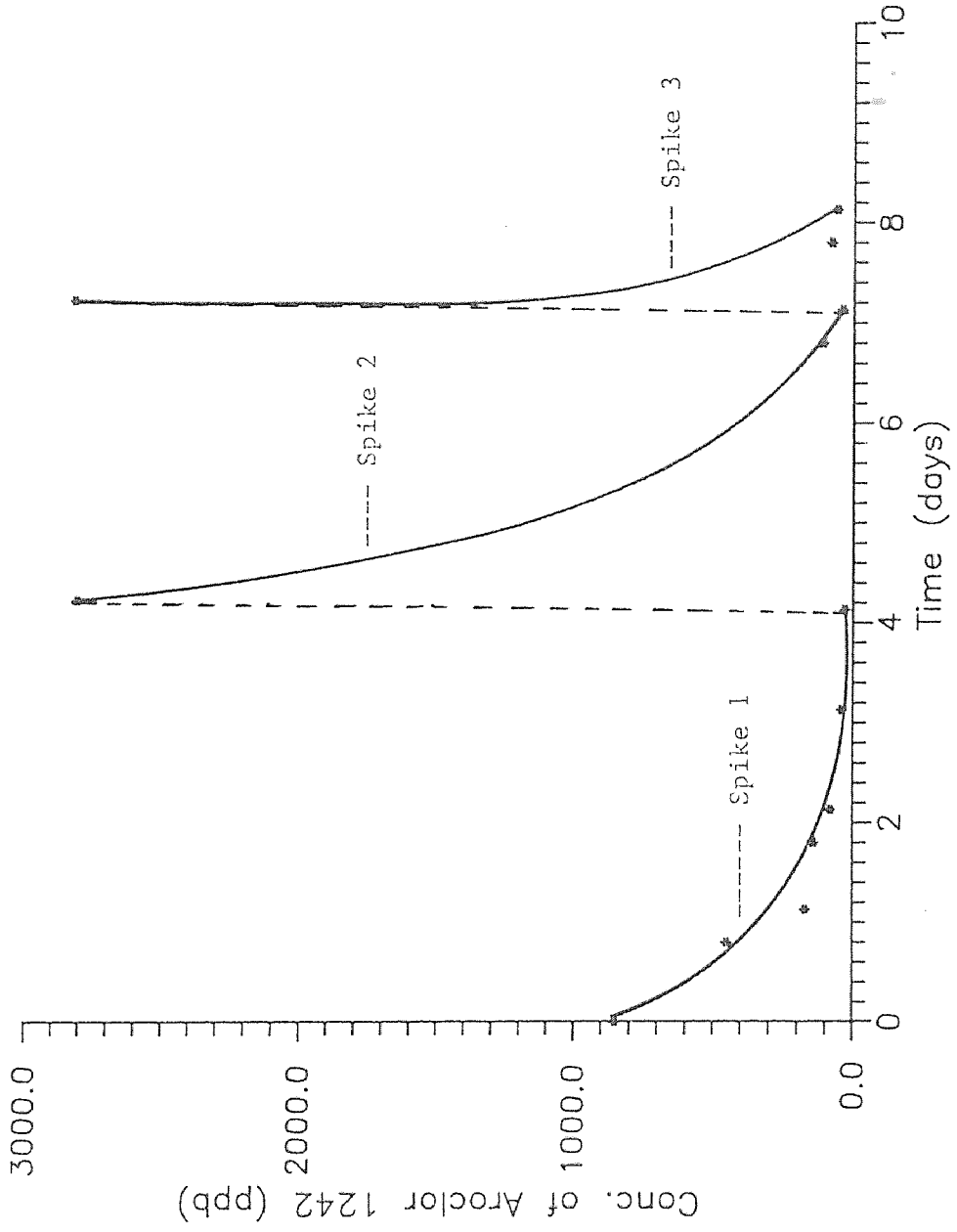

Fig 5. Removal of Aroclor 1242 using immobilized microorganisms.

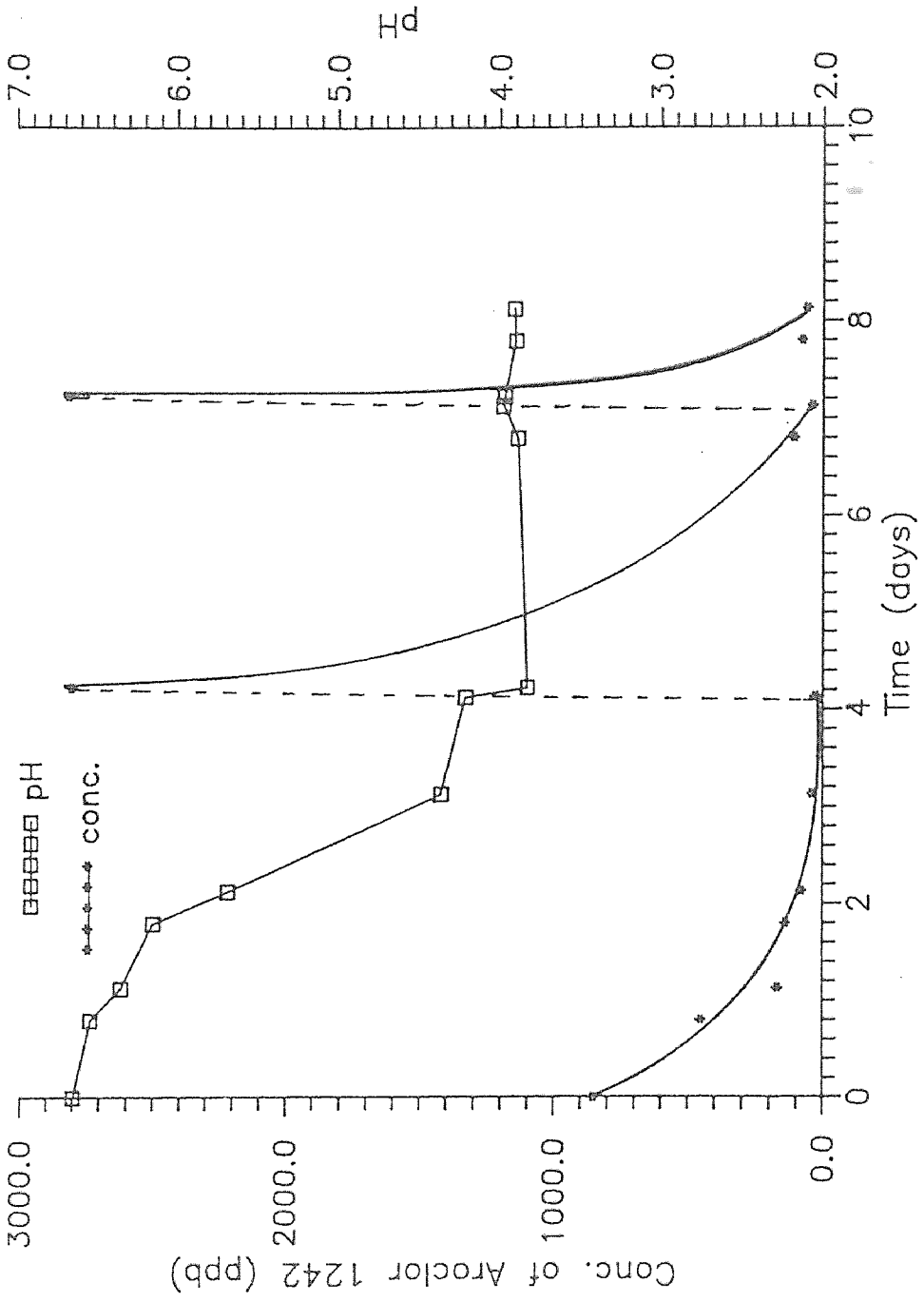

Fig 6. Variation of pH and concentration of Aroclor 1242 with time.

5.3.3 VARIATION OF O_2 UPTAKE WITH TIME:

Oxygen uptake rates were measured during the course of the run. As seen in Fig 7., initially the rate of oxygen consumption was low. The rate gradually increased over a period of two days and subsequently decreased after four days. As compared to the decrease in concentration of Aroclor 1242, the rate of oxygen uptake did not follow a comparative trend and hence it was difficult to correlate the two. However the increase in oxygen uptake rate over the first two days was also observed with other biodegradation studies conducted in the laboratory and is due to acclimation of the bacterial systems.

5.3.4 GENERATION OF FREE CHLORIDE

From 3.62 millimoles of free chloride at the start of the experiment the value increased to 4.22 millimoles at the end of 7 days resulting in a total increase of 17%, thereby substantiating that Aroclor 1242 was being biodegraded. Sample calculations of free chloride generated is shown in Appendix B.

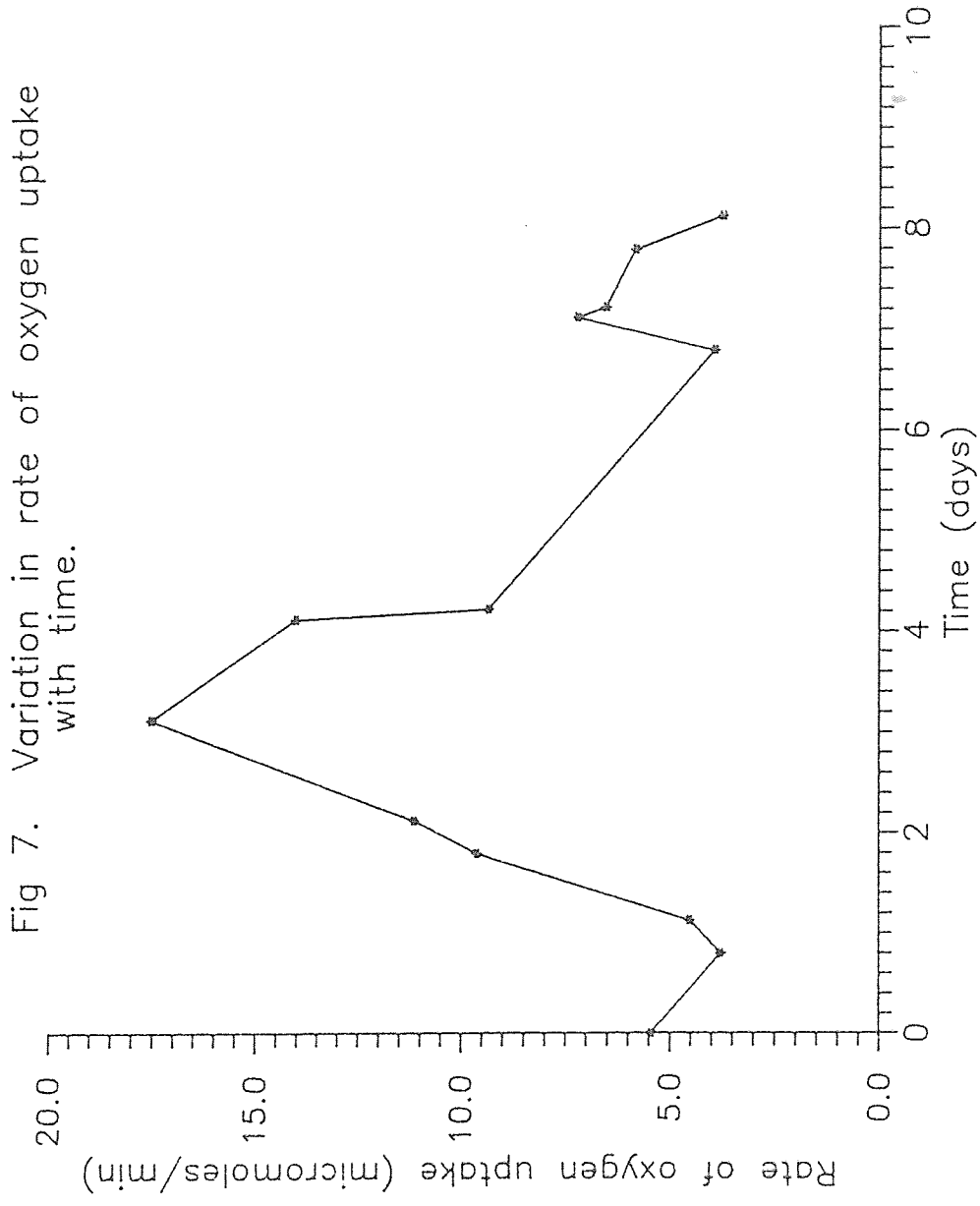

Fig 7. Variation in rate of oxygen uptake with time.

CHAPTER VI

CONCLUSIONS AND SUGGESTIONS

6.1 CONCLUSIONS

The very question of the feasibility of using calcium alginate immobilized activated sludge for treatment of aqueous waste, containing Aroclor 1242 has been established.

Biodegradation at a biomass loading of 50 g wet beads was not inhibited by any increase in the spiking concentrations. Continuous biodegradation of Aroclor 1242 was accomplished over a period of 8 days as seen in Fig. 5.

Total removal of Aroclor 1242 using recirculation reactor was 96, 98.6 and 98% for first, second and third spikes respectively, of which physical removal accounted for up to 25%. This suggested that biodegradation was the primary removal mechanism.

The use of nonionic surfactant, Triton X-405 enhanced the solubility of Aroclor 1242 by approximately 2000 times and also did not interfere with the analysis of the compound nor did have any effect on the microorganisms.

Generation of free chloride also substantiated that Aroclor 1242 was biodegraded.

6.2 SUGGESTIONS

In the present work, experiments in a batch recirculation reactor were conducted on a laboratory scale to assess the feasibility of immobilized mixed microbial population to biodegrade a model toxic compound Aroclor 1242. It is suggested that aqueous wastes containing less chlorinated Aroclors be biodegraded using this reactor setup. One of the important points to be considered in minimizing the problem of physical removal is that the materials to be used in building of a large scale reactor should be of either glass or teflon since the Aroclors does not adhere to these surfaces.

Precautions should be taken while using Aroclors and surfactants in the preparation of samples as they are established as carcinogens.

Use of low foaming surfactants will help reduce the problems of foaming in the system.

By identifying, isolating and immobilizing the specific microorganisms from activated sludge capable of biodegrading Aroclors, the rate of biodegradation may be enhanced.

Maintaining pH in the range suitable for bacterial dominance over fungus should also be considered. This pH control can be achieved by adding desirable amount of buffered solution.

APPENDIX A

SAMPLE CALCULATION FOR THE CONCENTRATION OF AROCLOR 1242:

The concentration of Aroclor 1242 was calculated using the method of sum of peak heights of the chromatogram.

Formula used:

$$S = \frac{M}{M_1} \times \frac{A}{A_1} \times S_1 \times D$$

where

S = concentration of sample extract

S_1 = concentration of standard

M = sum of peak heights of sample

M_1 = sum of peak heights of standard

A = attenuation of sample on GC

A_1 = attenuation of standard on GC

D = dilution factor of sample

Calculation for chromatogram (A)

S_1 = 500 ppb

M = 330 mm

M_1 = 228 mm

A = 256 x 1

A_1 = 128 x 1

D = 2

Using the above formula the concentration for chromatogram (A) is

S = 2800 ppb

APPENDIX B

CALCULATION FOR FREE CHLORIDE GENERATED USING SILVER NITRATE TEST

The following sample calculations for the estimation of free chloride generated at the start and end of the run for Aroclor 1242 is as shown below :

Volume of recycle stream.....2000 ml

Volume of 0.001 M $AgNO_3$.....9.05 ml

Volume of Aroclor 1242.....5.0 ml

Equivalents of free chloride present at the start of run =

$9.05 \times 1e^{-6} \times 2000 / 5 = 3.62$ millimoles Cl^-

Volume of recycle stream.....2000 ml

Volume of 0.001 M $AgNO_3$.....10.55 ml

Volume of Aroclor 1242.....5.0 ml

Equivalents of free chloride generated =

$10.55 \times 1e^{-6} \times 2000 / 5 = 4.22$ millimoles Cl^-

The difference in the Cl^- values indicated that Aroclor 1242 was being biodegraded.

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