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USE OF RECIRCULATION REACTOR TO STUDY BIODEGRADATION OF 2-CHLOROPHENOL

by
Kai-Chung Yang

Thesis submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirements for the degree of
Master of Science in Chemical Engineering
1988

APPROVAL SHEET

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ABSTRACT

Title of Thesis: Use of Recirculation Reactor to Study Biodegradation of 2-chlorophenol.

Kai-Chung Yang, Master of Science in Chemical Engineering 1988

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

The performance of immobilized cells in the treatment of hazardous waste was investigated using a recirculation flow reactor run in a batch mode. A mixed microbial population from a municipal wastewater treatment plant was immobilized in calcium alginate gel. The bio-oxidation ability of these microbes towards a model toxin (2-chlorophenol) was studied. The process parameters studied were as follows:

1. Flow rate of the recycle stream;
2. Biomass loading;
3. Spiking concentration of 2-chlorophenol;
4. Use of buffered vs. non-buffered defined medium.

Dissolved oxygen concentration was monitored using a flow Clark-type dissolved oxygen probe. Oxygen consumption was correlated with the biodegradation of 2-chlorophenol. Physical removal of 2-chlorophenol by air stripping in the absence of microorganisms was also determined and the removal rate compared with the biodegradation rate obtained. It was found that biodegradation was the primary removal mechanism.

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CHAPTER I

INTRODUCTION

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 the present work, 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 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 and cannot sustain frequently 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 [1]. 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 [1]. 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 [2]. 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 under severe operating conditions of flow.

In the present study, calcium alginate was investigated and accepted for the entrapment of cells. One of the many critical parameters which affects the kinetics of immobilized microbes is the diffusional or mass transfer resistance. 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. 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 [3].

In the case of 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. According to some studies [4], 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

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. Increasing the oxygen holding capacity of the medium has been attempted by the addition of hydrogen peroxide and perfluoro chemicals [8,9].

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 phenols are among the organic chemical pollutants that are most toxic, and hence, resistant to biodegradation. It has been reported that phenol is degraded with first order or Monod kinetics using free microorganisms [10], but substrate inhibition may take place with chlorinated phenols. 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 three competing removal mechanisms: adsorption, stripping and biodegradation [12]. The stripping rate is a direct function

of the thermodynamic equilibrium between the liquid and gas phases. Many of the organic priority pollutants are hydrophobic compounds with large activity coefficients. As a result, these compounds tend to volatilize from the aerated reactor if the vapor pressure is sufficiently high. Hence, aerobic biological treatment becomes a system of two competing removal mechanisms: biodegradation and stripping (neglecting adsorption). It is therefore necessary to investigate the primary removal mechanism since stripping is not a desirable phenomenon. Moreover, if the microorganisms are not acclimated to the compounds, then aeration may cause significant release of these substances into the atmosphere.

1.4 DESIGN CONSIDERATIONS

In the present study, a calcium alginate immobilized mixed microbial population was used to treat a model toxin (2-chlorophenol). Process parameters such as flow rate of the recycle stream, biomass loading, composition of nutrient media, and pH were investigated employing a packed bed recirculation flow reactor. Variation of these parameters has a pronounced effect on the rate of biodegradation.

Studies were also conducted to obtain a stable bead structure while having a viable population within the beads. This included the effect of calcium chloride concentration, sodium alginate concentration and curing time. Various flow rates and biomass loadings were studied to optimize the

specific substrate utilization of preacclimated microorganisms.

The recirculation reactor has been described by Chambers _et al._[13]. 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 2-chlorophenol has been investigated.

An attempt was made to define the operating windows with respect to substrate utilization and oxygen uptake. 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 main objective of this research work has been to investigate the fundamental process parameters which affect the performance of a recirculation bioreactor containing immobilized microorganisms in the treatment of hazardous waste. Use of a recirculation reactor run in batch has been made to:

1. Define the flow rate of the recycle stream above which external mass transfer resistance does not influence the biodegradation.
2. Optimize the loading density of biomass so as to have the most efficient use of biomass while operating at highest possible biodegradation rates.
3. Determine the concentration range of the model compound (2-chlorophenol) for which maximum substrate utilization can be achieved.
4. Define an appropriate composition of the nutrient medium.

CHAPTER III

LITERATURE SURVEY

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 [14] 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.

Lewandowski *et al.*[15] 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 microbial 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 and adsorption were negligible under the conditions of operation.

Westmeier and Rehm [16] 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.

Macaskie et al. [17] investigated the ability of polyacrylamide gel-immobilized cells of Citrobacter sp. to remove cadmium. Metal uptake was mediated by the activity of a cell bound phosphatase, induced during the pre-growth of the cells. They reported that the polyacrylamide gel, though suitable for laboratory scale experiments, would not be applicable to a large scale

process due to the economic considerations and toxicity of the gel precursors, and also due to the limited mechanical strength of the gel.

Klein *et al*. [18] investigated the kinetics of phenol degradation by free and immobilized *Candida tropicalis*. In both cases, the reaction was zero order with respect to phenol concentration over the range tested (< 1 g/l). Oxygen concentration was a major factor in controlling the degradation rate. An attempt was made to model this effect within the beads. The bead radius, number of cells per bead, specific activity of each cell, bulk oxygen concentration and oxygen diffusivity within the matrix were found to be the important variables.

Shaler and Klecka [19], studied the effects of dissolved oxygen concentration on the biodegradation of 2,4-dichlorophenoxyacetic acid. The relationship between the specific growth rate and concentrations of both the organic substrate and dissolved oxygen was found to follow Monod kinetics. They reported that dissolved oxygen concentrations below 1 mg/liter may be rate limiting for the biodegradation of chlorinated aromatic compounds such as 2,4-dichlorophenoxyacetic acid, which have a requirement for molecular oxygen as a co-substrate for metabolism.

Tanaka *et al*. [20] 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 [21] 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, oxygen was consumed faster than it could diffuse into the beads. At this point the cells had to compete for oxygen, and diffusion became the limiting factor for oxygen uptake.

Lurker et al. [22], in their investigation for atmospheric release of chlorinated organic compounds from the activated sludge process, found that over half of the chlorinated hydrocarbons emitted were released by air stripping from the aeration basins.

A laboratory scale activated sludge unit, which allowed a comparison of biological and physical removal

of hydrocarbons from acclimated biomass, was developed by Tischler *et al*. [23]. Studies of two industrial petrochemical wastewater samples showed that biological removal of the hydrocarbon was far more significant than removal by air stripping, which was only 0.22 and 0.31% of the total organic carbon of the waste.

In another study, Lewandowski and Armenante [24] reported that physical removal of 2-chlorophenol is a first order process, with a rate constant of 5.5×10^{-3} hr^{-1}. This value could also be predicted from thermodynamic consideration by assuming a 30% saturation of the air leaving the reactor.

In another study conducted in this laboratory [25], 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°C.

Chien and Sofer [4] studied the performance of immobilized yeast cell reactors. They studied a number of parameters including flow rate, amount and growth rate of yeast, bead size and type of medium. It was shown that variation in these parameters had a pronounced effect on the fermentation rate. The paper presents typical ranges

of the above parameters for the productivities of ethanol and demonstrates the patterns of changes that take place when bead size and reaction medium are varied. Saccharomyces cerevisiae cells were immobilized in calcium alginate beads for the production of ethanol. The productivity of immobilized yeast in an ordinary batch reactor (0.2 g ethanol/g yeast.hr) was two-thirds that of free cells suspended at an equivalent cell density (0.3 g ethanol/g yeast.hr). But in recirculation mode, different flow rate and bead sizes were used to optimize the productivity. The productivity of 3.34 mm beads at a flow rate of 8.8 liter hr^{-1} was 95 percent higher than that at 1.0 liter hr^{-1}. Maximum productivities of 0.34, 0.27 and 0.22 g ethanol/g yeast.hr were obtained for 9.2 percent yeast-immobilized beads of 3.34, 4.45 and 5.56 mm in diameter respectively.

Knudson et al. [26] have studied the influence of dissolved oxygen on substrate utilization kinetics of activated sludge. In their studies, they found that pure oxygen systems can be operated at higher specific utilization rates than the air system to obtain equivalent results. By increasing dissolved oxygen (DO) levels, pure oxygen-activated sludge systems (OAS) are able to maintain aerobic conditions throughout the floc particles, which results in increased sludge viability.

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) with continuous aeration. The culture was then centrifuged (International portable refrigerated centrifuge, Model PR-2) at 3000 rpm and 15°C to obtain concentrated pellets.

4.2 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°C before use.

4.3 DEFINED NUTRIENT MEDIUM

The composition of buffered defined medium used in this work as follows [23]:

Magnesium sulfate ....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.

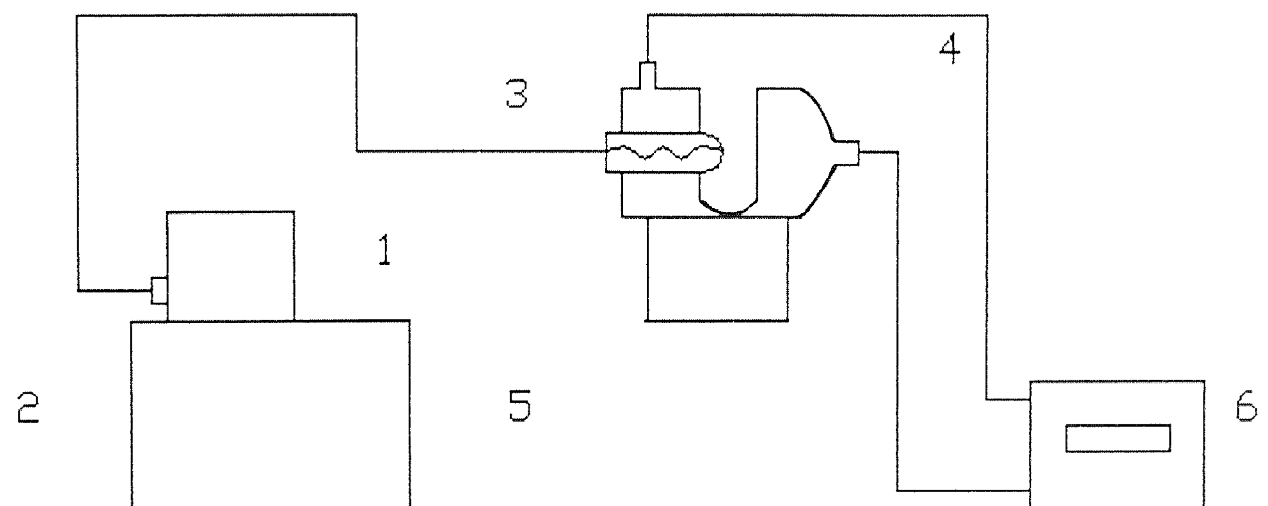
The composition of the nonbuffered defined medium is the same as buffered medium except that a slightly smaller amount of potassium phosphate (10 mg/liter) was used.

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

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 Clarke-type dissolved oxygen probe. Water at the required temperature (37°) 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 [24].

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 oxygen from air in



1. Amplifier
2. Chart Recorder
3. Oxygen Probe
4. Reactor
5. Magnetic Stirrer
6. Water Bath

Fig:1 EXPERIMENTAL SETUP OF A MICROASSAY REACTOR

water at 37°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°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.

4.5 BIODEGRADATION OF 2-CHLOROPHENOL IN RECIRCULATION REACTOR

4.5-1 Experimental setup and procedure

The scale-up from microassay reactor to recirculation reactor was 2000 times (0.05 g wet beads in microassay reactor to 100 g wet beads in recirculation reactor). The experimental setup of the reactor is shown in Figure 2. The reactor was 2.5" in diameter and 8" in length. The reservoir was 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).

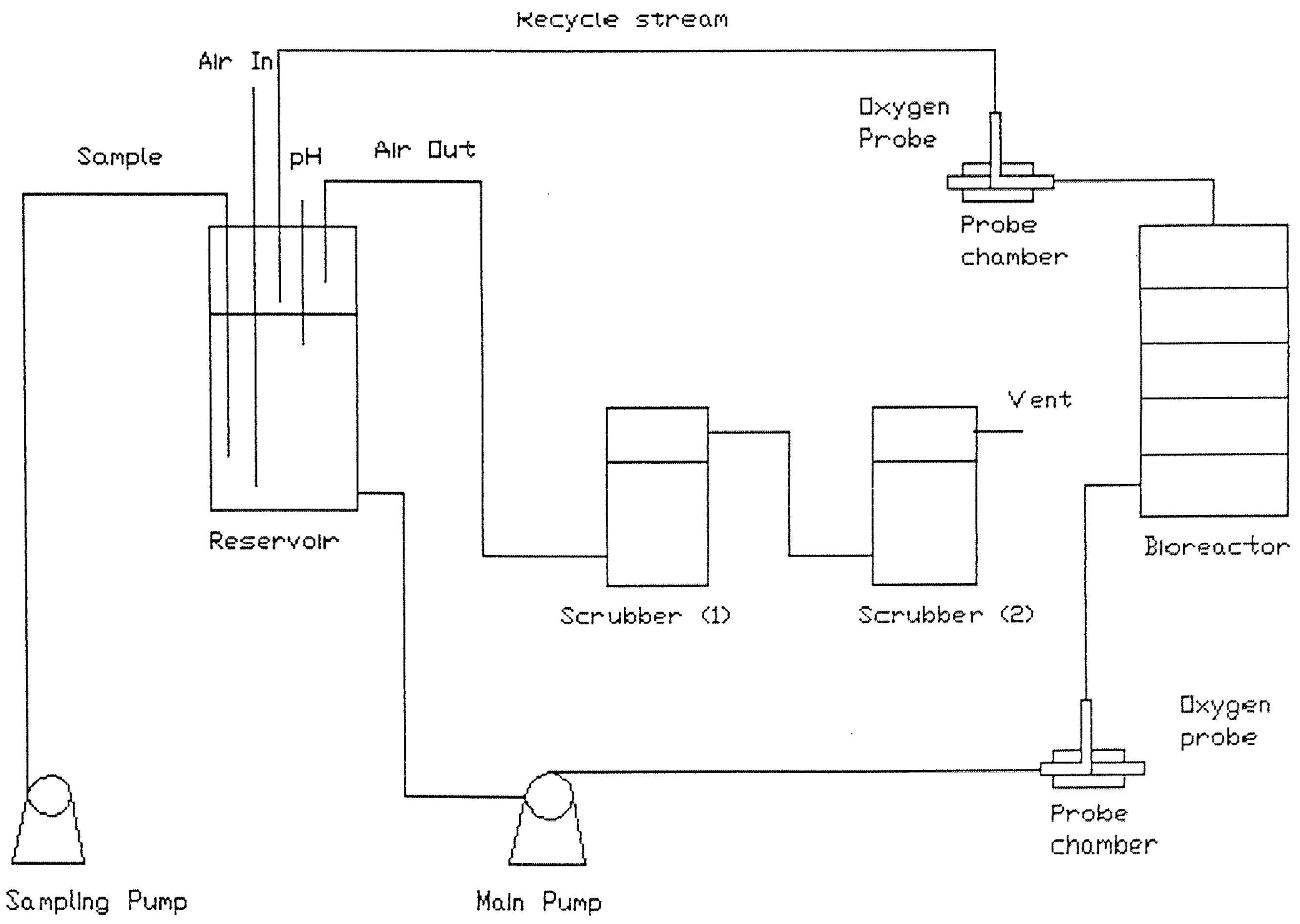


Fig:2 EXPERIMENTAL SETUP OF A RECIRCULATION REACTOR

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 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 3300) for concentration of 2-chlorophenol. The activity of the beads was also tested periodically by stopping the air supply to the reservoir. A starting concentration of 50 ppm 2-chlorophenol was used. After it had dropped to below 5 ppm, the system was again spiked with 50 ppm, and so on. Similar runs were done with spiking concentrations of 100 ppm and 150 ppm.

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. 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 [27]. The fall in the oxygen concentration in the system was continuously monitored using a strip chart recorder.

4.6 CONTROL RUN

Control runs were done under identical conditions of temperature and air flow but without biomass, to account for the removal of 2-chlorophenol by physical processes, stripping being the predominant mechanism.

4.7 FREE CHLORIDE DETERMINATION

Free chloride was determined by titration with a silver nitrate solution [28].

CHAPTER V

RESULTS AND DISCUSSION

5.1 BEAD STABILITY AND VIABILITY

Preliminary studies have shown that the method of immobilizing microorganisms in calcium alginate is very easy, practical and gives well defined integral beads.

Using a microassay reactor, parameters such as concentration of calcium chloride, concentration of sodium alginate and biomass concentration within the bead which influence both bead stability and viability were optimized with respect to oxygen uptake.

5.1-1 Effect of calcium chloride concentration on 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. Figure 3 shows the results, indicating that oxygen uptake rate when 1.0 M calcium chloride solution is used to cure the beads is about 70 percent of that when 0.1 M calcium chloride solution is used. Bead stability was unaffected by the concentration of calcium chloride and as a result, most of the experiments in the present study were conducted with beads cured in 0.2 M calcium chloride

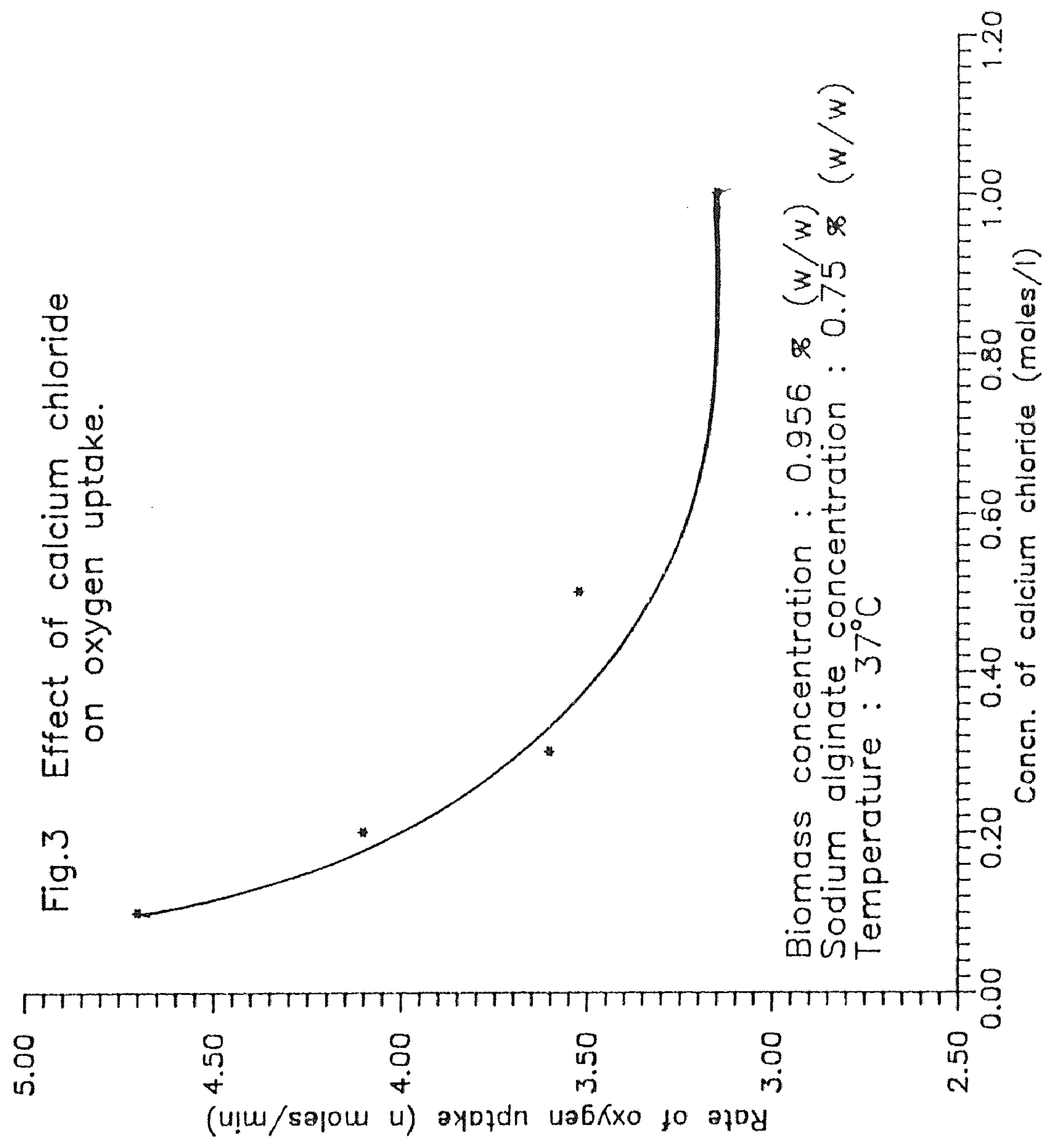


Fig.3 Effect of calcium chloride on oxygen uptake.
Biomass concentration : 0.956 % (w/w)
Sodium alginate concentration : 0.75 % (w/w)
Temperature : 37°C
Rate of oxygen uptake (n moles/min)
Concn. of calcium chloride (moles/l)
5.00
4.50
4.00
3.50
3.00
2.50
0.00
0.20
0.40
0.60
0.80
1.00
1.20

solution.

5.1-2 Effect of biomass concentration on oxygen uptake

This study was performed in order to find the optimum biomass concentration within the beads. The biomass concentration within the beads was changed by varying the ratio of water to bacterial pellets (see Methods section). The range of concentration studied was 5 to 40 mg biomass/gm of mixture.

Figure 4 shows changes in rates of oxygen consumption as a function of biomass concentration in the mixture. It is seen that the rates increase rapidly at lower concentrations but at higher concentrations the curve levels off. In the present study, a concentration of 25 mg biomass/gm of mixture was found to be optimal with respect to oxygen consumption and was used in most of the experiments.

5.1-3 Effect of sodium alginate concentration on oxygen uptake and bead stability

The concentration of sodium alginate is constrained at the lower bound by the inability to produce an integral bead below 0.75 percent (w/w), and at the upper bound by the difficulty of extruding beads above 1.5 percent (w/w).

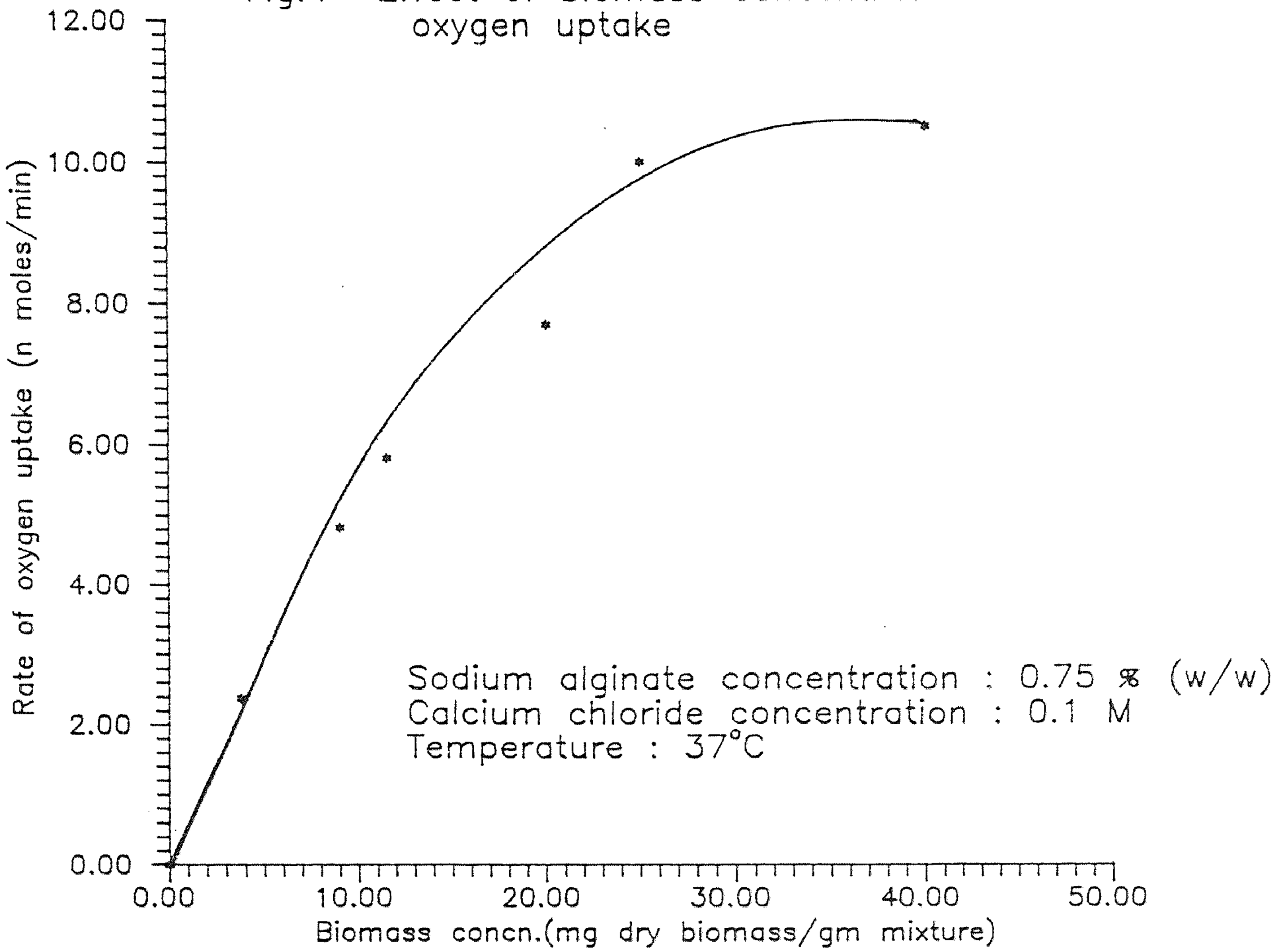


Fig.4 Effect of biomass concentration on oxygen uptake

As seen from Table 1, the oxygen uptake rate decreases with an increase in sodium alginate concentration. On the other hand, bead stability could be increased from 8 hours under high speed stirring for 0.75 percent alginate to 71 hours for 1.5 percent alginate. In the present study a concentration of 1.0 percent alginate was used because, in the recirculation configuration, the beads are not exposed to high mechanical shear as in the microassay reactor.

5.2 PHYSICAL REMOVAL OF 2-CHLOROPHENOL IN RECIRCULATION REACTOR

The initial control run on the physical removal (stripping) showed that 2-chlorophenol was removed by first order kinetics with a "K_La" value of 0.0575 hr^{-1} at room temperature and an air flow rate of 1.5 liter/min. Figure (5) shows a typical first order plot for removal of 100 ppm 2-chlorophenol by stripping.

Table 1: Effect of Sodium Alginate concentration on Bead Stability and Oxygen Uptake

Sodium Alginate % (w/w)	Beads Lasting Time (hours)	Total Oxygen Uptake (n moles)
0.75	8.00	3600
1.00	24.00	8352
1.25	36.00	14256
1.50	71.00	20022

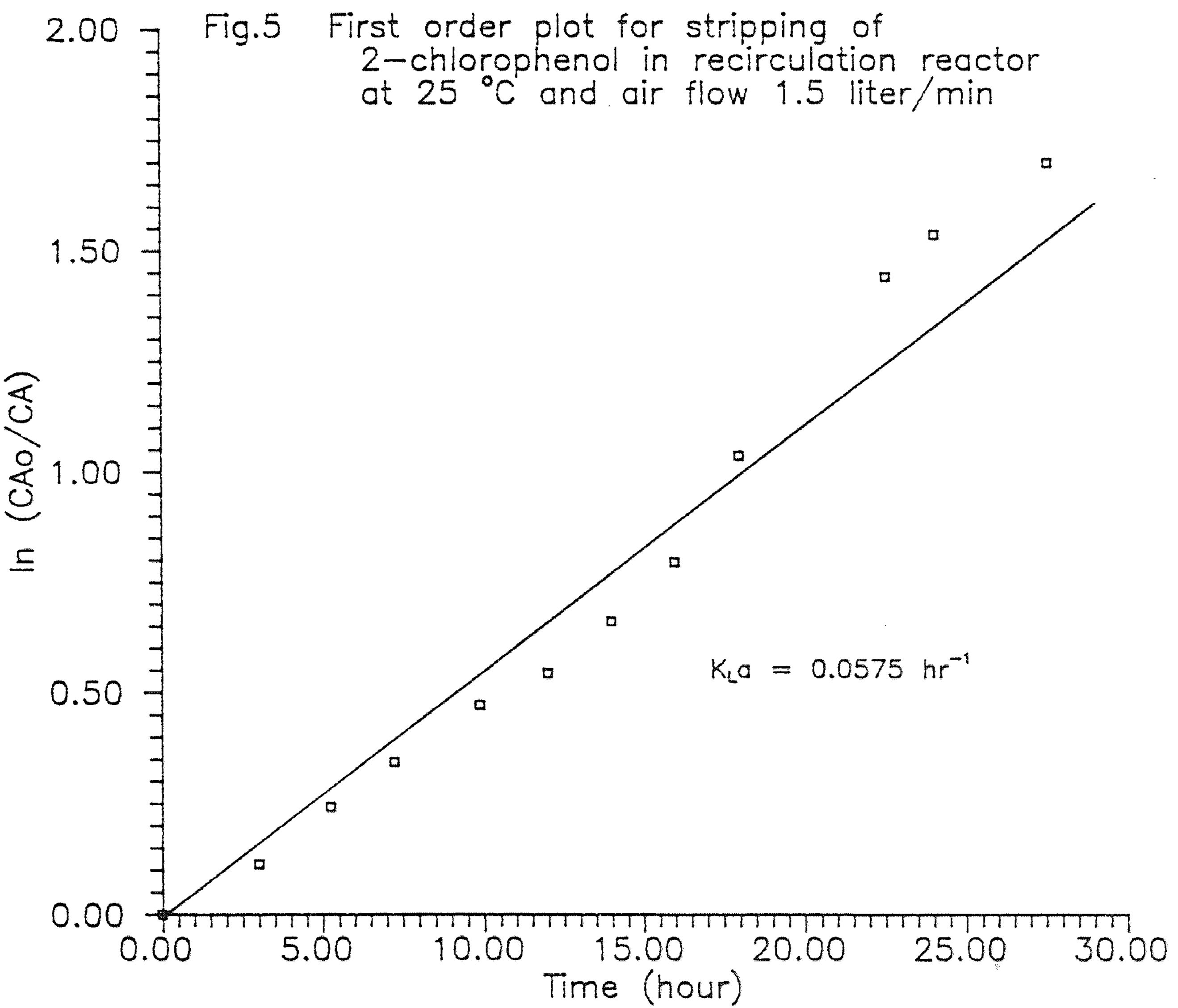


Fig.5 First order plot for stripping of 2-chlorophenol in recirculation reactor at 25 °C and air flow 1.5 liter/min

5.3 BIOLOGICAL REMOVAL OF 2-CHLOROPHENOL IN RECIRCULATION REACTOR

5.3-1 Effect of flow rate

The effect of flow rate was studied in the range of 100 to 1200 ml/min. This corresponds to a specific flow rate in the bioreactor of 4.93 to 59.20 $ml/min.cm^2$. Flow rate has a dual effect on the system. At low flow rates, the system may be under the limitation of external mass transfer, on the other hand, at higher flow rates the bead structure is endangered. As seen in Figure 6, within the range of flow rate studied, there was no significant change in the rate of biodegradation, which means the system is operating in a regime which is not influenced by external mass transfer.

As far as the bead stability is concerned, the operation was limited above a flow rate of 1200 ml/min, where the bead stability was reduced to only 48 hours. At flow rates below 600 ml/min, the system was in operation over a period of 28 days without any visible damage to the bead structure. In the present study, most of the experiments were conducted at a flow rate of 300 ml/min.

5.3-2 Effect of Biomass loading

The effect of biomass loading was studied in the range of 15 to 75 g wet beads. Table 2 shows the rate of

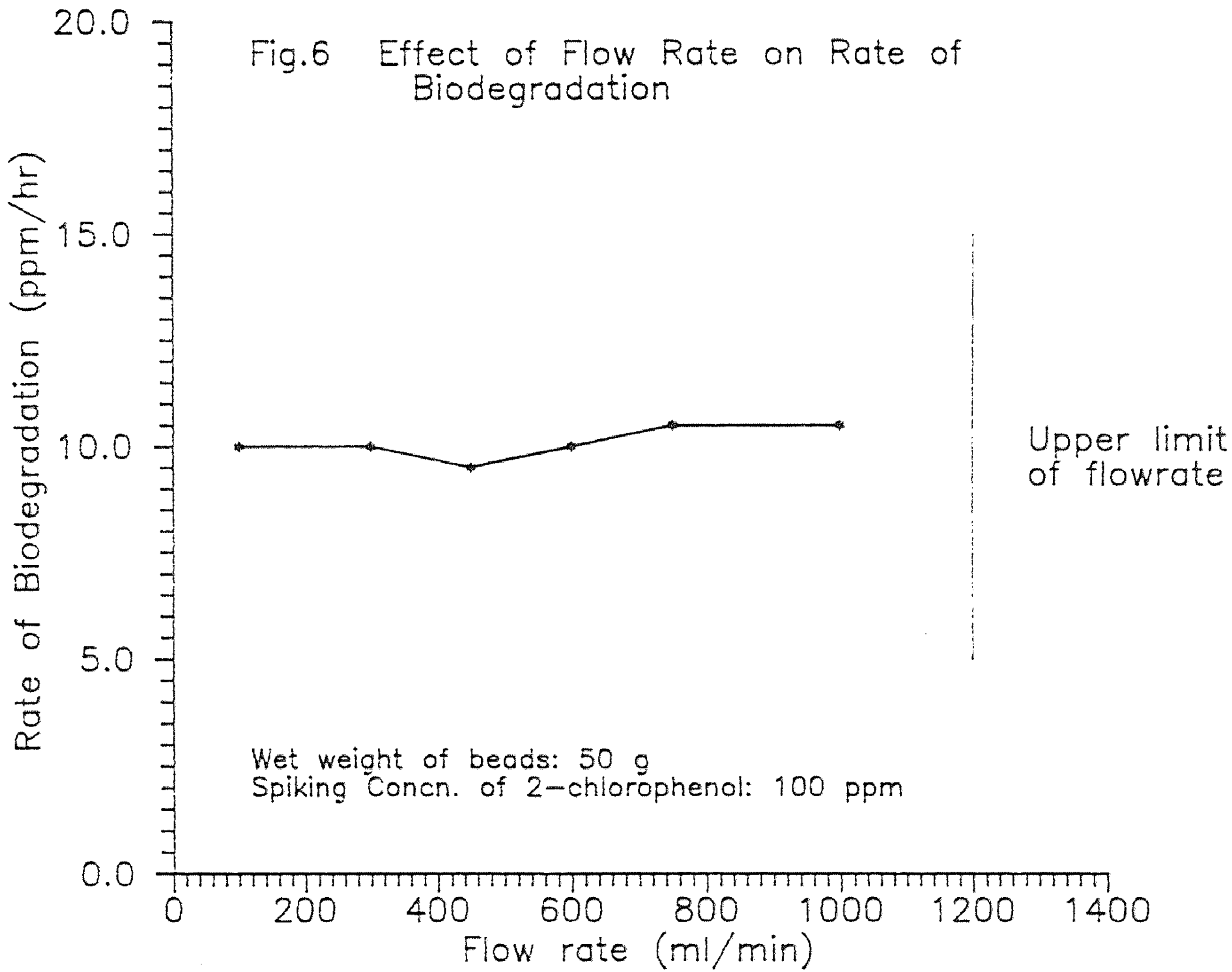


Fig.6 Effect of Flow Rate on Rate of Biodegradation
Rate of Biodegradation (ppm/hr)
20.0
15.0
10.0
5.0
0.0
0
200
400
600
800
1000
1200
1400
Flow rate (ml/min)
Upper limit of flowrate
Wet weight of beads: 50 g
Spiking Concn. of 2-chlorophenol: 100 ppm

Table 2: Effect of Biomass Loading on the Rate of Biodegradation:

Weight of beads gms	Biodegradation rate ppm/hr
15	3.5
30	5.5
50	10.0
75	12.5

biodegradation at various biomass loadings. Initially, by increasing the biomass loading, the rate of biodegradation increases proportionately, but in the range of 50 to 75 g, the increase in rate is not significant. Hence, in this range there exists an optimum value of biomass loading.

Increasing the biomass loading along the length of the reactor may result in inefficient use of the biomass which is located at the upper level due to lack of dissolved oxygen. Moreover, this kind of packing may result in an increase in the pressure drop in the system.

5.3-3 Effect of spiking concentration of 2-chlorophenol

Spiking concentrations of 50, 100 and 150 ppm were studied. As seen in Figure 7, the rate of biodegradation is almost the same for spiking concentrations of 50 and 100 ppm. With 150 ppm as the spiking concentration, it was observed that the biodegradation rate was reduced to 70 percent of that at 50 and 100 ppm. This clearly indicates an inhibitory effect at higher concentrations.

5.3-4 Use of buffered and nonbuffered medium.

It is known that the presence of nutrient medium is necessary for better activity of the microorganisms.

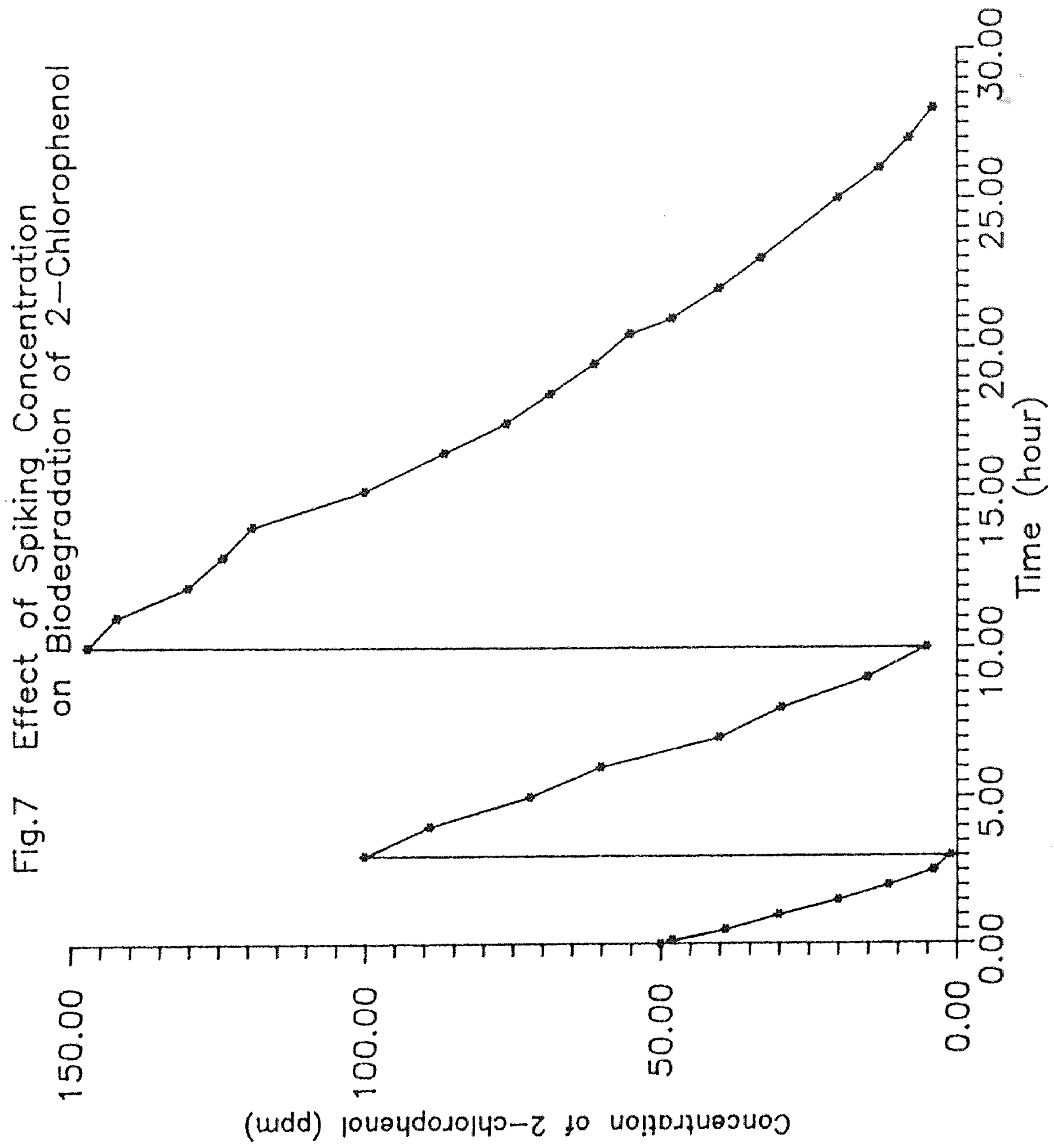


Fig.7 Effect of Spiking Concentration on Biodegradation of 2-Chlorophenol

Figure 8 indicates the comparative rates of oxygen uptake of immobilized microorganisms in distilled water and nutrient medium. It is seen that the rate of oxygen uptake in nutrient medium is 51 percent lower than that in distilled water.

Buffered medium (phosphate buffer pH 7.4) was used to control the pH during biodegradation but in the process, due to the high concentration of phosphate ions, the calcium alginate beads dissolved within a few days.

By using a nonbuffered medium (essentially no phosphate ions), the bead structure was intact even after one month of continuous operation. Also, as there was no control on pH during biodegradation, the pH dropped from 7.4 to 4.2 where it stabilized. This is due to mineralization of 2-chlorophenol, resulting in the generation of free chloride ions which were measured by titrimetry using silver nitrate.

Figure 9 shows the variation in pH and concentration of 2-chlorophenol with time in a nonbuffered medium. As it can be seen in the figure, the pH drops fast initially, then slowly, and finally levels off. It is also observed that the pH rises during the time when the concentration of 2-chlorophenol in the system is less than 5 ppm. This rise is because the biodegradation is slow and hence the production of free chloride is less during this time.

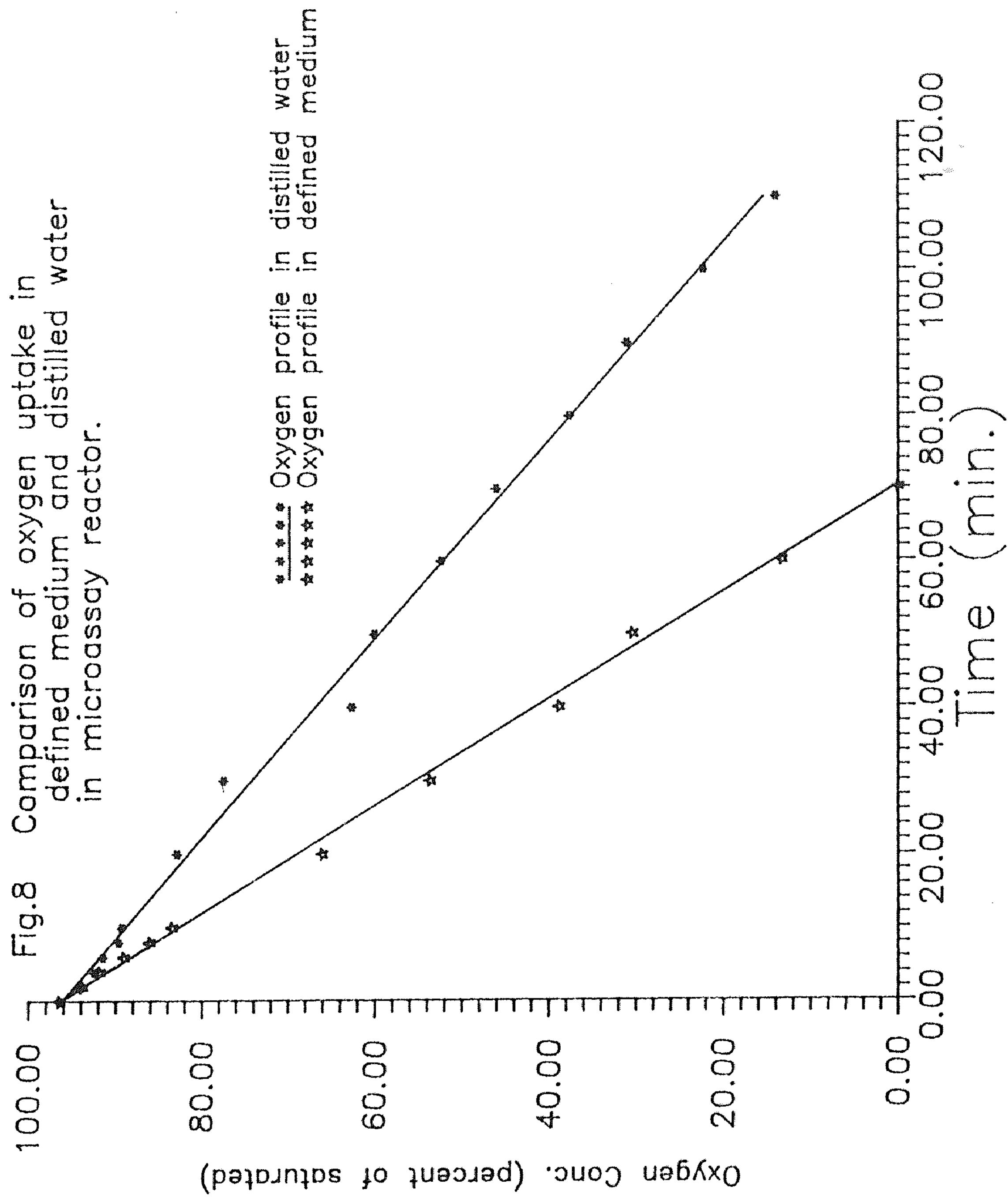


Fig.8 Comparison of oxygen uptake in defined medium and distilled water in microassay reactor.

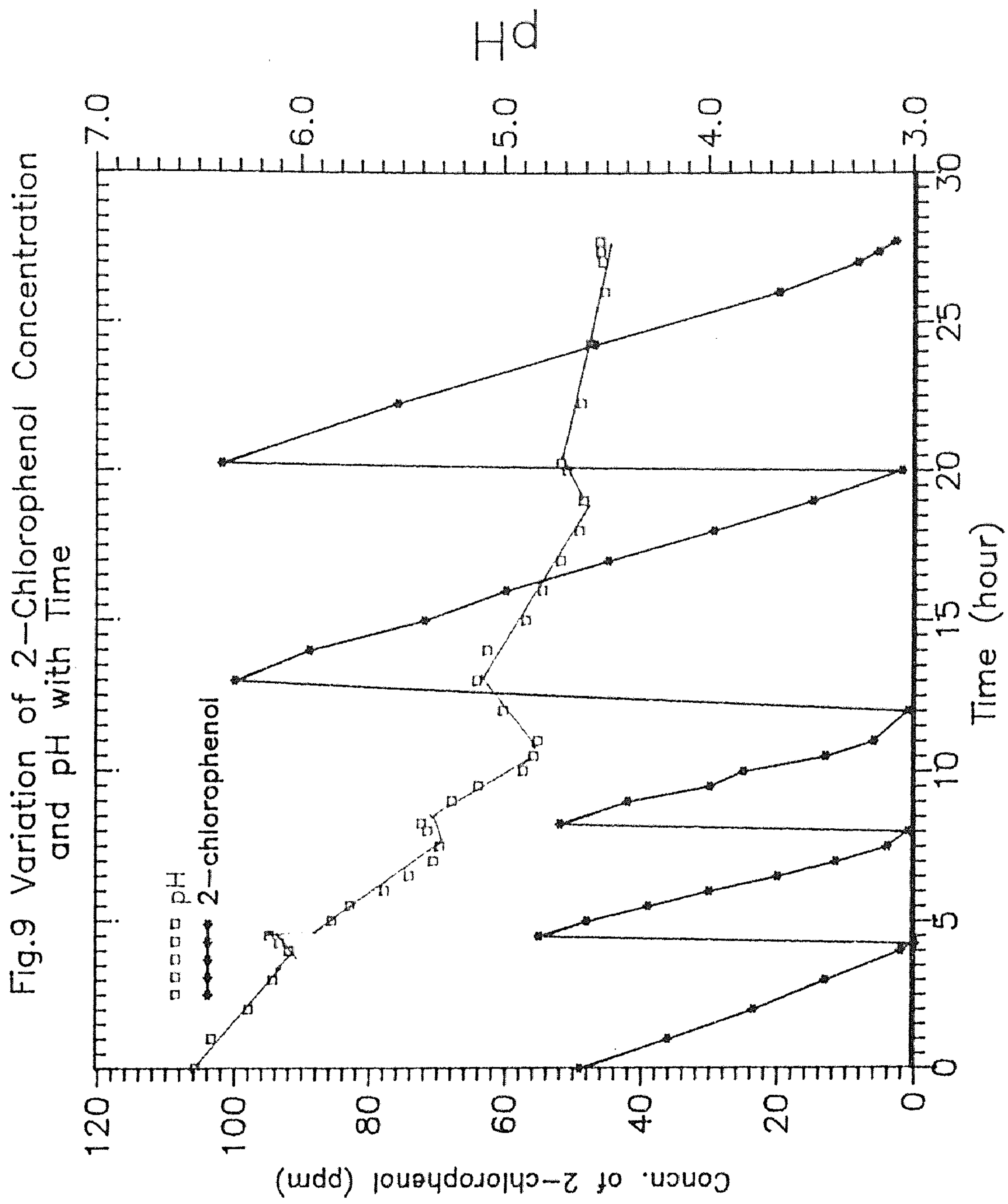


Fig.9 Variation of 2–Chlorophenol Concentration and pH with Time

5.4 MATERIAL BALANCE

An attempt was made to complete the material balance by following the consumption of both 2-chlorophenol and dissloved oxygen and the generation of free chloride.

For a particular spike of 100 ppm 2-chlorophenol, it was found that after accounting for physical removal, the balance on free chloride generated matched to 80 percent of 2-chlorophenol consumed (Appendix A). But for an overall run, the balance matched only to 60 percent, because of the difficulty in continuously monitoring the generation of free chloride. Figure 10 shows the mineralization of 2-chlorophenol for one complete run.

The amount of dissolved oxygen consumed was determined by following the rate of oxygen consumption during the entire run. For a particular spike of 50 ppm 2-chlorophenol after accounting for physical removal it was found that about 4.5 moles of oxygen were required for every mole of 2-chlorophenol biodegraded. However, from stoichiometry the requirement of oxygen is 6.5 moles for every mole of 2-chlorophenol consumed if complete mineralization is to take place.

In a nutshell, it could be said that biodegradation of 2-chlorophenol probably does not undergo complete mineralization, although no biodegradation products were detected on the gas chromatograph.

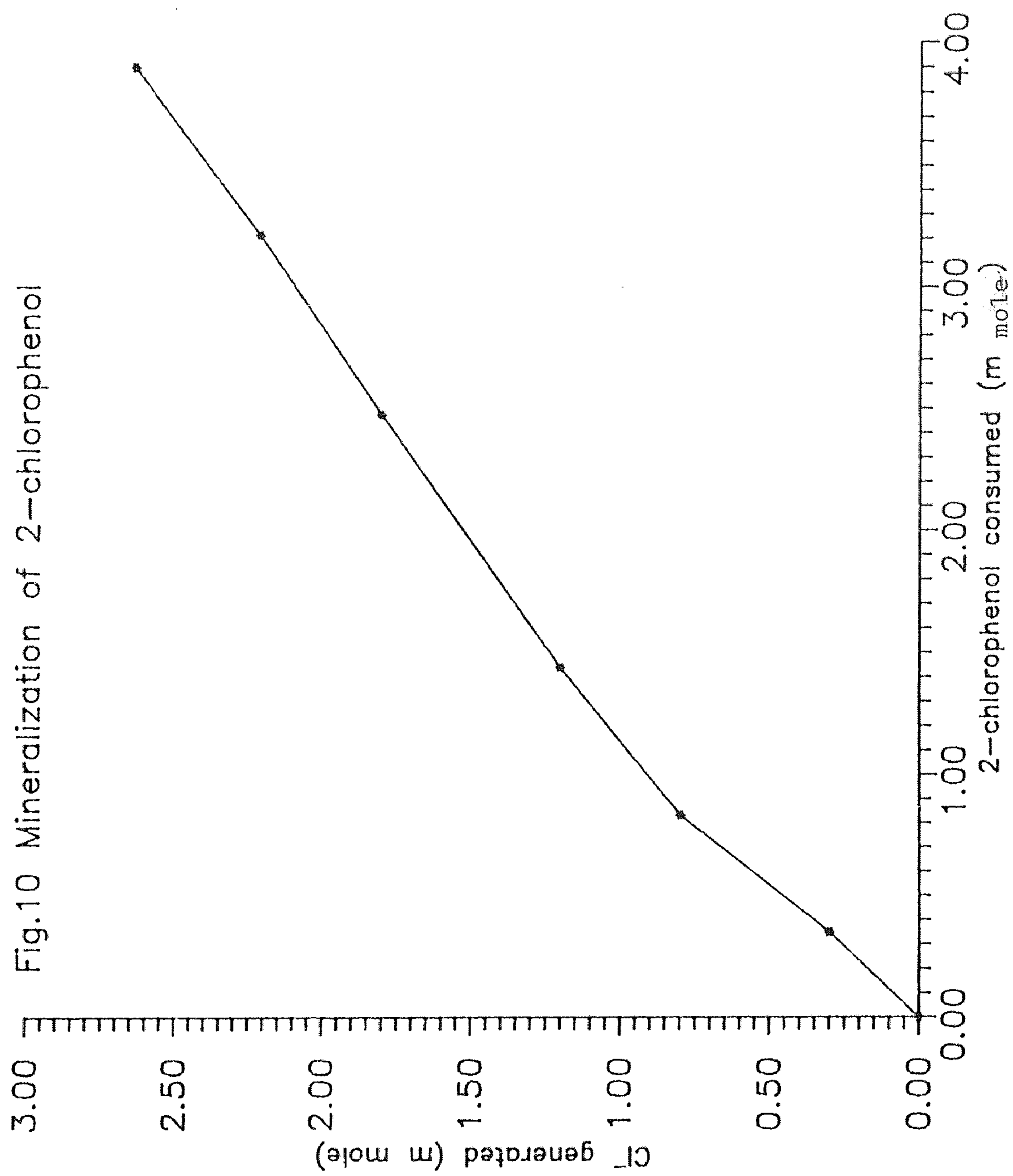


Fig.10 Mineralization of 2-chlorophenol

5.5 Kinetics

By linear regression analysis (Appendix B) it was found that the total removal fits a Monod type equation for constant biomass.

As seen in the figure (11) the data closely fits the Monod kinetics with Km = 19.61 ppm/hr, Ks = 2.93 ppm, S_o =56.5 ppm.

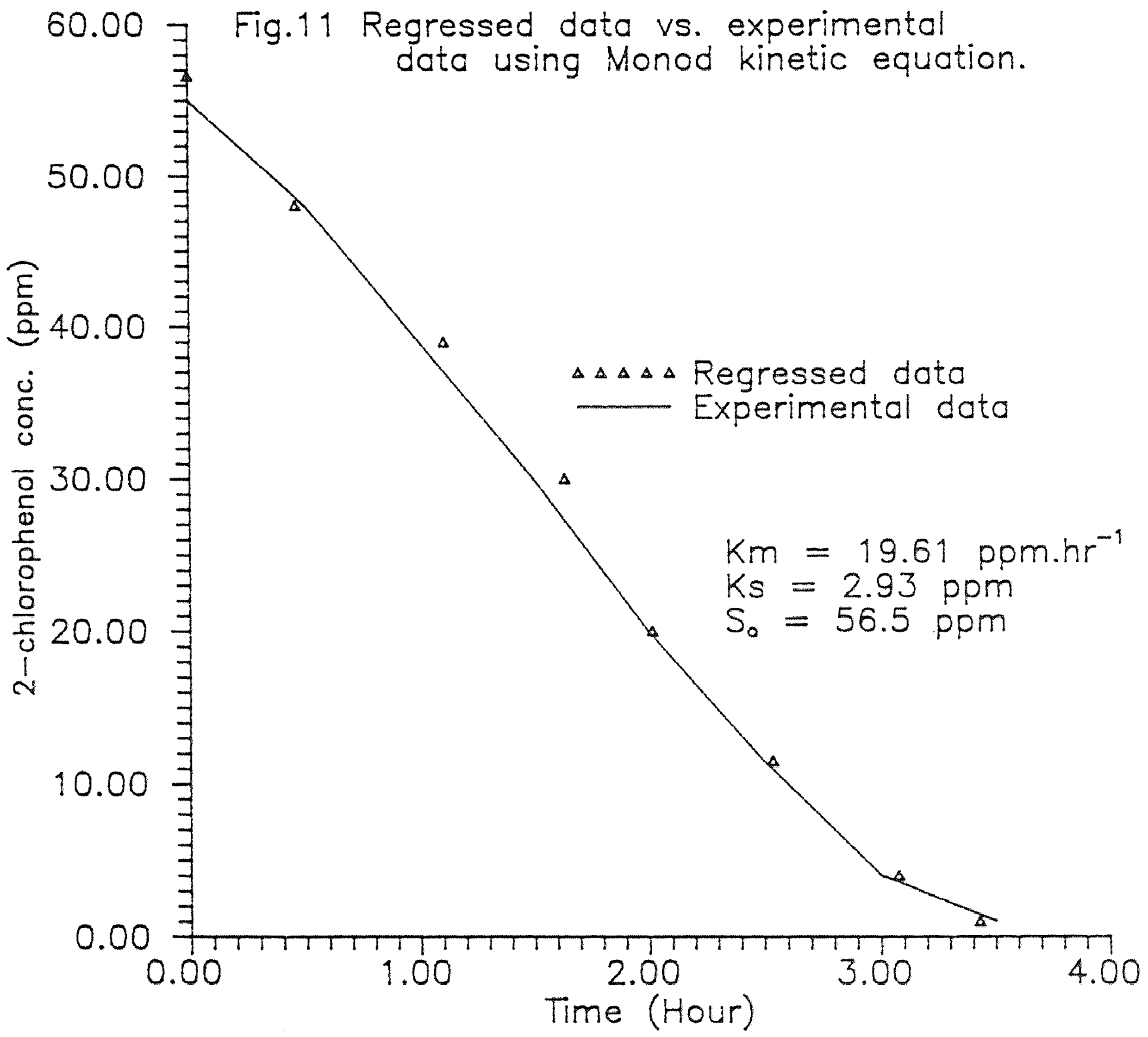


Fig.11 Regressed data vs. experimental data using Monod kinetic equation.

CHAPTER VI

CONCLUSIONS AND SUGGESTIONS

6.1 CONCLUSIONS

The use of calcium alginate immobilized activated sludge for treatment of aqueous waste, containing high concentration of toxic chemicals has been established. In the range of biomass concentration studied, the optimum oxygen consumption is attained at a concentration of 25 mg dry biomass/g of immobilizing gel. Increasing the alginate concentration has a pronounced effect on bead stability than an increase in calcium chloride concentration.

In the range of flow rate studied, the rate of biodegradation was not affected, but at higher flow rates the bead stability was endangered. A biodegradation rate of 12 ppm/hr was found to be maximum at a biomass loading of 75 g wet beads.

Biodegradation at a biomass loading of 75 g wet beads was not inhibited by any spiking concentrations below 120 ppm of 2-chlorophenol. Continuous biodegradation of 2-chlorophenol was accomplished over a

period of 28 days, as seen in Figure 12.

A novel and vital tool was observed in the form of "substrate dependent oxygen consumption" (Figure 13). Given a biomass, an unknown toxic chemical that induces substrate dependent oxygen consumption is confirmed to be biodegradable. The necessity of having such a tool is immense.

Both buffered and nonbuffered medium showed essentially the same rates of biodegradation. Although with buffered medium, the bead structure was destroyed in a few days.

A balance on free chloride generated, matched to 80 percent of 2-chlorophenol biodegraded. On the other hand, a balance on oxygen showed consumption of 4.5 moles of oxygen for every mole of 2-chlorophenol biodegraded, which is lower than the stoicmetric requirement.

The removal mechanism closely fits a Monod kinetics equation for constant biomass.

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 (2-chlorophenol). The effects of various parameters such

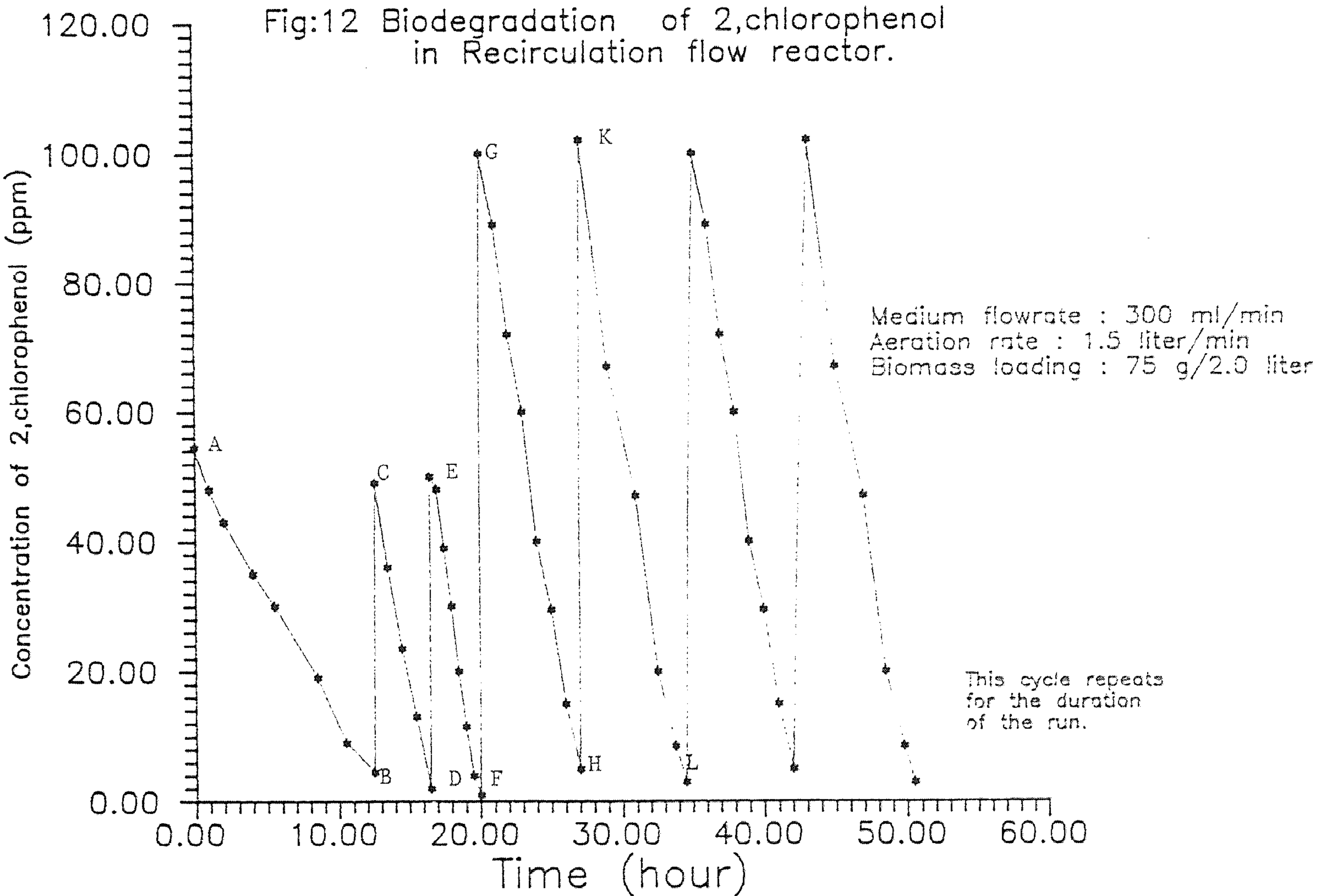


Fig:12 Biodegradation of 2,chlorophenol in Recirculation flow reactor.

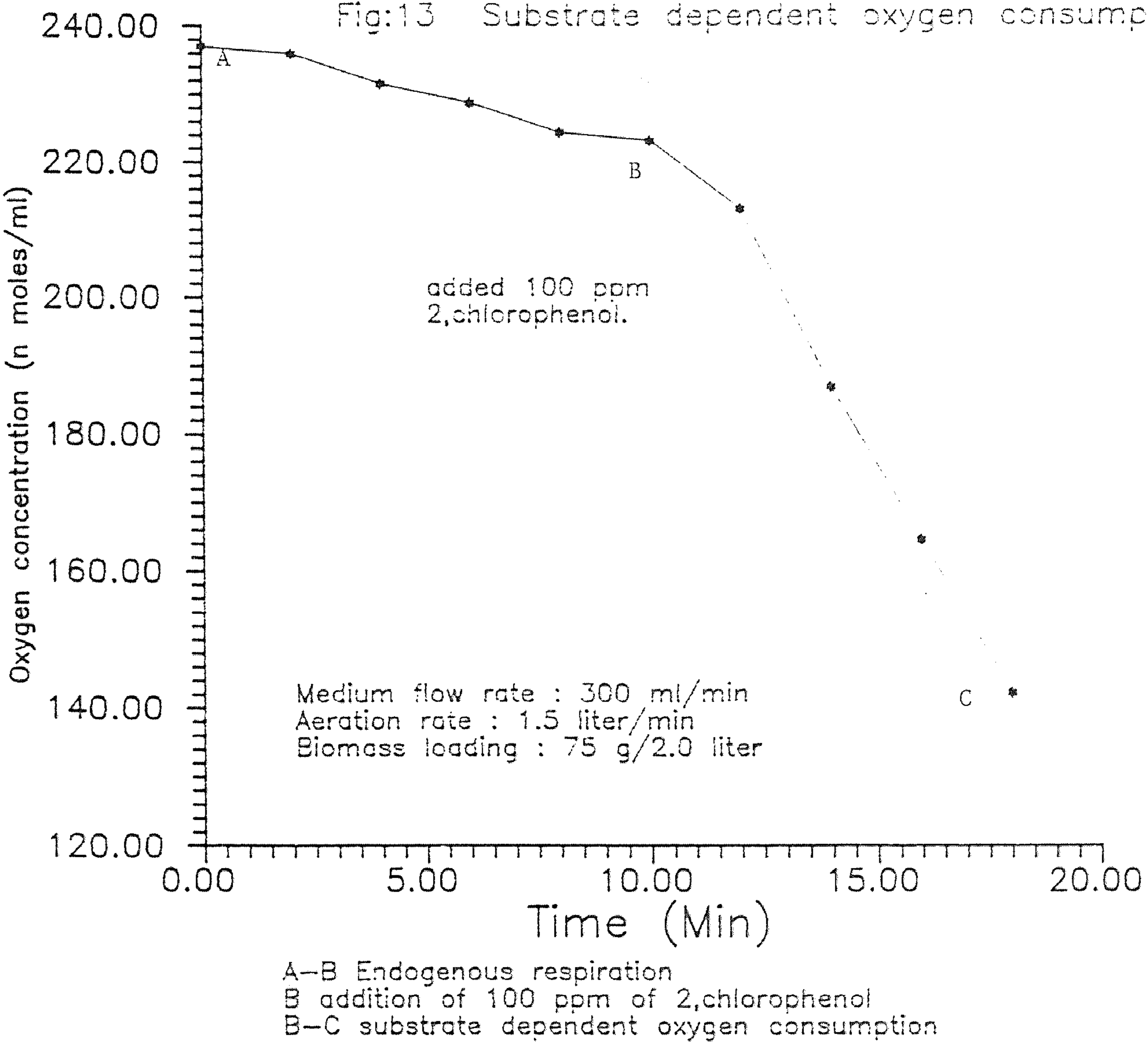


Fig:13 Substrate dependent oxygen consumption

as flow rate, biomass loading, spiking concentration of 2-chlorophenol and nutrient medium composition were studied. This information will be vital in reactor design.

The next logical step will be to demonstrate the technology on a larger scale. A continuous flow system needs to be investigated. The system should be a microprocessor based, computer controlled unit, which will continuously run and control the reactor from a remote location.

A detailed economic analysis of the above system is necessary.

Finally, the objective should be to treat a stream containing mixture of toxic chemicals.

APPENDIX A

CALCULATION FOR FREE CHLORIDE GENERATED USING SILVER NITRATE TEST

Following are the sample calculations for the estimation of free chloride generated during two consecutive spikings of 2-chlorophenol.

Volume of recycle stream .... 2.0 liter

Volume of scrubber solution (0.5 M NaOH).... 1.0 liter

At the end of first spike

4.6 ml of 0.005 M $AgNO_3$ = 10 ml of recycle stream

1.7 ml of 0.005 M $AgNO_3$ = 10 ml of recycle stream

At the end of second spike

5.2 ml of 0.005 M $AgNO_3$ = 10 ml of recycle stream

1.8 ml of 0.005 M $AgNO_3$ = 10 ml of recycle stream

$M_1 * V_1 = M_1 * V_1$

Equivalents of free chloride generated during one spike in recycle stream:

0.005M * (5.2-4.6)ml * 2.0 liter / 10 ml = 0.55 m mole

Equivalents of free chloride in scrubber solution:

0.005M * (1.8-1.7)ml * 1.0 liter / 10 ml = 0.05 m mole

Total equivalents of free chloride generated during one spike:

(0.55 +0.05) m mole =0.6 m mole

Moles of 2-chlorophenol added = 1.075 m moles

Moles of 2-chlorophenol stripped out = 0.33 m moles

Moles of 2-chlorophenol biodegraded = 0.745 m moles

$$\frac{\text{moles of free chloride generated}}{\text{moles of 2-chlorophenol biodegraded}} = \frac{0.6}{0.745} = 0.8$$

APPENDIX B

The Monod expression for specific substrate utilization based on active biomass can be given as

$$U_a = \frac{k * S}{Ks + S}$$

where

U_a = Specific substrate utilization rate ($time^{-1}$)

k = Maximum substrate utilization rate ($time^{-1}$)

Ks = Half-velocity coefficient (mass/vol.)

S = Substrate concentration (mass/vol.)

For a reactor configuration operating under essentially constant biomass, the total substrate utilization can be given as

$$R_b = \frac{Km * S}{Ks + S}$$

where

R_b = Rate of biodegradation of the substrate.

Km = Maximum substrate utilization rate.(mass/vol.time)

$$\frac{ds}{dt} = \frac{Km * S}{Ks + S} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad I$$

Integrating (I)

$$\frac{Ks}{Km} * \ln\left(\frac{S_o}{S}\right) + \frac{1}{Km}(S_o - S) = t \quad \ldots\ldots\ldots \quad II$$

Solving for Ks and Km by linear regression, we have to

minimize

$$\underline{S} = \quad (S_i - S)^2 = \quad (t_i - t)^2$$

$$\underline{S} = \quad [t_i - (\frac{Ks}{Km} * \ln S_o + \frac{S_o}{Km}) + \frac{Ks}{Km} * \ln S + \frac{1}{Km}],$$

Let

$$a = \frac{Ks}{Km} * \ln S_o + \frac{S_o}{Km} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad III$$

$$b = \frac{Ks}{Km} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad IV$$

$$c = \frac{1}{Km} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad V$$

$$\underline{S} = \quad (t_i - a + b * \ln S_i + c * S_i)^2$$

$$\frac{\partial \underline{S}}{\partial a} = 2 * \quad (t_i - a + b * \ln S_i + c * S_i) * (-1)$$
$$= 0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad VI$$

$$\frac{\partial \underline{S}}{\partial b} = 2 * \quad (t_i - a + b * \ln S_i + c * S_i) * (\ln S_i)$$
$$= 0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad VII$$

$$\frac{\partial \underline{S}}{\partial c} = 2 * \quad (t_i - a + b * \ln S_i + c * S_i) * (S_i)$$
$$= 0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad VIII$$

Equation VI,VII, VIII are to solve for a, b, and c.

The value of a, b, and c were found to be

$a = 3.487$

$b = 0.149$

$c = 0.051$

therefore

$$Km = 19.61 \text{ ppm/hr}$$

$$Ks = 2.93 \text{ ppm}$$

$$S_o = 56.5 \text{ ppm}$$

BIBLIOGRAPHY

1. Dwyer, D.F., Krumme, M.L., Boyd, S.A., and Tiedje, J.M., 1986. "Kinetics of Phenol Degradation by an Immobilized Methanogenic Consortium". Appl. Environ. Microbiol., 52, 345-351.

2. Kennedy, J.F. and Cabral, J.M.S., 1983. "Immobilized Living Cells and their Applications". Appl. Biochem. Bioeng., 4, 189-280.

3. Linko, Y.Y. and Linko, P., 1985. "Immobilized Living Cell Biotechnology". Proceedings of the 16th FEBS Congress, Part C, 191-196.

4. Nan K. Chien and Sam S. Sofer, 1985. "Flowrate and Bead Size as Critical Parameters for Immobilized Yeast Reactors". Enzyme. Microb. Technol., 7, 538-542.

5. Kierstan, M. and Buckle, C., 1977. "The Immobilization of Microbial Cells, Subcellular Organelles and Enzymes in Calcium Alginate Gels". Biotech. Bioeng., 19, 387-397.

6. Gaudy, A.F. (Jr) and Gaudy, E.T., 1980. "Microbiology for Environmental Scientists and Engineers". McGraw Hill Book Co., New York, NY.

7. Enfors, S.O. and Mattiason, B., 1983. "Oxygenation of Processes Involving Immobilized Cells", in Immobilized Cells and Organelles, Vol. II, B.

Mattiason, Ed.. CRC Press, Boca Raton, FL. Chap. 3, 41-60.

8. Adlercreutz, P. and Mattiason, B., 1982. "Oxygen Supply to Immobilized Cells. 3 Oxygen supply by Hemoglobin or Emulsions of Perfluorochemicals", European Journal of Applied Microbilogy and Biotechnology, 16, 165-170.

9. Larson, R.J., Clinckemaille, G.G. and Van Belle, L., 1981. "Effects of Temperature and Dissolved Oxygen on Biodegradation of Nitrilotriacetate". Water. Res., 15, 615-620.

10. Pak, D., "The Biodegradation of Phenolics using Mixed Liquors from Passaic Valley Sewerage Commission Plant, Newark, N.J." M.S. Thesis, New Jersey Institute of Technology, Newark, N.J. 07102, 1985.

11. Chung J. Kim and Walter J. Maier, 1986. "Acclimation and Biodegradation of Chlorinated Organic Compounds in the Presence of Alternate Substrates". Journal WPCF., 58, 157-164.

12. Lewandowski, G., Knox, D. and Pai, P. "The Implications of Vapor-Liquid Equilibrium in the Biodegradation of Hazardous Wastes", 1987. Dept. of Chemical Eng., Chemistry and Environmental Science. New Jersey Institute of Technology, Newark, NJ. 07102. Unpublished Work.

13. Chambers R. P. *et al.*, 1972 Enzyme Engineering ,

Wiley-interscience. 24.

14. Shamat, N.A. and Maier, W.J., 1980. "Kinetics of Biodegradation of Chlorinated Organics". Journal WPCF. 52, 2158-2166.

15. Lewandowski, G., Adamowitz, D., Boyle, P., Gneiding, L., Kim, K., McMullen, N.,' Pak, D. and Salerno, S.A "Comparison of the Microbial Response of Mixed Liquors from Different Treatment Plants to Industrial Organic Chemicals". Presented at the International Conference on Innovative Biological Treatment of Toxic Wastewaters, June 25, 1986, Arlington, VA.

16. Westmeier, F. and Rehm, H.J., 1985. " Biodegradation of 4-Chlorophenol by Entrapped Alcaligenes sp. A7-2". Appl. Microbiol. Biotechnol. 22, 301-308.

17. Macaskie, L.E., Wates, J.M. and Dean, A.C.R., 1987. "Cadmium Accumulation by a Citrobacter sp. Immobilized on Gel and Solid Supports: Applicability to the Treatment of Liquid Wastes containing Heavy Metal Cations". Biotech. Bioeng., 30, 66-73.

18. Klein, J., Hackel, U., and Wagner, F., 1979. "Phenol Degradation by Candida tropicalis whole cells Entrapped in Polymeric Ionic Networks", in Immobilized Microbial Cells, K. Venkatsubramanian Ed.. ACS Symposium Series 106, 101-118.

19. Shaler, T.A. and Klecka, G.M., 1986. "Effects of Dissolved Oxygen Concentrations on Biodegradation of 2,4-dichlorophenoxyacetic acid". Applied and

Environmental Microbiology, 51, 950-955.

20. Tanaka, H., Matsmura, M. and Veliky, I., 1984. "Diffusion Characteristics of Substrates in Calcium Alginate Gel Beads". Biotech. Bioeng., 26, 53-58.

21. Gosmann, B. and Rehm, H.J., 1986. "Oxygen Uptake of Microorganisms Entrapped in Calcium Alginate Gel". Appl. Microbiol. Biotechnol., 23, 163-167.

22. Lurker, P.A., Clark, C.S. and Elia, V.J., 1982. "Atmospheric Release of Chlorinated Organic Compounds from the Activated Sludge Process". Journal WPCF, 54, 1566-1573.

23. Tischler, L.F. et al., 1979. "Evaluation of the Potential for Air Stripping of Hydrocarbons During the Activated Sludge Wastewater Treatment". Paper presented at WPCF, 52nd Annual Conference, Houston, TX.

24. Lewandowski, G.A. and Armenante, P.M. "Reactor Design using a White Rot Fungus for Hazardous Waste Treatment". Paper presented at the Workshop on Detoxification of Recalcitrant Organics by Naturally occuring and Genetically Engineered Microorganisms, October 19, 1987, Newark, NJ.

25. Lakhwala S.F. Lodaya P.M., Yang K.C., Lewandowski, G.A., Sam S.S., 1988. "Design of Toxic Waste Treatment Bioreactor: Viability Studies of Microorganisms Entrapped in Alginate Gel". Presented at the International Conference on Physiochemical

and Biological Detoxification of Hazardous Wastes. Altantic City N.J.

26. Knudson, M.K., Williamson, K.J., and Nelson P.O., "Influence of Dissolved on Substrate Utilization Kinetics of Activated Sludge". Journal WPCF, 54, 52-59.

27. Camp, C.E., "Preliminary Systems and Process Design of a Microsomal Bioreactor", M.S. Thesis, University of Oklahoma, 1985.

28. Greenberg, A.E., Trussell, R.R., and Clesceri, L.S., "Standard Methods for Examination of Water and wastewater" 1985.