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Biodegradation of styrene in a recirculation reactor using immobilized activated sludge

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ABSTRACT

Title of Thesis: Biodegradation of Styrene in a Recirculation
Reactor Using Immobilized Activated Sludge

Shreyans Dilip Shah, Master of Science in Chemical Engineering
1991

Thesis Directed by: Sam S. Sofer, Professor
Department of Chem. Engg.,
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The performance of immobilized cells in the biological treatment of hazardous waste was investigated using a recirculating flow reactor in batch mode. A mixed culture activated sludge from a municipal waste water treatment plant was immobilized in calcium alginate gel. The bio-oxidation ability of these microbes towards a model toxin (styrene) was studied under closed system using dilute H_2O_2 solution as an oxygen source.

The process parameters studied were as follows:

1. Effect of flow rate of recycle stream,
2. Effect of biomass loading,
3. Effect of spiking concentration of styrene, and
4. Flow pattern and average residence time within the reactor.

Dissolved oxygen concentration was monitored by a Clark type dissolved oxygen probe and also by an oxygen electrode. Biodegradation rates of styrene with two different oxygen sources, air and H_2O_2 , were determined and compared. Abiotic losses of styrene were taken into account. It was found that biodegradation was the primary removal mechanism when H_2O_2 was used as oxygen source, whereas stripping was the primary removal mechanism in the case of air. Considerable adsorption of styrene on the alginate was observed initially, but all the styrene desorbed to the bulk slowly and biodegraded finally.

BIODEGRADATION OF STYRENE IN A RECIRCULATION
REACTOR USING IMMOBILIZED ACTIVATED SLUDGE

by
Shreyans Dilip Shah

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
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Dedicated

To

My Parents

For Their Understanding When Entropy Was High
And For Their Encouragement When Energy Was Low
During My Academic Advancement

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CHAPTER I

INTRODUCTION

In the petrochemical industry styrene is used in large quantities mainly as a starting material for synthetic polymers such as polystyrene and styrene-butadiene rubber. It is also used as a solvent in the polymer processing industry and consequently is present in many industrial effluents. Airborne emissions of styrene often cause problems, even at low concentrations (less than 1 volume per million volumes [vpm]), due to malodorous properties of the compound. Styrene with its toxicological profile has been labelled as a priority pollutant by the USEPA.

Continuous release of xenobiotics in general, and that of listed priority pollutant organic compounds in particular, poses a severe threat to the well-being of the human race. For the past two decades, researchers have engaged themselves in finding the ways and means of containing the releases on one hand, and removing or converting the already released toxic compounds as non-toxic compounds on the other. Removal methods have been either physical, chemical or biological. Removal of styrene from industrial wastewaters may be accomplished using styrene degrading bacteria as biocatalyst.

In the past, biological reactors for the treatment of aqueous waste containing toxic compounds have typically utilized activated sludge in suspended form. The use of immobilized microorganisms has been so far limited to the selection of new immobilization 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 population has been investigated for the biodegradation of styrene.

1.1 IMMOBILIZATION AND ITS ADVANTAGES

Application of immobilized bacterial cells to biodegradation has been a subject of intensive and rigorous study in recent years. The use of immobilized microorganisms has many advantages over the conventional free cell system [1] in the treatment of aqueous wastes. Washout of biomass is one of the most common problems encountered in free-cell biological treatment of toxic organic chemicals. This problem becomes a main concern when pure cultures are used. Furthermore, the free cell system is very sensitive to varying input conditions. Long residence times are required so that the organisms can become acclimated and evolve a population that is compatible with the feed.

Most of these conditions can be significantly improved using immobilized microorganisms. Such a system facilitates separation of liquid from bacteria and offers a greater

degree of operational flexibility. Immobilized cells are much more resistant to high concentrations of toxic chemicals [2]. In addition, the cell density of an immobilized cell 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 reusable biomass.

Numerous methods have been developed for immobilized biocatalyst preparation [2]. 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 be able to withstand 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 [3]. 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 to the surface of solid supports offers an advantage because the support is stronger than gel matrices.

Diffusion of oxygen and substrate is no longer a major problem, as growth is on the surface. The disadvantages are that the microorganisms can't 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 used for the entrapment of cells. In work done prior to this at the NJIT Biotechnology Laboratory, the physical stability and activity of alginate beads have been optimized with respect to parameters such as biomass concentration in the bead, concentrations of sodium alginate and calcium chloride used for curing the beads, and temperature of operation [4].

1.2 MASS TRANSFER CHARACTERISTICS

One of the many critical parameters which affects the kinetics of immobilized microbes is the diffusional or mass transfer effect. Cell entrapment in alginate is a rather simple and non-toxic method for immobilization, but the gel may create a diffusional barrier for both toxic substrate and oxygen. The average pore size of this gel (natural polysaccharide) is estimated to be larger than that of polyvinyl alcohol and other polymeric gels. Calcium alginate gel is therefore used mainly as a carrier for the immobilization of whole cells. On the other hand, large pore size can cause problems by allowing the entrapped enzymes or

cells to leak out. Polymeric gels have smaller pore size and can be used to overcome leakage of cells and enzymes, but they offer more resistance to diffusion of substrates. It is reported that calcium alginate gel provides little barrier to diffusion of neutral substrates up to a molecular weight of 5,000 [5].

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 [6] 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 may still be limited by intraparticle diffusional resistance.

1.3 REQUIREMENTS OF DISSOLVED OXYGEN

In a system utilizing aerobic microorganisms for biodegradation of organic compounds, dissolved oxygen requirements are of great importance. Biodegradation of an organic compound such as styrene requires oxygen as co-substrate for metabolism [7]. Aerobic microorganisms also utilize oxygen primarily as the terminal electron acceptor for aerobic respiration. Generally bacterial respiration does not appear to be affected above a critical dissolved

oxygen concentration. The critical dissolved oxygen level 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 [8]. Relatively little is known about the influence of dissolved oxygen on the microbial degradation of organic chemicals. 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 substrate and products through immobilized matrices. 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 [9]. 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. Addition of hydrogen peroxide and perfluoro chemicals have been attempted to increase the oxygen holding capacity of the medium [9,10].

1.4 ACTIVATED SLUDGE AND BIODEGRADATION

Biological treatment, and the activated sludge process specifically, is 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 organic aromatic compounds such as benzene, toluene, xylene, styrene, etc. The diversity of the microbial flora serves as a good environment for developing cultures that can biodegrade synthetic organics in general and simple organic ring compounds in particular.

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 styrene can be derived from activated sludge [11,12,18].

In general, biological treatment systems involve three competing removal mechanisms: adsorption, stripping and biodegradation. 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. The vapor pressure of styrene at room temperature (25⁰C) is 6.176 mm Hg. Thus the biological treatment involves a system

of three competing removal mechanisms: biodegradation, adsorption and stripping.

1.5 DESIGN CONSIDERATIONS

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

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 the process 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 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 in equilibrium with the bulk substrate concentration. Based on this principle,

the performance of a recirculation reactor to study biodegradation of styrene has been investigated.

CHAPTER II

OBJECTIVES

The primary objective of this research has been to investigate the critical and fundamental process parameters which affect the performance of a recirculation bioreactor using immobilized microorganisms in the treatment of a hazardous toxic waste such as styrene. The specific objectives of this research have been to:

1. Determine the primary mechanism for the removal of styrene,
2. Design the recirculation bioreactor with optimum flow rate of the recycle stream with respect to external mass transfer resistance and bead stability,
3. Optimize the loading density of biomass to achieve the highest possible biodegradation rates,
4. Define a suitable concentration range of the model compound (styrene) for which maximum substrate utilization can be achieved, and
5. Study of the flow pattern and residence time distribution in the reactor.

CHAPTER III

LITERATURE SURVEY

Activated sludge has been recognized as one of the most versatile methods for wastewater treatment. Beginning from the "gestation period" to the development of "aerated lagoons and ditches", Sawyer [14] has discussed the complete evolution process in terms of 12 milestones. The ultimate fate of pollutant organic chemicals in the ecosystem, and particularly in the aquatic environment, has been a focus of extensive research. There has been a growing interest in obtaining precise information on biodegradation patterns and rates of specific chemicals in wastewater treatment systems to characterize decay rates in the environment. Most of the literature to date on biodegradation of simple organic ring compounds such as benzene toluene, xylene, styrene, etc., deals with the isolation and identification of bacterial species. Moreover, most of the time it deals with the use of free microorganisms.

Styrene monomer is a widely used, important material for synthetic resins, such as polystyrene, and polystyrene-butadiene rubber. However, not all styrene present in the environment is of anthropogenic. Styrene is also known to be formed in nature by the decarboxylation of cinnamic acid by microorganisms such as Penicillium, Aspergillus, and

Saccharomyces. Styrene has been reported as biologically degradable in mammalian tissues [15].

Mammalian metabolism of styrene has been studied quite extensively in view of the intensive industrial use of styrene and its possible toxic and carcinogenic properties [16]. The first step in the major pathway of mammalian styrene metabolism is the oxidation to styrene oxide. Microbial degradation of styrene, however, is not well understood. The first attempt to isolate styrene degrading microorganisms from soil samples was futile as reported by Omori et al. [18], though methyl styrene had been shown to be metabolized [17] during their earlier study for a variety of alkenyl-substituted aromatics for utilization by soil microorganisms. Subsequently, Sielicki et al. [19] described styrene-utilizing mixed culture. The metabolites were traced and identified, and they proposed two degradative pathways for styrene: one was via β -phenethyl alcohol (β -PA) and phenylacetic acid and the other was via low molecular weight oligomer. The isolation of pure cultures degrading styrene was first reported by Shirai and Hisatsuka [12], who isolated 31 strains.

Tabaka et al. [20] studied the degradability and acclimation rates of 96 compounds. The compounds studied included: monocyclic and polycyclic aromatic hydrocarbons and their chlorine substituted counterparts, phenolics and a host of pesticides and insecticides.

Stover and Kincannon [21] studied the fate of specific organic compounds during conventional biological treatment. They also included the effects of stripping and adsorption while estimating the removals.

The technique of using immobilized microorganisms for treatment of hazardous and toxic wastes has been recognized as a promising method [4, 22, 23, 24].

Westmeier and Rehm [25] 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 protected the cells against the high concentrations of 4-chlorophenol and allowed the 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 the 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.

Klein et al. [26] 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.

Chien and Sofer [6] studied the performance of immobilized yeast cell reactors. They studied a number of parameters such as flow rate, yeast growth rate, bead size and type of medium. It was found 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. Different flow rates and bead sizes were used to optimize the productivity of Saccharomyces cerevisiae cells which were immobilized in the calcium alginate gel.

Lodaya [27], of this Laboratory, studied the biodegradation of benzene and again of a BTX mixture using activated sludge immobilized in calcium alginate gel and on celite carrier. He found that the maximum substrate (benzene) utilization rate (K_M) was almost six times higher in case of alginate gel compared to celite carrier. He also reported that the mass transfer resistance for benzene was negligible for the cross sectional velocity of flow above 2.5 cm/min for both immobilization techniques.

The diffusion characteristics of several substrates into and out of calcium alginate gel beads has been studied

in detail by Tanaka et al. [28]. They found that the diffusion coefficients of most of the substrates (with molecular weight less than 20,000) into and from calcium alginate gel beads were the same as that of water systems. These results suggest that these substrates can diffuse easily into and out of calcium alginate gel beads. They also 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.

Earlier in this laboratory, Yang [29] studied the biodegradation of 2-chlorophenol using immobilized mixed culture. He found that higher concentrations (> 150 ppm) of the substrate (2-chlorophenol) had an inhibitory effect on the rate of biodegradation. He also reported that the total removal fits Monod kinetics.

In another study also conducted in this laboratory [30] it was found that microorganisms in immobilized form could withstand almost seven times higher concentrations of 2-chlorophenol, compared to free form. 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.

Other comparative studies of biological and physical removal of organic compounds from activated sludge processes have also been found in the literature [31, 32].

Biodegradation of benzene and related aromatic hydrocarbons has been attempted by Weber et al., [33] among others [34, 35]. They studied removal of nine organic compounds including benzene, toluene and o-xylene in activated sludge treatment systems. Addition of powdered activated charcoal to enhance removal of the above toxic compounds by biodegradation was evaluated. They concluded that though the removal of biodegradable compounds did not change much, significant removals of poorly biodegradable and non-biodegradable compounds were noticed.

CHAPTER IV

MATERIALS AND EXPERIMENTAL METHODS

4.1 MICROORGANISMS

Activated sludge (mixed microbial population) from the Parsippany-Troy Hills (N.J.) Wastewater Treatment Plant was used in this study. The microorganisms were acclimated at room temperature, with styrene (approximately 100 ppm) as the only carbon source, successively over five days (two spikes per day) with continuous aeration. The culture was then centrifuged (International portable refrigerated centrifuge, Model PR-2) at 3000 rpm and 6⁰C with a rotor of 10.5 inch diameter for 5 minutes to obtain concentrated pellets. These pellets were stored at 4⁰C and used to prepare the beads.

4.2 MEASUREMENT OF DRY BIOMASS

A known weight of pellets obtained after centrifugation was dried at 105⁰C for 25 hours in an electric oven (Blue M Electric Company, Model Stabil Therm). The dry weight so obtained was then combusted at 700⁰C in an electric furnace (Hevi-Duty Electric Co., Milwaukee, Wis, USA, Model 051-PT) for about 6 hr to find the amount of ash content in the pellets.

The dry biomass so obtained was expressed as dry biomass per unit weight of pellets. The pellets used, had typically a concentration of 45 mg dry biomass per gram of pellets.

4.3 IMMOBILIZATION

The entrapment of microorganisms 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 along with sodium chloride (0.5% w/w) in a blender. Sodium alginate (1.0% w/w) was then added slowly to the mixture, with continuous stirring to obtain a homogeneous cell suspension, which was then extruded as discrete droplets in a slowly stirred solution of 0.1 M calcium chloride with the help of a syringe pump. 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 solution for at least 24 hours at 4⁰C before use.

4.4 DEFINED NUTRIENT MEDIUM

The composition of the defined nutrient medium used in this study was as follows:

Magnesium chloride	100 mg
Magnesium sulfate	10 mg

Ferric chloride	0.5 mg
Potassium phosphate	10 mg
Water	100 ml

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

4.5 MICROASSAY REACTOR FOR VIABILITY STUDIES

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. This reactor has been a valuable tool in enzyme kinetic studies because the data produced are economical, accurate and reproducible.

The experimental setup is shown in the Figure 1. The microassay reactor consists of a 1.9 ml water jacketed reaction vessel with a small magnetic stirring bar. The concentration of oxygen was monitored by a Clark-type dissolved oxygen probe. Water at the required temperature (25 to 27⁰C) was circulated in the jacket through a water bath (Haake, TYP F-4391). The uniform oxygen concentration was maintained by magnetic stirrer. Sufficient mixing was allowed to overcome any mass transfer resistance. The dissolved oxygen concentration was recorded by a chart

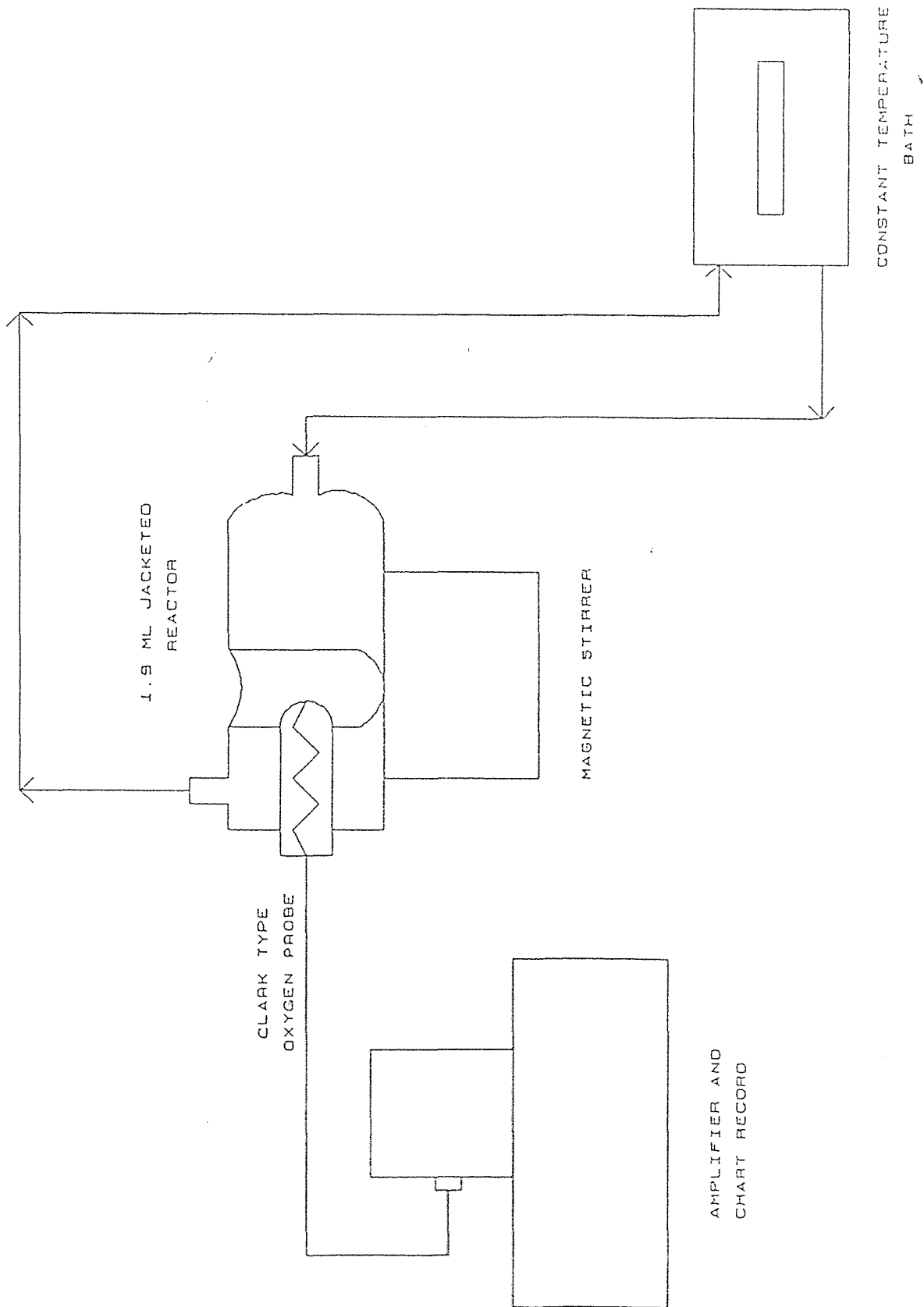


FIGURE 1. EXPERIMENTAL SETUP OF A MICROASSAY REACTOR

recorder assembly (Model RD2030, Omega Engineering Inc., CT, USA.)

Before the start of each run, the reactor was washed successively with methanol and distilled water. It was then rinsed several times with sterile defined medium. Sterile defined medium 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 25⁰C was estimated to be 220 nmole/ml. After saturation with air, four beads weighing a total of 0.04 g (\pm 3.0%) were shocked at 42⁰C 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 at the top, and the concentration of dissolved oxygen monitored on the strip chart recorder.

4.6 SHAKER FLASK EXPERIMENT

Shaker flask tests were conducted for the feasibility study. 50 ppm styrene in 100 ml defined medium along with 2.8 g biomass pellets (free cells) were placed in 250 ml flasks. Control runs were performed without biomass. 0.75 ml of 3% H₂O₂ was added to the reaction mixture and the flasks were put into a shaker bath (Environ Shaker, Model, Lab-Line) under identical conditions at a speed of 150 rpm and 32⁰C. Samples were taken periodically and analyzed on the gas chromatograph for styrene concentration.

4.7 BIODEGRADATION OF STYRENE IN RECIRCULATION REACTOR

4.7-1 Experimental Setup and Procedure

The recirculation reactor used in this study has been described and used in earlier studies [13, 29]. The scale-up of biomass loading from microassay reactor to recirculation reactor was 2500 times (0.04 g wet beads in microassay reactor to 100 g in recirculation reactor).

The experimental setup of the recirculation reactor is shown in Figure 2. The bioreactor was 2.5 inches (6.35 cm) in diameter and 8 inches long (20.3 cm). Thus the total volume of reactor was 640 ml. The reservoir was 4.5 inches (11.4 cm) in diameter and 10 inches (25.4 cm) long. The total reaction volume was 2 liters. The reactor was packed with 100 gm biomass beads on the perforated two stage plastic supports as shown in Figure 2. The reaction mixture was circulated between reservoir and reactor using a centrifugal pump (FMI Lab Pump, Model QD-1, Fluid Engineering inc., Oyster Bay, NY) at a flow rate of 290 ml/min. The reactor contained a thermometer and pH probe (Model 250, Ciba Corning Diagnostic Limited, UK). Liquid samples were taken periodically from the reservoir and analyzed by gas chromatography for styrene. All experiments

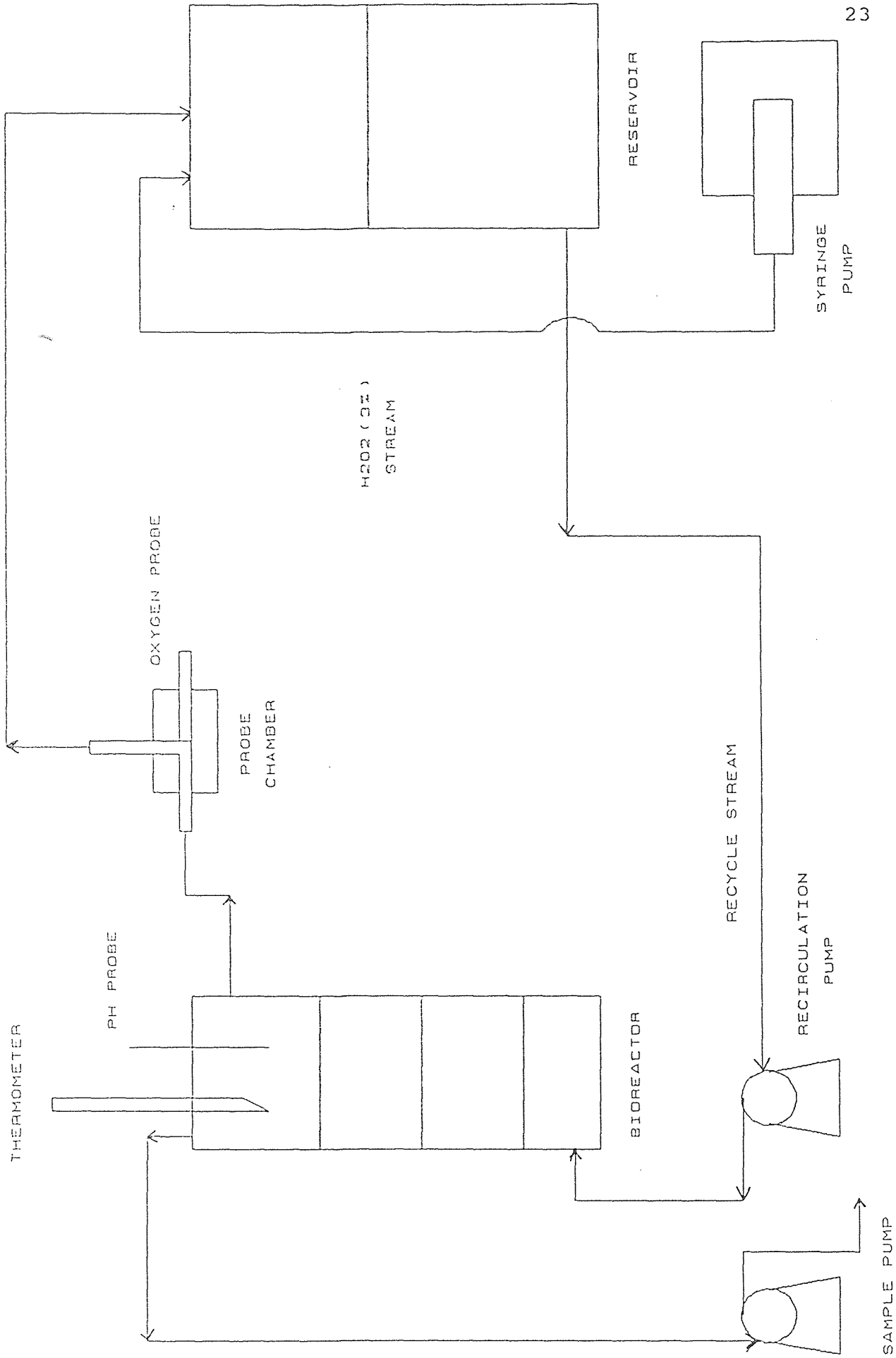


FIGURE 2. RECIRCULATION REACTOR

in the recirculation reactor were done at room temperature (25-27°C)

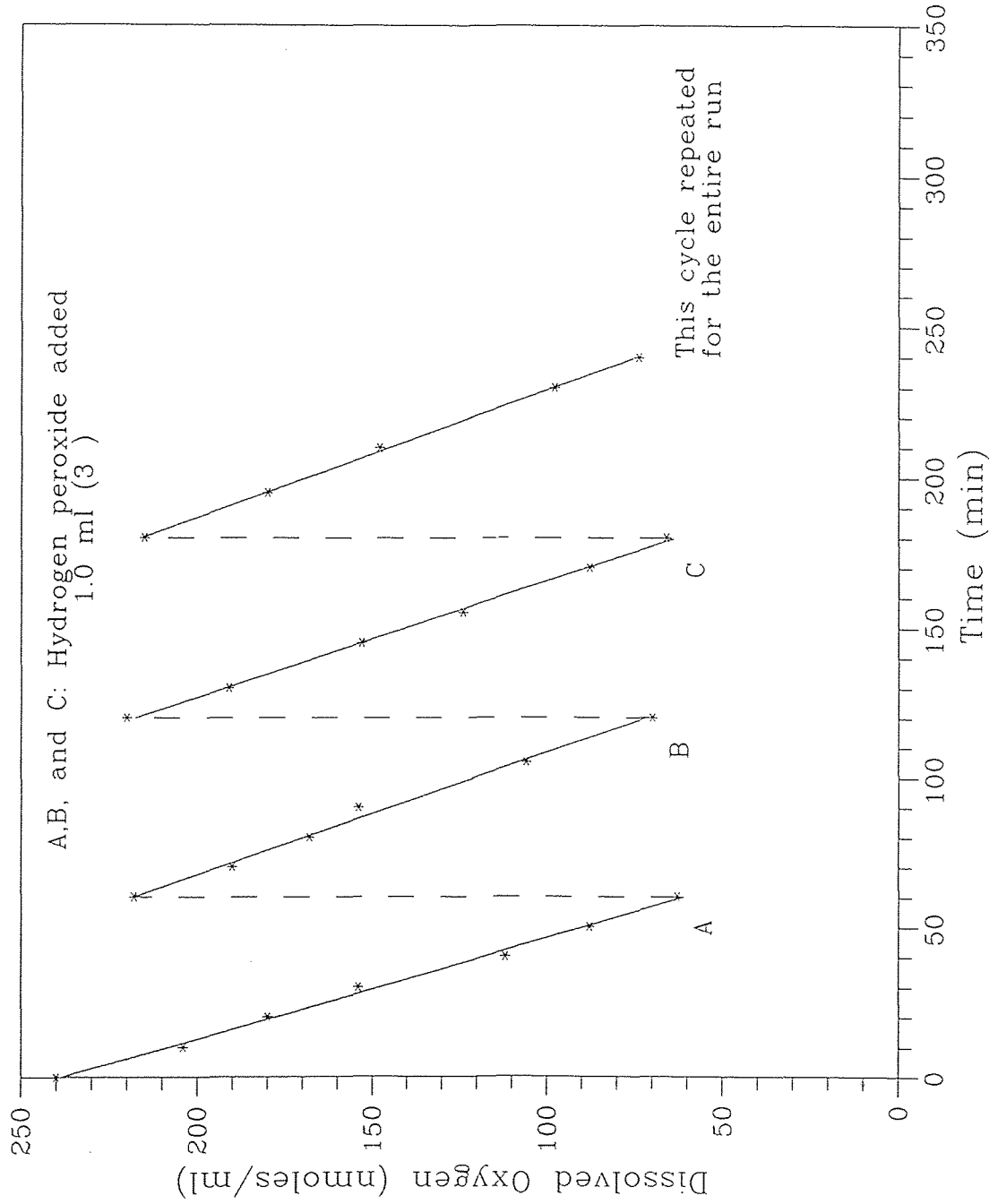
A starting concentration of 50 ppm styrene was used. After it had dropped to below 1 ppm, the system was again spiked with 50 ppm, and so on. Similar runs were done with spiking concentrations of 75 and 100 ppm.

4.7-2 SUPPLY AND MEASUREMENT OF DISSOLVED OXYGEN

Aeration as a means of maintaining the dissolved oxygen (DO) was performed but because of the slow biodegradation rate, stripping proved to be a primary means of styrene removal. So to obtain the system having least stripping effect, H₂O₂ was used as an oxygen source.

Catalase, a terminal respiratory enzyme, is present practically in all aerobic living cells and it breaks down the H₂O₂ into H₂O and O₂ [36]. A small volume of very dilute solution of H₂O₂ (30 g/l) was injected periodically by pump (Model 7013, Cole-Palmer Instrument Co., IL) to provide the dissolved oxygen needed for aerobic biodegradation. It can be seen from Figure 3 that periodic addition of H₂O₂ (1.0 ml/hr) was enough to keep the minimum dissolved oxygen concentration of 2 mg/l in the reactor. Control studies with no biomass shows that this does not result in chemical oxidation of styrene. The oxygen probe was connected through a signal conditioner and amplifier to a chart recorder assembly. Moreover, oxygen concentration in ppm was also

Fig. 3 Periodic Injection of H_2O_2 in Recirculation Reactor with styrene concentration of 50 ppm to Maintain Dissolved Oxygen Concentration

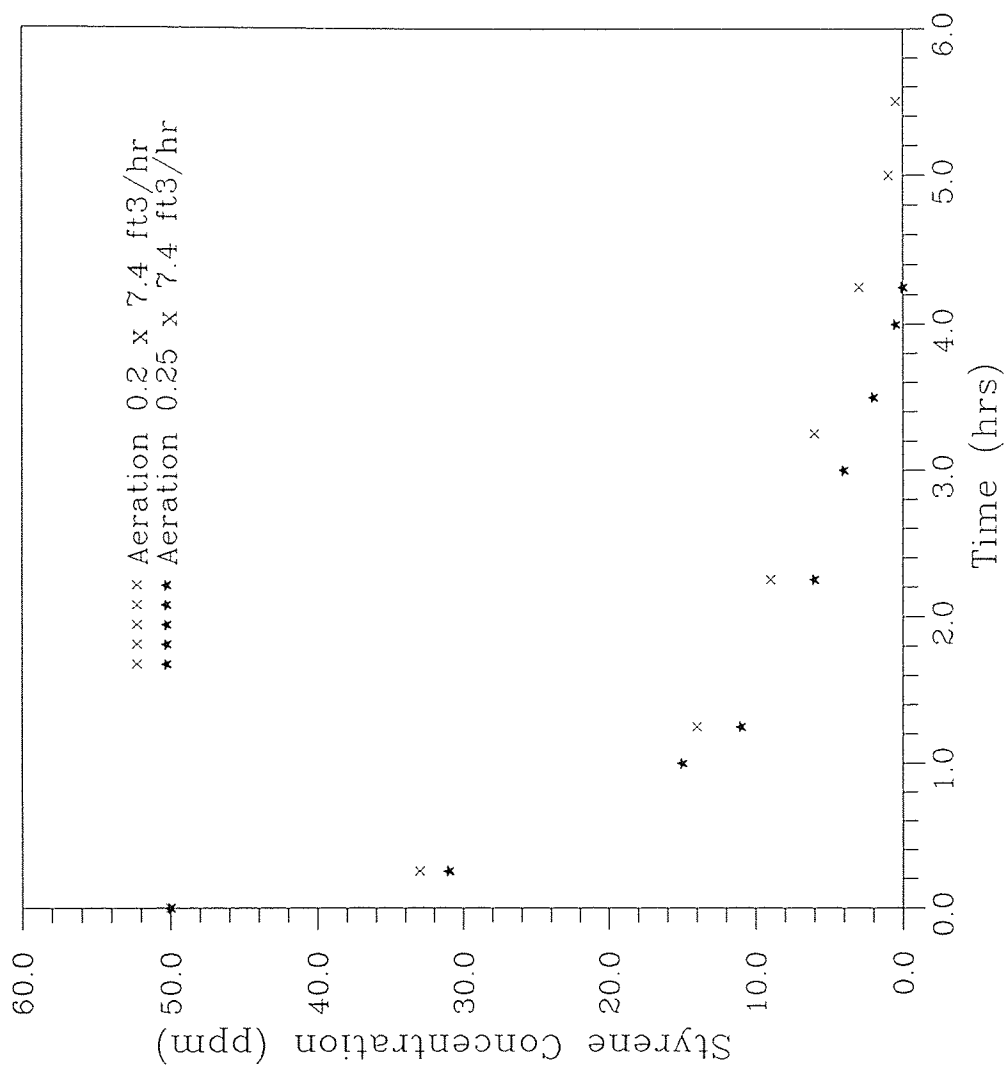


measured along with periodic cycle of H_2O_2 injection, using O_2 electrode (Orion Research, Model 97-08-00). Conditions under which H_2O_2 will oxidize simple ring compounds have been discussed in the literature [37]. Given that a very small quantity of H_2O_2 is added, and the very short half life due to large quantities of the catalase present in the activated sludge, it can be safely assumed that there is no chemical oxidation of styrene.

4.8 MEASUREMENTS FOR PHYSICAL REMOVAL (CONTROL RUN)

Styrene is known to be a volatile compound and has malodorous smell even at very low concentrations (< 1 vpm). Hence aeration of any kind may result physical removal being the primary mode of removal, rather than biological removal. As seen from the Figure 4, simple aeration at low flow rates is enough to remove the styrene completely in five hours. Thus most of the experiments were conducted using H_2O_2 . Control runs were done under the identical conditions of temperature and oxygen supply, either from air or H_2O_2 , but with alginate beads without biomass. The purpose of the control runs is to find out the removal of styrene by abiotic losses, particularly, by stripping.

Fig. 4 Effect of Air Flow Rate on
Physical Removal of Styrene



4.9 METHOD OF ANALYSIS

A Perkin Elmer gas chromatograph (model 8500 series) was used for analysis of styrene. The column used for this purpose was 6 ft x 1/8 inch stainless steel column (SUPELCO) packed with 5% SP-1200 containing 1.75% Bentone-34 on 100/120 Supelcoport^R mesh. For calibration purposes, a known quantity of styrene was added to the defined medium and stirred for one hour in the 500 ml stoppered flask. This solution was then diluted depending on the desired initial concentrations. Above 15 ppm, injection volume was 2 μ l and below the 15 ppm concentration it was 5 μ l.

4.10 RESIDENCE TIME DISTRIBUTION AND FLOW PATTERN STUDY

An experiment using the mixing-cup method [38] was done to study the flow pattern and residence time distribution within the recirculation reactor at a flow rate of 290 ml/min, at which most of the experiments were conducted.

Isoterge^R was injected in the reactor as a tracer and the output of the tracer was collected at known time intervals in different beakers. The collected samples were analyzed on a UV Spectrometer (Bausch and Laumb Inc., Model Spectronic 20D) at a wavelength of 470 nm for the concentration measurement of tracer (Isoterge^R) concentration.

CHAPTER V

RESULTS AND DISCUSSION

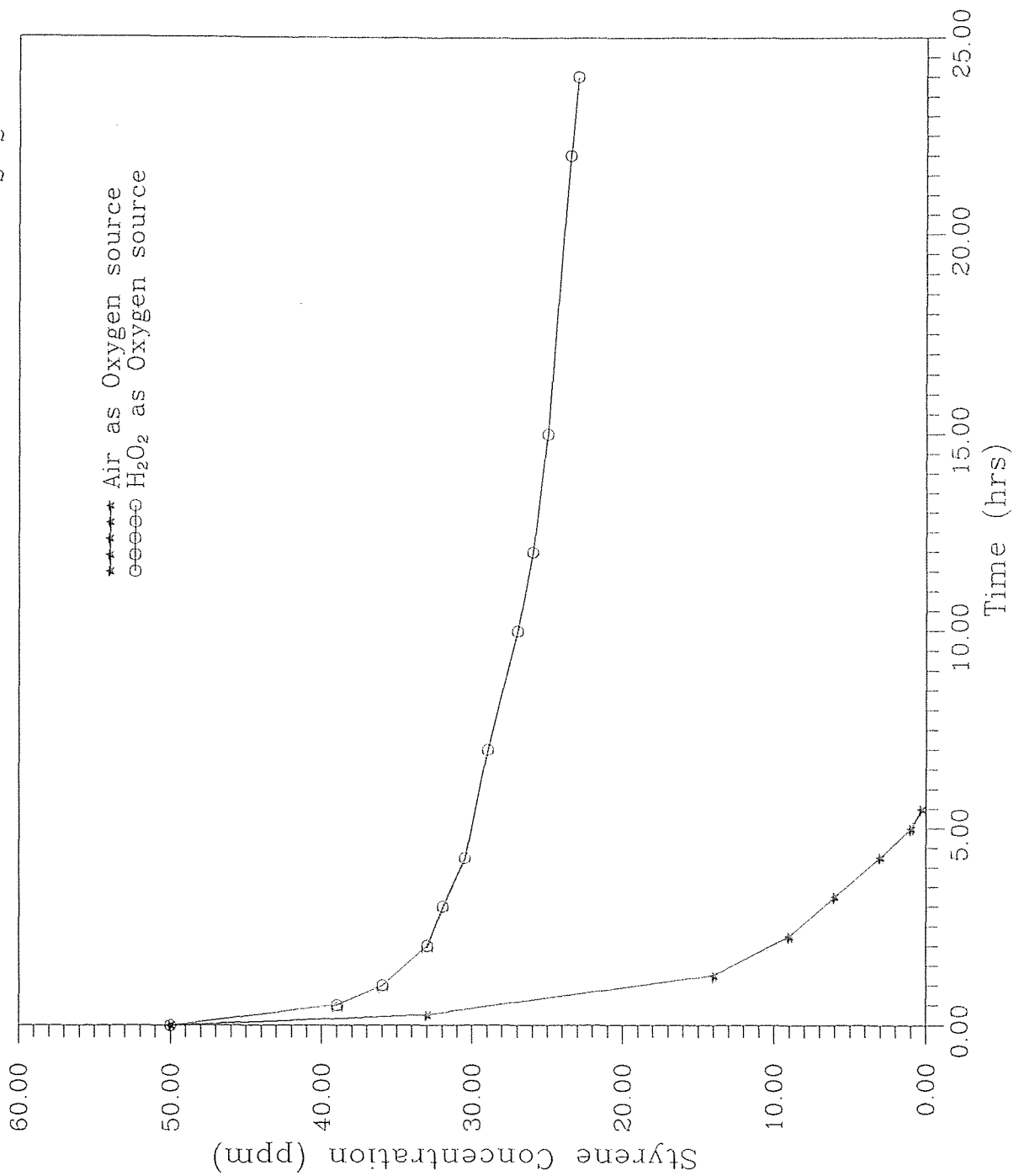
5.1 EFFECT OF MODE OF OXYGEN SUPPLY

In most biodegradation processes of nontoxic, nonvolatile or less volatile organic compounds, aeration is the typical mode of the oxygen supply. Though the styrene is not highly volatile or toxic, its malodorous properties, even at low concentration (<1 vpm), causes problems. Initially biodegradation studies with aeration at different flow rates of air were conducted, which resulted in high evaporation and/or entrainment loss.

5.1-1 AIR VS H₂O₂ AS OXYGEN SOURCE

Use of air as a means of oxygen supply makes the system open (susceptible to physical loss mainly by evaporation of substrate) while use of H₂O₂ provides an essentially closed system. Figure 5 shows the physical removal of styrene for both types of oxygen sources under identical conditions. It is seen that with air supplied at $0.2 \times 7.4 \text{ ft}^3/\text{hr}$, the concentration of styrene is reduced from 50 ppm to less than 1 ppm in 5.5 hours whereas during the same time period, with 1.0 ml/hr of 3% H₂O₂, it is reduced to only 29 ppm. Moreover, after adsorption, physical removal (evaporation)

Fig. 5 Comparison of Physical Removals of Styrene in Recirculation Reactor With Air and H₂O₂



of styrene is very high for air (open system) compared to H_2O_2 (closed system).

5.1-2 BIODEGRADATION OF STYRENE USING AIR AND H_2O_2

One additional possibility with the use of H_2O_2 is the removal of styrene by oxidation reaction. Therefore net biodegradation in both the cases was measured. As seen in Figure 6 for a starting concentration of 50 ppm, the net biodegradation rate is 1.6 ppm/hr for the open system and 1.67 ppm/hr for the closed system. These values are within experimental error.

5.2 EFFECT OF STARTING CONCENTRATION ON STYRENE ADSORPTION

Figure 7 shows the adsorption of styrene with respect to its starting concentration. As seen from the figure, styrene concentration drops about 40 to 55% immediately after the injection of 40, 50, 75 and 100 ppm styrene in the reservoir. Here we can assume that after an equilibrium reaches between the styrene in the bulk and styrene within the beads, the decrease in the styrene concentration is due to biodegradation and other abiotic losses such as stripping and reaction with H_2O_2 .

One can argue that any adsorption taking place is not chemisorption but physical adsorption, because repeated spiking of styrene concentrations were made and every time immediate concentration drop or adsorption was about 40 to

Fig. 6 Biodegradation of Styrene in Recirculation Reactor With Air and H_2O_2

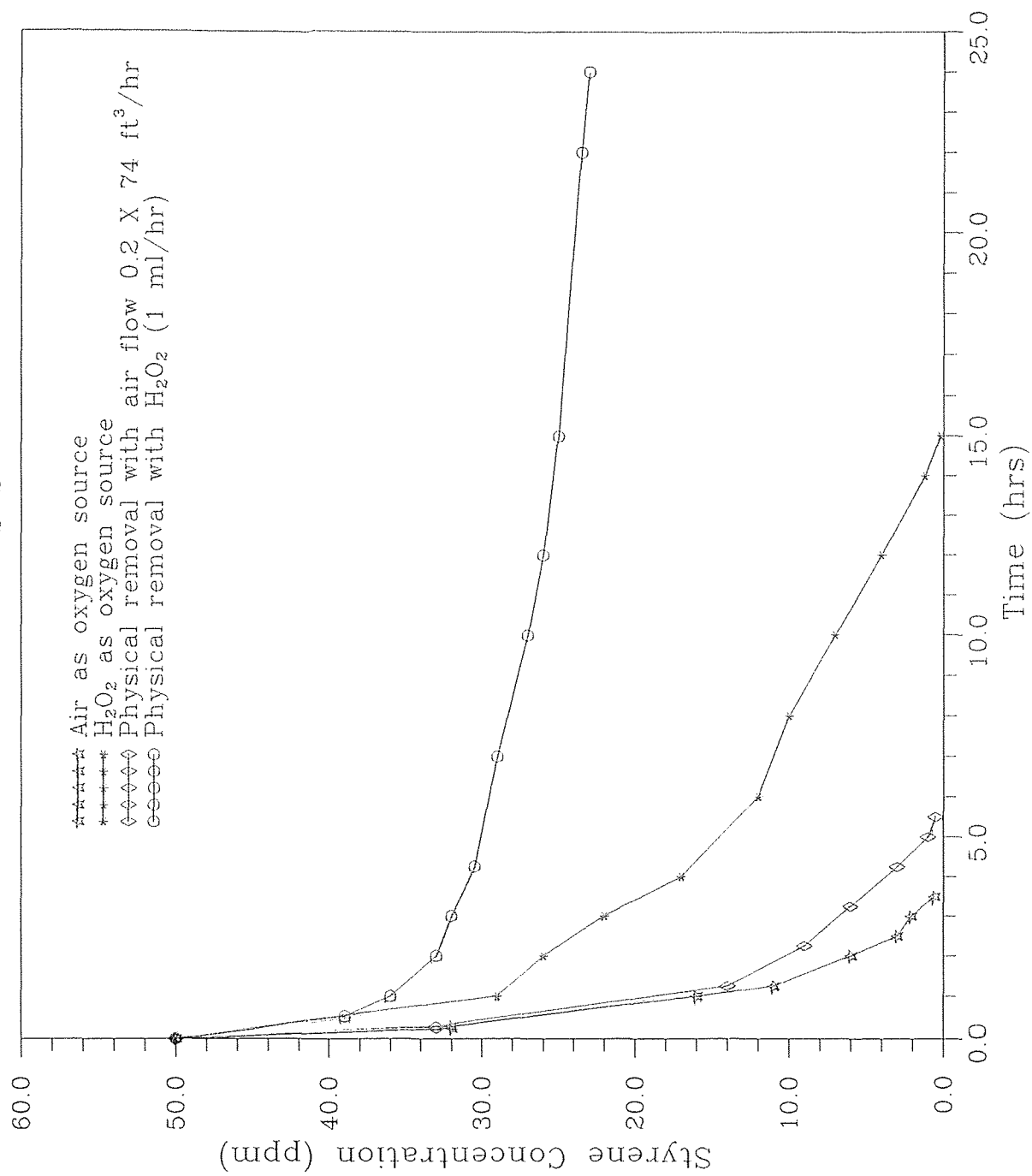
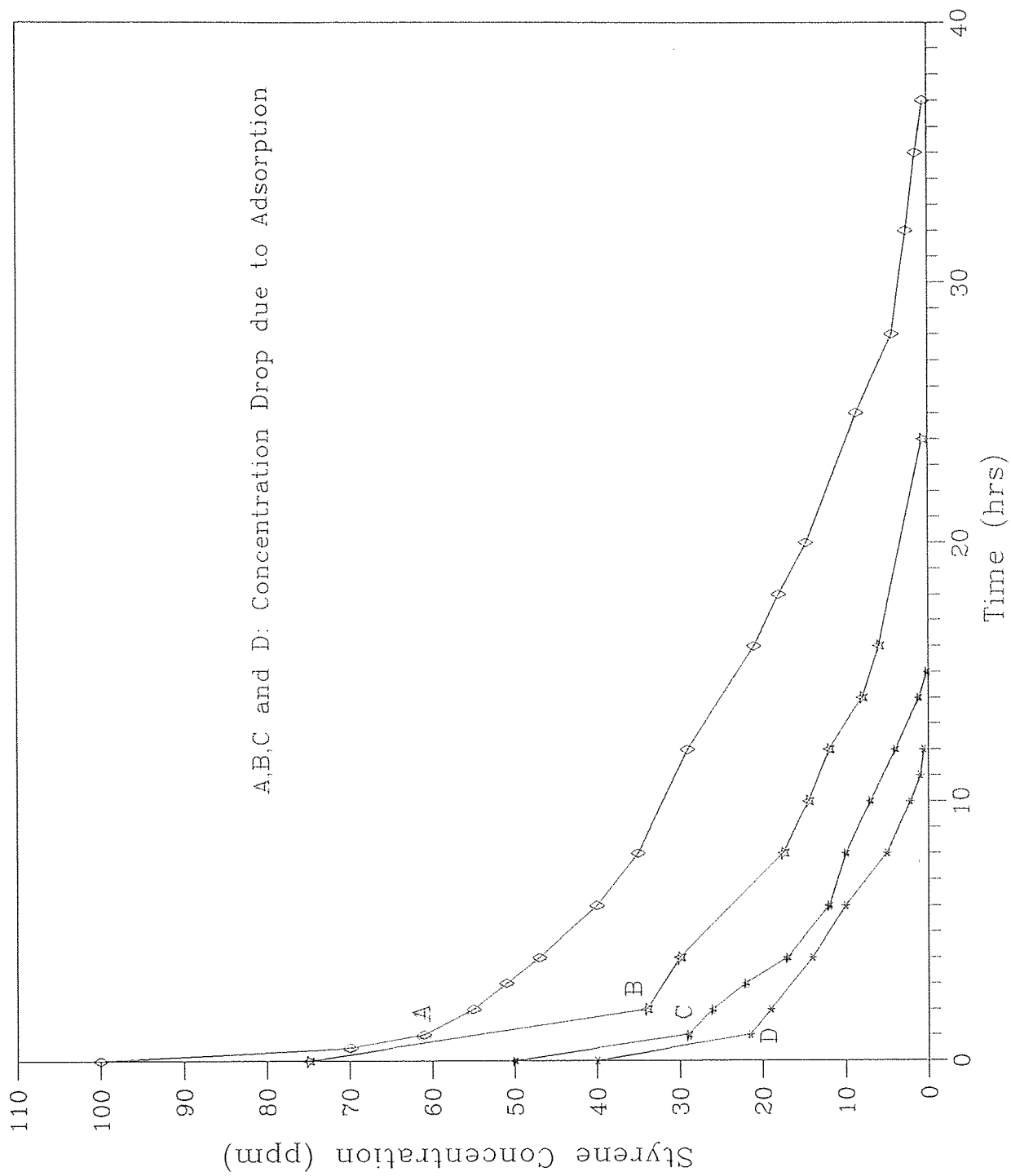


Fig. 7 Effect of Starting Concentration of Styrene on Adsorption



50%. If there had been chemisorption, with every spike of styrene, available active sites on the beads for styrene chemisorption would have decreased as a result immediate percentage drop of styrene concentration would be less. But that did not happen during entire run which confirms the absence of chemisorption.

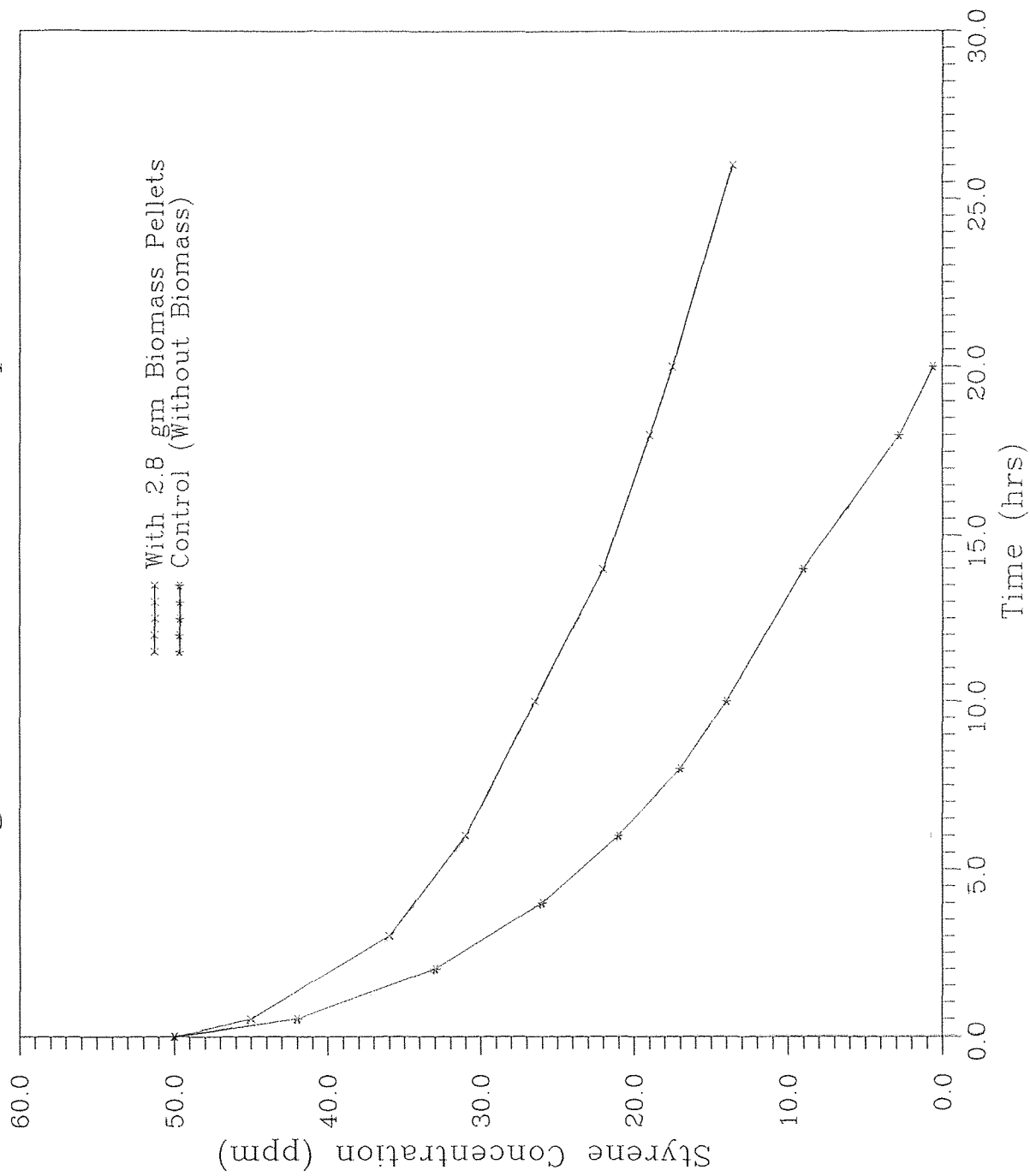
5.3 SHAKER FLASK TESTS (FREE CELLS)

Shaker flask test results are plotted in Fig. 8, which indicates no significant adsorption of styrene when mixed culture is used in the free form. Moreover, here physical removal by evaporation is also higher compared to the immobilized form in recirculation reactor. Higher physical removal may be because of higher bulk concentration of styrene in the absence of adsorption. The average biodegradation rate of styrene observed was 0.85 ppm/hr. The lower average biodegradation rate may be due to a lower dissolved oxygen level. These low levels are due to only one injection of H_2O_2 (0.75 ml) given in the beginning to saturate the mixture with dissolved oxygen.

5.4 PHYSICAL REMOVAL OF STYRENE IN RECIRCULATION REACTOR

The physical removal (particularly stripping) of styrene during the control run (without biomass) showed that after initial adsorption styrene was removed physically by neither first order nor second order at room temperature and

Fig. 8 Shaker Flask Experiment



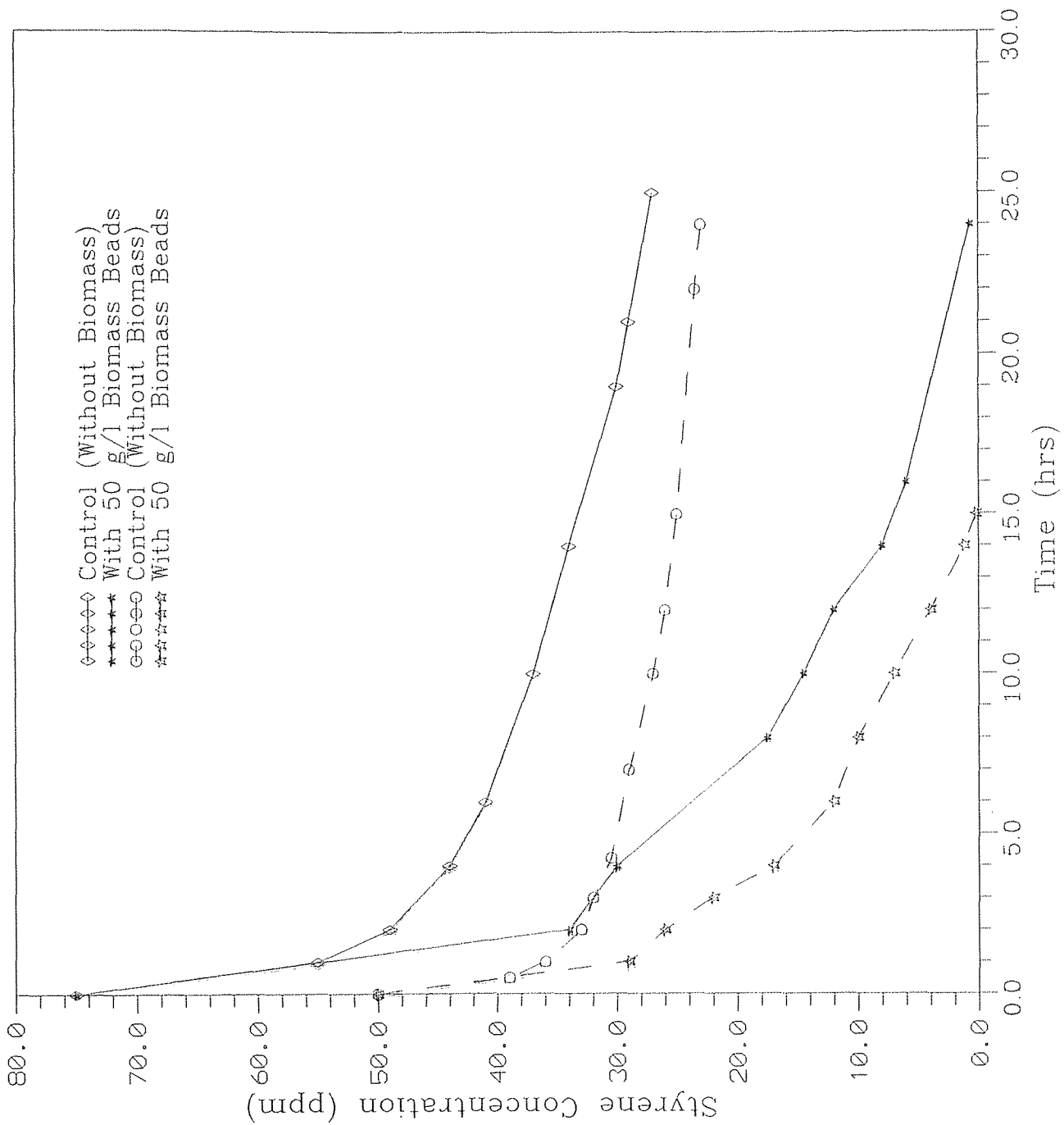
with H₂O₂ supply of 1.0 ml/hr. This is comprehensible since all the styrene in the reactor does not remain in the bulk but some of it remains in equilibrium with styrene on the bead surface that makes the physical removal mechanism somewhat complex, and physical removal rate is slow. A comparison of physical removal and total removal of styrene at 50 ppm and 75 ppm is shown in the Figure 9. Starting from a concentration of 75 ppm, up to 55% of the styrene was adsorbed on the alginate beads within 2 hours, though this concentration drop also includes the dilution because of the beads' volume in the reactor. The approximate volume of the 100 gm beads was 165 ml, compared to a total liquid volume of 2000 ml. After an equilibrium is reached between the styrene in the bulk and styrene within the bead, a comparison of two removal rates shows that about 32% styrene is lost by abiotic means (evaporation and reaction with hydrogen peroxide), and the remaining 68% is degraded biologically. Similarly for 50 ppm styrene up to 42% initial drop in concentration, 72% styrene degraded biologically. The remaining 28% are abiotic losses.

5.5 BIOLOGICAL REMOVAL OF STYRENE IN RECIRCULATION REACTOR

5.5-1 EFFECT OF FLOW RATE ON RATE OF BIODEGRADATION

The flow rate (recirculation rate) is an important parameter for the recirculation reactor. It has a dual

Fig. 9 A Comparison of Physical and Total Removal of Styrene in Recirculation Reactor



effect on the system. At low flow rates, the system may be under the limitation of external mass transfer, whereas at very high flow rates, channeling causes inappropriate use of reactor volume and the bead structure can also be damaged. Therefore, optimization of flow rate is necessary. Table 1 shows that above the flow rate of 75 ml/l there is no apparent effect of external mass transfer on the rate of biodegradation.

In the present study, most of the experiments were conducted at a cross sectional velocity of 9.2 cm/min (flow rate 290 ml/min) at which beads remained stable for about 40-45 days.

5.5-2 EFFECT OF BIOMASS LOADING ON THE RATE OF BIODEGRADATION

The effect of biomass loading on the rate of biodegradation has been studied with different biomass loading and concentration of styrene. The results are tabulated in Table 2. The values of specific apparent rates from Table 2 suggest that with an increase in biomass loading, the apparent rate of biodegradation does not increase proportionately. In the range of 50 to 100 gram biobeads the specific apparent rate is higher. 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 insufficient use of the biomass,

TABLE : 2 EFFECT OF BIOMASS LOADING AND SPIKING
CONCENTRATION ON BIODEGRADATION RATE

Reactor volume: 2 l
Flow rate : 290 ml/min
1 g wet bead = 11.25 mg dry biomass

Biobeads loading (wet beads) (g)	Spiking concentration of Styrene (ppm)	Apparent K_M (ppm/hr)	Specific Apparent K_M (ppm/hr/g dry biomass)
50	50	2.27	4.04×10^{-3}
100	50	3.33	2.96×10^{-3}
	75	3.10	2.75×10^{-3}
200	50	5.00	2.22×10^{-3}
	75	4.69	2.08×10^{-3}

particularly at the upper level due to lack of dissolved oxygen.

5.5-3 EFFECT OF SPIKING CONCENTRATIONS OF STYRENE ON THE BIODEGRADATION RATE

Spiking concentrations of 40, 50, 75 and 100 ppm were studied with the 50 g/l biomass beads. Results are shown in Figure 10. It is clear from the figure that the apparent biodegradation rate is almost the same, 3.33 ppm/hr, for spiking concentrations of 40 and 50 ppm. With 75 ppm and 100 ppm spiking concentration, biodegradation rate drops to 6.5 and 19% respectively compared to biodegradation rate for 40 and 50 ppm. This clearly indicates an inhibitory effect at higher concentration of styrene.

5.6 EFFECT OF STYRENE CONCENTRATION AND MEDIUM ON OXYGEN UPTAKE IN MICROASSAY REACTOR

Using a microassay reactor, oxygen uptake with respect to styrene concentration was measured. In this case biomass used was in free form. The results are shown in the Table 3. It is clear that highest oxygen uptake is 3.70 nmole/ml min when styrene concentration is 150 ppm. Without the styrene (endogenous respiration), oxygen uptake is 2.15 nmole/ml min. Oxygen uptake at constant biomass is directly related to rate of biodegradation. In the free form, biodegradation is highest when styrene concentration is 150 ppm.

Fig. 10 Effect of Spiking Concentration on Biodegradation of Styrene

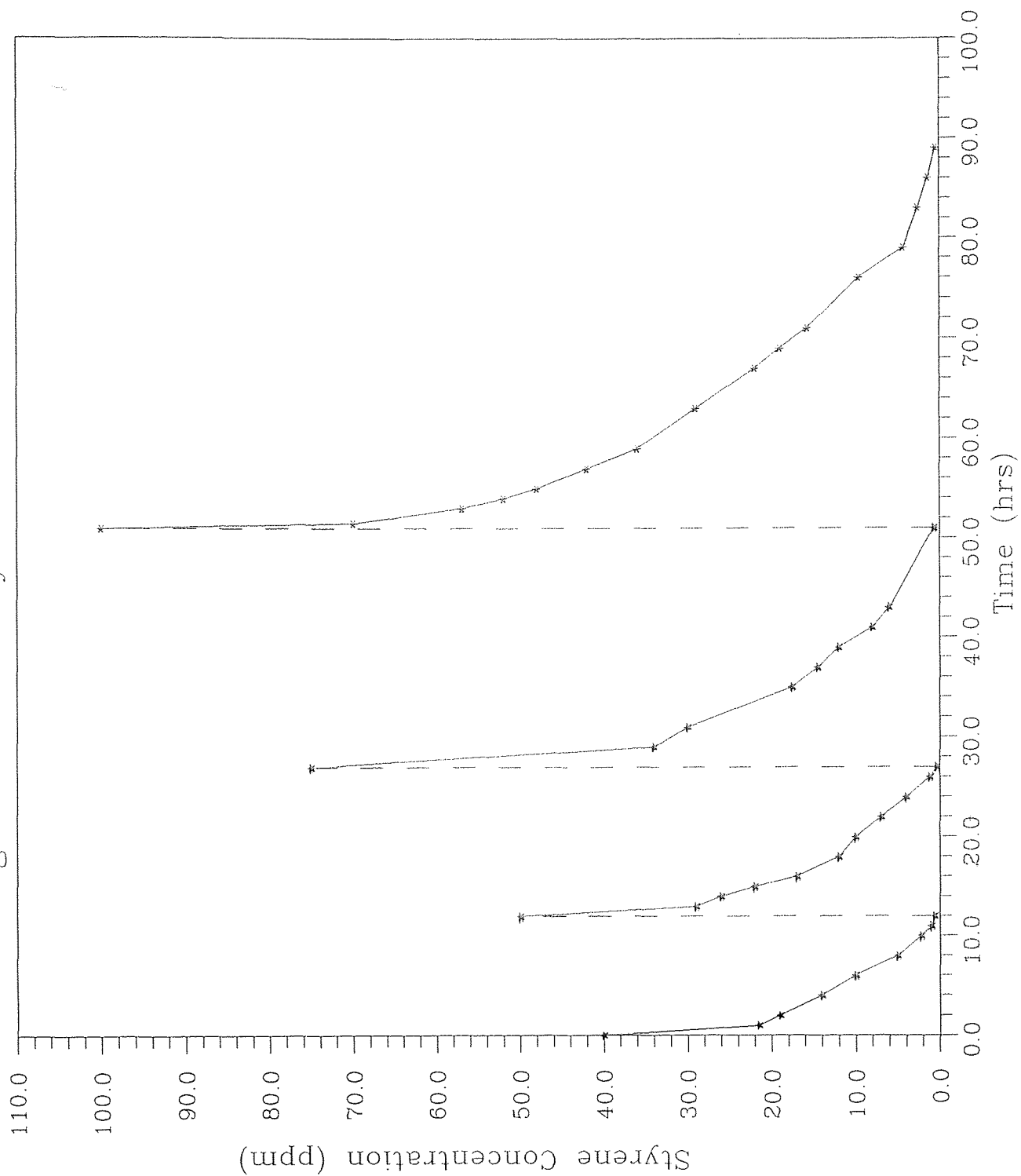


TABLE : 3 EFFECT OF STYRENE CONCENTRATION ON RATE
OF OXYGEN UPTAKE IN MICROASSAY REACTOR

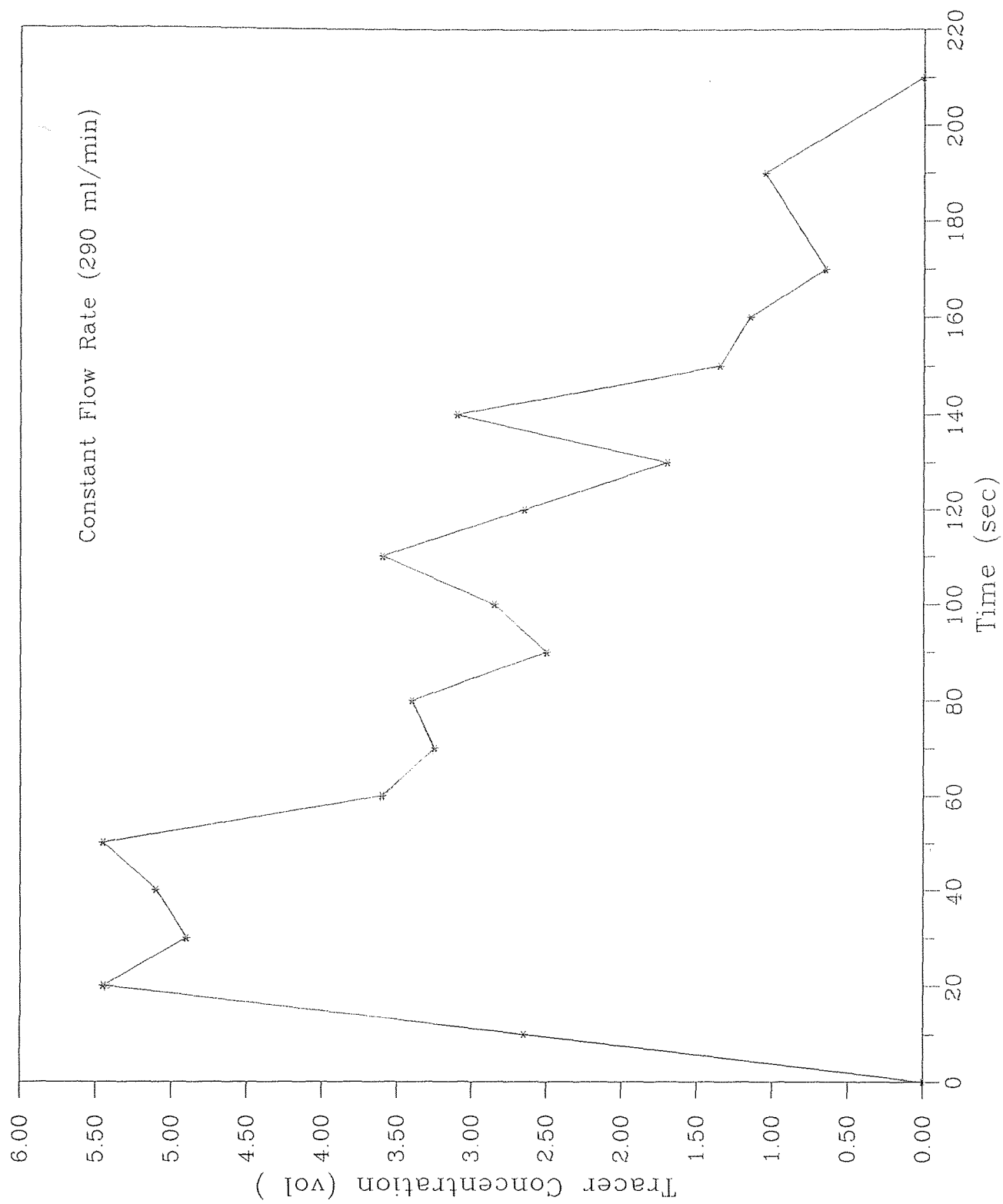
Styrene Concentration (ppm)	Rate of Oxygen Uptake (nmole/ml min)
300	2.68
150	3.70
75	2.47
0 (without styrene)	2.15

During another set of experiments it was also observed that with a styrene concentration of 150 ppm in distilled water, the rate of oxygen consumption was 2.6 nmole/ml min whereas for defined medium it was 3.75 nmole/ml min.

5.7 FLOW PATTERN WITHIN THE REACTOR

Figure 11 shows the stimulus response of an impulse (delta function) [38] input. From the graph it is clear that flow patterns within the reactor involve back-mixing. Generally back-mixing is not desirable, particularly for fast continuous reactions. But in this case, the biodegradation of styrene is very slow compared to the average residence time and the reactor is in recirculation mode. Moreover, we have to optimize with the linear velocity to overcome any diffusional resistance and the bead stability. The mean residence time of the fluid in the reactor is 99 seconds. Back mixing is therefore not a primary concern.

Fig. 11 Flow Pattern Within the Reactor



CHAPTER VI

CONCLUSIONS

The results of the experiments indicate that the use of calcium alginate immobilized activated sludge has potential to become a promising technology in hazardous waste treatment. The major advantage of this technique is that there is essentially no cell washout and no sophisticated separation technique is necessary to recover cells for reuse.

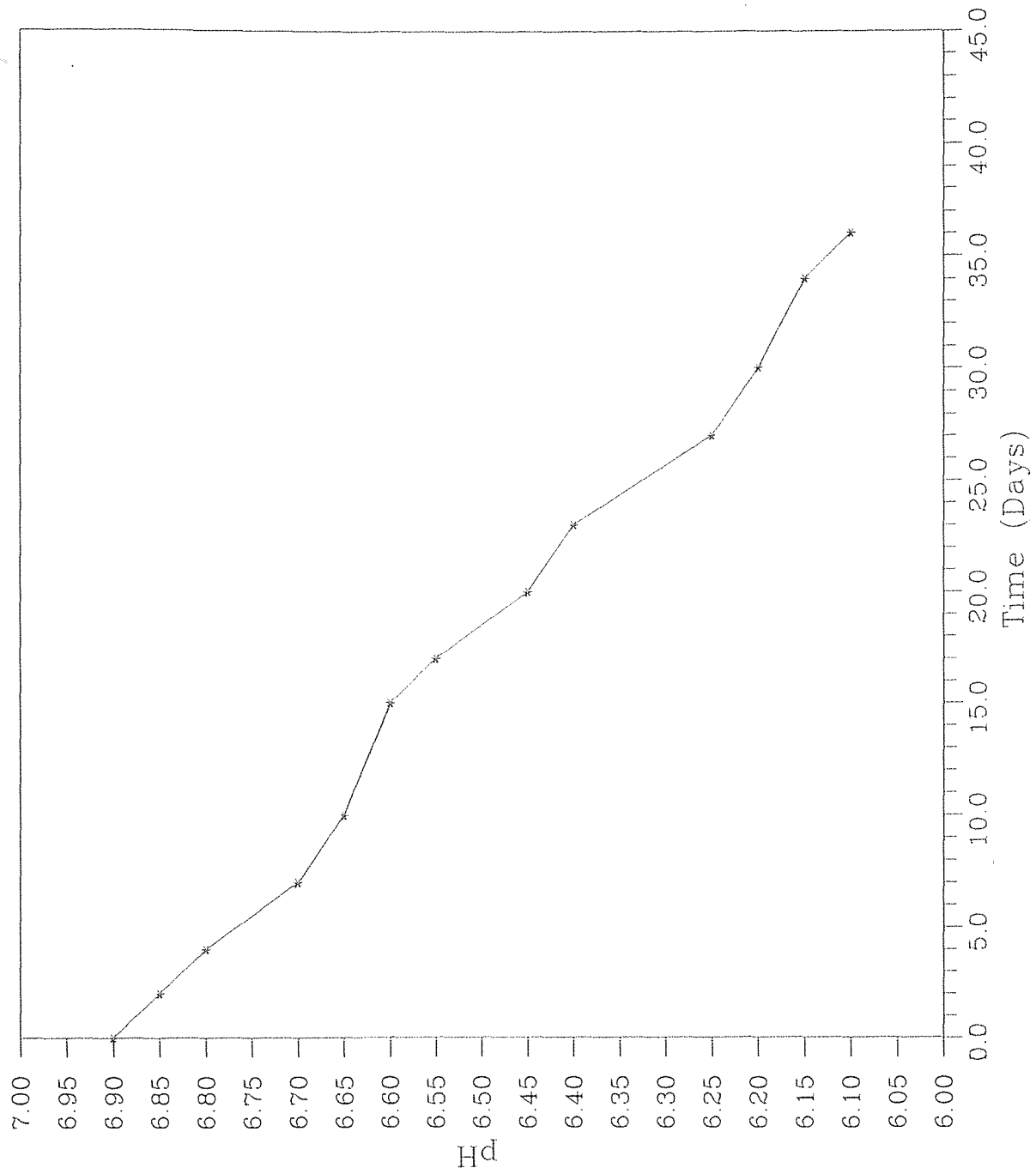
With H_2O_2 it is possible to have biological removal of styrene as the primary mode of removal, as opposed to stripping in case of air bubbling

In the range of flow rates studied, the rate of biodegradation was not affected much above the flow rate of 100 ml/min. This means above flow rates of 100 ml/min, there is no significant external mass transfer limitations for those conditions.

With 50 g/l biomass beads and a recirculation rate of 290 ml/min, after initial adsorption, a maximum biodegradation rate of 2.4 ppm/hr was achieved, with 40 and 50 ppm of styrene concentration. High localized cell density gives high activity for a given volume.

The decrease in the pH value with time (Figure 12) of the reaction mixture suggests that the accumulation of biodegradation products such as carbon dioxide.

Fig. 12 pH Record in Recirculation Reactor
for Styrene Biodegradation



A tracer stimulus-response curve indicates the flow within the reactor is highly back-mixed.

CHAPTER VII

SUGGESTIONS FOR FURTHER RESEARCH

The work presented here was conducted on a laboratory scale to assess the feasibility for the use of immobilized mixed microbial population in hazardous waste treatment. The next logical step would be scaling it up to an intermediate scale before a possible pilot scale demonstration.

The waste stream treated was made up in the laboratory. For the technique to be of any practical importance, a treatment of a real waste stream containing styrene and similar aromatics would be necessary.

The influence of the adsorption of styrene on the beads should be studied in detail and adsorption equilibria should be evaluated. Taking the adsorption effect into the account, there is a need for developing a biokinetic model that would enable us to predict any inhibitory effect due to adsorption and hence to change in process parameters accordingly to nullify that effect.

The development of a process control scheme whereby the oxygen consumption by the microorganisms can be monitored and supplied as needed is desirable.

For the feasibility of the process in the real world, a continuous flow system needs to be investigated.

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