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A combined gel entrapped and membrane attached microbial process for biodegrading organic compounds

New Jersey Institute of Technology, 1991
A COMBINED GEL-ENTRAPPED AND MEMBRANE-ATTACHED MICROBIAL PROCESS FOR BIODEGRADING ORGANIC COMPOUNDS

BY

FAYAZ LAKHWALA

Dissertation 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 Doctor of Engineering Science

1991
Title of Dissertation: A Combined Gel-Entrapped and Membrane-Attached Microbial Process for Biodegrading Organic Compounds

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ABSTRACT

A comparative study has been performed between two bioreactors treating phenol, one using calcium alginate entrapped microorganisms, and the other using microorganisms attached on the surface of a polymeric membrane. Laboratory experiments conducted in batch recirculation mode, as well as a continuous feed mode, showed that the calcium alginate bio-bead reactor is more efficient in treating high feed concentrations of phenol, while the membrane bioreactor is more effective in the lower concentration regime (less than 150 ppm). In batch recirculation mode, the reaction rates (expressed as mg phenol/hr*gm dry cells) in the membrane reactor are an order of magnitude higher at feed concentrations of phenol below 100 ppm. Biodegradation rates for the two reactors match at around 250 ppm phenol concentration, and at about 1000 ppm phenol concentration the reaction rates in the calcium alginate bioreactor are 7.5 times the corresponding rates in the membrane reactor.

In continuous feed mode the alginate reactor requires 5.5 times more volume than the membrane bioreactor for close
to 90% degradation of a feed stream containing 50 ppm phenol. At 1000 ppm feed concentration of phenol, the membrane reactor requires 3.4 times larger volume than the alginate bioreactor for the same degradation.

Due to substrate inhibition, and an inability to sustain a high biomass concentration, the membrane reactor shows poor efficiencies at high phenol concentrations. At low phenol concentrations, the apparent reaction rates in the alginate bioreactor decrease due to diffusional resistance of the gel matrix, while reaction rates in the membrane bioreactor remain high from essentially no internal diffusional resistance.

At high phenol concentrations (above 250 ppm), the degradation rates in the alginate reactor show strong dependence on dissolved oxygen levels, indicating a DO limited operating regime. The degradation rates in the membrane bioreactor also show oxygen dependency, not due to intrinsic kinetics, but more due to the transport effects.

Results indicate that a combined reactor system (employing both reactor types) can be more effective over a wide range of phenol concentrations than those where only entrapped or attached microbial reactors are used for bioremediation.
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Among my family I thank my parents for standing by me throughout my long academic career with sacrifices and
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1.1 Bioreactors in Hazardous Waste Treatment

Biological treatment of organic wastes is a technology which originated in older times primarily to treat domestic wastes. But, as it is true with any other technology, this field also needs to be developed for the present day needs and demands. Lately, the thrust in research has been to extend this technology to treat highly toxic, and much more complex industrial wastes. Biological treatment of numerous organic compounds has been realized in the last two decades but not many processes other than the conventional activated sludge treatment have been designed and implemented on a large scale for specific applications. Due to the complexity of the waste stream, the design and development of high efficiency bioreactors to treat industrial wastes has been difficult as well as challenging [1].

The kinetics of most biological processes can be described by the Monod model which states that at higher substrate concentrations, the reaction rate is maximum (zero order), while at a lower substrate concentration the reaction rate becomes slow (first order) [2]. Processes treating organic wastes require that the concentration of organic compounds in the effluent be low due to strict
environmental regulations. To treat a highly concentrated organic waste stream down to such a low concentration with one reactor system requires long reaction and residence times, large reactor volumes, and is hence not economical. Primarily because of changing reaction rates, a combination of two or more reactors may be much more favorable. This combination may either be of the same reactors operated in different configurations (PFR, CSTR etc.) or in the case of reactors using immobilized microbes, the biomass may be immobilized in different ways in a series of reactors, or in a system of reactors using different biocatalyst altogether.

Technologies and concepts developed in the field of chemical reactor engineering can be applied for the design and control of these reactors. But, when dealing with bioreactors, theories and practice have to be adapted to the peculiarity of the biocatalyst.

1.2 Immobilization: Types and Advantages

One of the major changes in bioreactor designs in the last two decades has been the incorporation of immobilization techniques. Using immobilized microorganisms is more advantageous than using free microorganisms for numerous reasons [3-5]. The recovery and subsequent reuse of biomass is easily accomplished due to immobilization. Choice
of different operating modes for reactors is possible and most important, upon immobilization there may be a desirable change in biological and chemical activity of the biomass [6].

Several immobilization techniques of cells on the surfaces of solid inert supports, and cells inside semi-permeable polymeric membranes are in use. In general, enzymes and microbes can be immobilized in two ways. One is by entrapment in a gel or polymer matrix (like alginate, carageenan, and polyurethane) which is permeable to the nutrients, oxygen, and the substrates. A second is by attachment on the surface of inert supports such as diatomaceous earth, glass beads, and polymeric membranes [7-9].

Both methods have their advantages and disadvantages. When the biomass is entrapped, the external matrix protects it from high concentrations of organic compounds which could be inhibitory. The disadvantage is that in the region of low substrate concentration, the reaction rates decrease due to added resistance to mass transfer.

When biomass is attached on the external surface of a support, it is in direct contact with the bulk stream concentration of the substrate provided the biomass does not create a protective slimy coat. Such a system is unsuitable for treating high concentrations of an inhibitory compound.
But in the low concentration regime this system can provide better degradation rates due to the absence of external mass transfer resistance.

A big disadvantage for systems using entrapped biomass is that the biomass grows, and eventually breaks open the matrix. This can be controlled by removing the fixed nitrogen from the stream that is being treated by some physical or chemical pre-treatment. By doing so the biomass growth will be minimized, but not eliminated, and this may also reduce the reaction rates.

When the biomass is attached on the external surface, the biofilm growth is self regulated. The biofilm increases to a point beyond which it cannot sustain additional growth. This is due to insufficient diffusive transport of the substrate for the maintenance energy of the bacteria. The excess biomass is also removed continuously through a process called "sloughing". Consequently the bioreactor will never collapse due to excessive growth as in the earlier case, and can be operated over a much longer time.

1.3 Operating Windows

Efficient bioreactor designs must achieve optimal interactions between the cells and the culture media by providing good heat and mass transfer between the different
phases. As a result of poor or inefficient heat and mass transfer, a reactor can be operated within many operating windows.

For a bioreactor treating organic wastes under aerobic conditions, three important parameters which govern the intrinsic kinetics are the concentration of dissolved oxygen (DO), the biomass concentration, and the substrate concentration. Figure 1.1 shows conceptually, the dependence of dissolved oxygen and substrate concentration on the reaction rate assuming that biomass concentration is high. For a concentration of 50 ppm organic substrate, the reaction rate increases as the concentration of DO is increased initially. This region corresponds to an operating regime limited by DO. In such a situation a kinetic model using organic substrate alone as a rate limiting parameter cannot be used, and will not represent the true kinetics. After a certain point, the reaction rate maximizes and is no more influenced by the DO. At this stage the reaction rate is limited by substrate concentration, which if increased from 100 to 250 ppm for example, will result in higher reaction rates.

A similar relationship exists between the biomass concentration and DO when a non-inhibitory substrate is present in excess. As seen in Figure 1.2, an initial increase in biomass concentration increases the reaction
rates indicating a biomass limited operating window. The reaction rates do not increase after a point, where the system becomes DO limited. At this point the reaction rates can be enhanced by increasing the concentration of DO. If the substrate is in excess and DO is limiting, a change in DO source from air to pure oxygen should increase the reaction rates by five times.

In all of the above cases, the rate limiting compound may be either present in small quantities or its availability at the reaction site may be limited by external transport effects and/or internal diffusional effects. The availability of both nutrients and the oxygen can be made possible by proper design of the biocatalyst and optimal design and operation of the bioreactor. In general, the modeling and subsequent scale-up of bioreactors has been more complex due to poor understanding of the rate limiting steps which keep changing as the micro-environment near the biocatalyst changes.

1.4 External Transport Effects

External transport effects depend strongly on linear velocity or the superficial mass velocity of the feed to the reactor. This parameter controls the rate of transfer of nutrients from the bulk phase to the surface of the
biocatalyst. Depending on the type of substrate and its bulk concentration, the linear velocity can be varied to operate under a high mass transfer resisting environment (for high substrate concentrations) or a low mass transfer resisting environment (for lower concentrations of substrate).

For a system operating under the limitation of external mass transfer, an increase in linear velocity will increase the substrate concentration on the surface of the biocatalyst, and this in turn will increase the reaction rates (Figure 1.3, curve A, 50 ppm). Here it is assumed that internal diffusional effects within the pellet are minimal. The rate will increase up to a point where the solid-liquid film resistance around the bead is completely eliminated, and a maximum rate is obtained. At this stage the reaction is kinetically controlled if internal resistance is negligible, and further increases in reaction rates can be obtained by increasing the bulk concentration of DO or concentration of a non-inhibitory substrate (Figure 1.3, curve B, 100 ppm).

In the case of an inhibitory compound like monochlorophenol, a high bulk concentration combined with high linear velocity will increase the substrate concentration at the biocatalyst surface to a point where inhibition may come into play [10]. This will decrease the reaction rates as seen in Figure 1.3, curve C (500 ppm).
1.5 Internal Diffusional Effects

Assuming that the external transport resistance has been overcome, transport inside the biocatalyst may still be a rate limiting factor. In general conventional biological processes treating organic wastes are slow processes, and are not limited by external and internal transport effects. But, when the biomass is immobilized the local cell density increases by more than an order of magnitude. Consequently the internal transport effects become important in reactor design. The substrates and the nutrients have to diffuse across the external matrix, and into the biofilm. This depends on the characteristics of the substrate, the physical and chemical properties of external matrix, and the biofilm thickness.

The physical parameter critical here is the biofilm thickness. In the case where the biomass is entrapped inside a bead, the biofilm thickness can be changed by varying the bead diameter. The diffusion path across the biofilm for the substrates and the nutrients can be reduced, and the reaction rates can be increased as seen in Figure 1.4. Finally at a critical biofilm thickness internal diffusion is not a limiting factor, and the reaction becomes kinetically controlled.

Other factors which affect internal transport are the porosity of the external matrix, and the diffusivity of the
substrate in the gel. The choice of gel matrix depends on the molecular weight, and other physical and chemical properties of the substrate.

1.6 Biofilm Reactors

Many biofilm reactors and biofilm models have been studied to understand the kinetics of substrate utilization, and biofilm development. Most of these reactors use inert inorganic supports like silica, activated carbon, and diatomaceous earth to grow the biofilm on the surface. These biocatalysts are then used in conventional-packed bed or fluidized-bed reactors for biodegradation.

In fluidized-bed configuration the shear rates are high, and significantly effect the biofilm development. This has been one of the factors which has been overlooked in developing models for steady-state biofilms. Moreover, the rate of biofilm loss due to shear increases as the total mass increases, and is also a function of particle size and density. In packed-bed configuration the losses are significant but not as high as in fluidized-bed configuration due to low linear velocities.

In view of the above limitations a design which will provide tangential flow of medium across the biofilm will help reduce the losses due to shear. A spiral cross section for flow like the one used in this study will reduce the
loss and improve reactor performance.

1.7 Design Considerations

The performance of immobilized cell bioreactors depends not only on the relevant microbial or enzymatic kinetics, but also on the physical process parameters. The reaction kinetics depend on parameters like biomass concentration, DO, and substrate concentration. These parameters, among others, control the inherent kinetics, and have been investigated in this research.

Even though the reaction kinetics are maximized/optimized, the process efficiency is dictated by choice of appropriate reactor configurations, and residence times. These are the other parameters which have been investigated.
FIG. 1.1 CONCEPTUAL OPERATING WINDOW: REACTION RATE AS A FUNCTION OF SUBSTRATE AND DISSOLVED OXYGEN CONCENTRATIONS
FIG. 1.2 CONCEPTUAL OPERATING WINDOW: REACTION RATE AS A FUNCTION OF BIOMASS AND DISSOLVED OXYGEN CONCENTRATIONS

![Diagram showing reaction rate as a function of biomass and dissolved oxygen concentrations.](image)
FIG 1.3 CONCEPTUAL OPERATING WINDOW:
EFFECT OF FLOW RATE AND SUBSTRATE
CONCENTRATION ON REACTION RATE

FLOW RATE

REACTION RATE

C  500 ppm
B  100 ppm
A  50 ppm
FIG. 1.4 CONCEPTUAL OPERATING WINDOW: EFFECT OF BEAD SIZE AND FLOW RATE ON REACTION RATE
CHAPTER II

LITERATURE SURVEY

Scientists and engineers have exploited their fields of expertise in developing high performance bioreactors. The literature survey for this work was done to identify and cite approaches taken by members of both the communities in design and development of high efficiency bioreactors. Engineers with new and improved reactor configurations and more realistic mathematical models have contributed in developing designs which can be easily scaled and put into commercial applications.

Biochemists and scientists on the other hand have probed into more fundamental aspects of this technology, and have developed better biocatalysts, and have defined appropriate environments for the biological catalysts to perform specific functions. In the present work a literature review was concentrated in the area of bioreactors which use immobilized biocatalysts.

Immobilization by entrapment within a semi-permeable membrane, and by attachment to the surface of an inert support are two major categories of immobilization. When the biocatalyst is entrapped inside a matrix, the selection
criteria are primarily based on the pore size of matrix. With a proper pore size the biocatalyst can be localized and the diffusion of nutrients and products across the matrix can be controlled as described by Tanaka et al. [11].

In the case of immobilization by attachment on the surface of inert supports, the immobilization process is believed to be both passive and active. The initial bacterial layer formed on the membrane is due to weak Van der Waals forces of attraction between the membrane and the bacteria. This initial adhesion is presumed to be reversible and is influenced by the cell surface hydrophobicity, and the electrostatic potential. The total interaction energy of a particle as a function of its separation distance from the surface is a sum of van der Waals attraction ($G_A$), and the electrostatic attraction ($G_E$). The van der Waals attraction is the result of intermolecular interaction forces which can, to a certain extent be related to the surface hydrophobicity. $G_E$ on the other hand depends on the surface potential of the bacteria and the solid support, as well as on the electrolyte strength of the medium. This is called passive attachment and constitutes only a monolayer bacterial film [12].

Later, when the carbon source is provided, the microorganisms secrete exo-polysaccharides and attach more
biomass through an active process which develops the biofilm as described by Wollersheim [13].

Unlike forced immobilization as in the case of the entrapment method, parameters like microbe-surface interaction, surface porosity, pore size opening, and hydrophylic/hydrophobic characteristics of the surface control the immobilization process.

One of the major concerns while using immobilized cell technology has been the transfer of oxygen across the biofilm. Due to high local cell density, the requirements of DO are more than that required for a conventional free cell system. It has been hypothesized that DO limitations also occur in conventional activated sludge systems wherein an anoxic core forms inside the biological floc, void of sufficient DO [14].

In an interesting study Adlercreutz and Mattiasson have investigated [15] the use of hemoglobin and emulsions of perfluoro chemicals to increase the oxygen carrying capacity to immobilized cells. Here the role of hemoglobin is not very different from its role in animals. Perfluoro chemicals are nonpolar and chemically inert organic compounds in which gases like carbon dioxide and oxygen have high solubilities. Both methods have their advantages and disadvantages, but to make the process economically more
realistic the authors concluded that perfluoro chemicals were better than hemoglobin.

Even if the oxygen carrying capacity is increased, factors like bioparticle or pellet size can limit the availability of oxygen to the cell entrapped systems. Chen and Humphrey [16] have determined that a simple relationship could be used for estimating the critical particle diameter. Here critical is defined as reaching some limiting value of oxygen at the center of the pellet. By solving for zero and first order approximations of Michaelis Menten kinetics they determined the critical radius for given conditions of biomass density, diffusivity, and specific respiration rate. The drawback in this model is that the effects of substrate on specific rate of oxygen consumption were not accounted for.

The supply and availability of oxygen at reaction sites within the bead or bioparticle is mainly dependent on diffusivity of oxygen in supports entrapping whole cells. Sato and Toda [17] found that diffusivity of oxygen in agar gel was 70% of that in water. Adlercreutz [18] concluded that diffusion of oxygen in calcium alginate gel was 25% of that in water.

Besides the gel, diffusion of oxygen across the biofilm is also important. Tomlinson and Snaddon [19]
reported oxygen diffusivity in microbial slimes from 62 to 83% of that in water.

In another study Sun et al. [20] used a random pore model to determine the dependence of oxygen diffusivity on cell concentration when entrapped in alginate gel. By increasing the cell density four times, they observed a 73% decrease in effective diffusivity.

Toda et al. [21] also demonstrated that by using a shallow flow bioreactor the production of acetic acid from ethanol by immobilized Acetobacter aceti M7 could be significantly enhanced. By using a shallow flow bioreactor, high oxygen transfer rates were achieved which were good enough to meet the oxygen uptake rate for oxidation of ethanol.

Livingston and Chase [22] modeled phenol degradation in a fluidized bed bioreactor. The model described simultaneous diffusion and reaction of both phenol and oxygen in the reactor packed with calcined diatomaceous earth particles to which the bacteria were attached. The model predicted a transition from phenol to oxygen limiting kinetics. This is the first time that such a model has been developed. Alternatively, by conducting well controlled experiments, limiting zones for every parameter can be determined. The authors found that at a critical ratio in
the range of 0.9 to 1.1 (phenol/oxygen) a transition from phenol to DO limiting kinetics occurred. It should however be noted that a similar relation may exist between the biomass concentration and the oxygen or the substrate as a limiting factor. Also, the model may predict erroneous critical ratios because of hindered diffusivities of both phenol and oxygen into the biofilm.

From these studies it is evident that supply of oxygen to immobilized cells is most important, and at many times the limiting step in the process.

Besides optimizing the parameters which affect the inherent kinetics, an economically and technically feasible process cannot be designed without the choice of proper reactor configuration. Many different reactor configurations have been designed and studied. These new configurations overcome physical limitations of the process and are aimed at achieving better interactions between the biocatalyst and the medium. To a certain extent the choice of reactor configuration also depends on the apparent kinetics of the system. An appropriate reactor configuration is not the one which will maximize the reaction rates, it’s the one which will also minimize the cost of treatment of a unit volume of the waste stream.

Hamoda and Al-Haddad [23] determined the feasibility and design parameters for using an Aerated Submerged Fixed
Film (ASFF) reactor to treat petroleum refinery wastewater. The critical design parameter in their study was oxygen transfer. They demonstrated that a high surface area to volume ratio along with efficient oxygen transport can significantly enhance the organic removal from the wastewater.

Wagner and Hempel [24] studied biodegradation of naphthalene 2-sulfonate by sand immobilized Pseudomonas in a three phase airlift-loop reactor. With this reactor good fluidization characteristics, as well as a good hold back of the biocatalyst inside the reactor were achieved. Their configuration allows continuous inoculation of the microbial strain to the reactor.

Brauer [25] has given an excellent review of unique bioreactor designs which are different from the conventional stirred vessel bioreactors. He has grouped these bioreactors in five different groups, and discussed the advantages and disadvantages of these bioreactors. The new designs discussed bring about enhanced mixing, continuous renewal of the interfacial area, do not allow build up of dead volume and sedimentation in the bioreactor, and finally allow a high biomass loading. From fundamental research of bioreactors he has proposed three rules to be observed for design of bioreactors. Prevent rotational motion of the
biosuspension, generate motion of the biosuspension in the axial or radial direction, and prevent fluid flow in big spaces.

In another study de Gooijer et al. [26] observed and modeled a series of stirred tank reactors containing immobilized biocatalyst beads, and obeying Michaelis-Menten kinetics to obtain an optimum design. They defined optimum as the smallest total reactor volume needed to accomplish a specific conversion. They demonstrated that a cascade of reactors gave optimum design over one single reactor to achieve a given conversion.

Membrane reactors have not been used extensively for biological treatment of wastewater except those in the form of rotating biological contactors (RBC). Membrane reactors have however been used a great deal for immobilizing enzymes and in other biochemical applications.

In a recent study Uttapap et al.[27] used a microporous membrane bioreactor to prepare enzyme free glucose for a continuous culture. The primary function of the membrane was to filter out the enzyme by immobilization and recover it for recycling.

Hausser et al.[28] used a microporous membrane bioreactor to immobilize a two-enzyme (Fungal α-Amylase and Glucoamylase) system for the production of maltose
containing corn syrups. They reported high conversions at very short residence times compared to those reported for bed type reactors. They attributed this behavior to essentially no diffusional resistance, better flow profiles, and a more effective enzyme loading in the membrane reactor over the bed type reactors.
CHAPTER III

OBJECTIVES

The objectives of this research were as follows:

1. To develop, design and study two different immobilized cell bioreactor systems for the treatment of aqueous organic wastes.

2. To demonstrate that the choice of immobilization method can significantly influence the performance of bioreactors.

3. To define and determine various operating windows with respect to the rate limiting parameters for both reactors.

4. To determine the efficiencies and limitations of the two designs by varying critical process parameters like linear velocity, DO level, and substrate concentration.

5. One of the two systems studied (membrane attached microbes) was also developed in this study, and the objectives here were to select a suitable membrane, as well as come up with a reactor configuration which would be optimum from the point of view of performance, design, operation and scale up.
6. Finally the aim was to compare and contrast the two systems on an equal basis, and to determine whether a combination of the two systems would be optimum from the point of view of cost and performance in the treatment of organic wastes.
CHAPTER IV

MATERIALS AND EXPERIMENTAL METHODS

4.1 MICROORGANISMS

Fifty liters of activated sludge were obtained from the Parsippany Water Pollution Control Plant (NJ). The sludge was sieved through 297 um opening screen, and washed two times with 0.5 % saline. The sludge was then acclimated with one 100 ppm phenol spike per day for five days, at room temperature (27°C+/- 2). It was then centrifuged at 3000 rpm and 4°C (IEC model PR-2 centrifuge), and the pellets so obtained were stored at 4°C, and were used for inoculating the bioreactor, and to make the biobeads.

Dry biomass content (dry weight of cells) of the sludge was determined by drying five samples of pellets in the oven at 120°C for 24 hours. The dry biomass concentration varied from 44 to 66 mg dry biomass/g pellet.

4.2 NUTRIENT MEDIUM

The nutrient medium used was developed in an earlier study [22], and it consisted of magnesium chloride (100 mg), manganese sulfate (10 mg), ferric chloride (0.5 mg),
potassium phosphate (10 mg), and tap water (100 ml for trace elements). The above solution was then diluted to 1000 ml by adding distilled water. The pH was adjusted to 7.2 using mineral acid or base. Fixed nitrogen was excluded from the medium to minimize the growth of biomass during the experiments.

4.3 PRELIMINARY STUDIES AND SELECTION OF MEMBRANE

The objective of this preliminary study was to determine the possibility of attaching chemical waste degrading microorganisms (acclimated to phenol) to two different biosupport materials developed by FMC's Biosupport Group, Pine Brook, NJ. The membrane is in the form of a sheet, and is about 1.5 mm thick.

The gray biosupport material (FMCG) and the white biosupport material (FMCW) were punched into small circular discs of equal weight, and about 5 mm in diameter. Following this, the discs were soaked in water and subjected to vacuum (three times) for removing air bubbles present in the disc material.

The physical and chemical properties of the two biosupport membranes are listed in Table 4.1.
4.3.1 Shaker Flask Experiments

Erlenmeyer flasks of 200 ml capacity containing nutrient medium were used in this study. Fifteen discs of white membrane were put into four Erlenmeyer flasks each containing 100 ml nutrient medium supplemented with 100 ppm phenol as the sole carbon source. Two flasks were inoculated with 100 mg pellets of activated sludge, and the other two flasks were kept as control flasks (without biomass). On a similar basis four other flasks were used with the gray membrane. An additional Erlenmeyer flask containing 100 ml nutrient medium supplemented with 100 ppm phenol was also kept to account for any loss of phenol by volatilization. These flasks were mounted on a shaker-incubator (Lab-Line, Model 3528, reciprocating type) at room temperature (25 to 27°C), and 120 rpm. Periodically liquid samples were withdrawn and analyzed on the gas chromatograph (GC) for removal of phenol.

The attachment and growth of microorganisms on the biosupports were determined by measuring oxygen consumption rates in the microassay reactor (see Figure 4.1), by weighing the discs, and by microscopic observations.

The microassay reactor is a small jacketed vessel of 1.8 ml capacity, with a small oxygen probe as described by Lakhwala et al.[29]. In the present work, the reactor was
used in batch mode to obtain quick and easy measurements of dissolved oxygen consumption rates. The microassay reactor was filled with nutrient medium (without carbon source) and saturated with oxygen by bubbling air. Oxygen consumption rates of the microorganisms attached on the surface of membrane were determined by adding membrane discs, and measuring the drop in oxygen concentration on a chart recorder.

4.4 CALCIUM ALGINATE BEAD REACTOR

4.4.1 Immobilization of Microorganisms by Entrapment in Calcium Alginate Gel

For a given batch of 50 g pellets a typical procedure for making beads was as follows. Wet bacterial pellets and 0.5% saline solution were taken in a ratio of 2:5 by weight in a blender. Sodium alginate (0.75% w/w) was then added slowly to the mixture with continuous stirring over a period of 2 to 3 minutes 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 to 3.5 mm in diameter. The beads were then cured in calcium chloride for 24 hours at 4°C before use.

4.4.2 Experimental Set-up of the Calcium Alginate Bio-Bead Reactor

Experimental setup of the recirculation reactor (batch mode) is shown in Figure 4.2. The reactor is 11.5 cm in diameter and 25.5 cm long. The reservoir is 6.5 cm in diameter and 20.5 cm long. The total reaction volume is 2 liters. The reaction medium is circulated between the substrate reservoir and the reactor using a centrifugal pump. The linear velocities were maintained high enough to overcome the solid/liquid film resistance to mass transfer by recirculating the stream at 1.2 l/min.

Temperature and pH were monitored inside the reactor. All experiments were done at room temperature (25 to 27°C), and the DO levels were maintained by sparging air/O2 or injecting H2O2.

For continuous flow experiments, the reactor used was the same as in batch mode (see Figure 4.3). The reactor volume was recirculated as before between the reactor and the reactor reservoir. Feed was pumped in from a larger feed reservoir at the bottom of the reactor, and the effluent was
withdrawn from the top of the reactor. The DO levels were maintained as in batch mode with either air, pure O_2 or H_2O_2.

### 4.5 MICROPOROUS MEMBRANE REACTOR

#### 4.5.1 Membrane Module

The membrane reactor developed in this study offers many advantages. Unlike the bed/column type reactor, this configuration allows no room for channeling, and diffusion limitation is restricted only to the biofilm attached on the membrane. Moreover, due to the low substrate concentration being treated, the biofilm does not grow extensively. Another advantage is that, the membrane has a high surface area and high porosity which allows a higher biomass loading, and hence less required reactor volumes.

The polymeric membrane used to immobilize the microorganisms was a 1 inch wide strip, wound in a spiral configuration. Two modules, one 12 feet and the other 36 feet in length of membrane were studied. The membrane type was FMCG because it showed better performance as explained later in the text. The inner surface of the membrane has projecting ribs throughout the length which provides a
spacing between adjacent spirally wound layers. The height of the ribs can be changed to vary the spacing. Two spacings, one of 0.3 mm and the other of 1.2 mm were studied. The spirally wound membrane is supported between two plastic plates which gives it mechanical strength and rigidity.

Initially two different membranes were screened on a smaller scale to determine their performance. The membrane selected for most part of the reactor study (FMCG) had a pore size of 1.5 um and is more hydrophylic. The average porosity of the membrane was 60%.

4.5.2 Experimental Set-up of the Membrane Reactor

Figure 4.4 shows experimental set up of the membrane reactor. The membrane module is fixed between two steel plates and a plastic housing which forms the complete reactor. The steel plates have openings for inlet and outlet. The inlet and outlet connections can be reversed depending on the direction of flow i.e. inside-out (going in at the center and out at the periphery) or outside-in (going in at the periphery and out at the center of the loop). A high pressure pump was used to pump the feed from the reservoir in the inside-out direction.

For batch recirculation experiments, the total working volume was 500 ml, 250 ml in the reservoir and 250 ml in the
reactor with a 36 ft long membrane strip, and 0.3 mm spacing module. For continuous flow experiments, the reactor volume was 250 ml. With the 12 ft long, 1.2 mm spacing module the reaction volume was 1000 ml (200 ml in the reactor and 800 ml in the reservoir) for batch experiments, and 200 ml for continuous flow experiments.

4.5.3 Immobilization of Microorganisms by Attachment on the Membrane

The membrane reactor was flushed with nutrient medium (pH 7.2) to assure that pH inside the reactor was around neutral. The reservoir was filled with 600 ml nutrient medium. After adequate recirculation and removal of any air bubbles inside the reactor, the feed pump was stopped. One gram by weight of bacterial pellets was then added to the reservoir and mixed vigorously. A 100 ul reservoir sample was analyzed on a Coulter multisizer for cell counts. The medium along with cells was then recycled through the reactor at a flow rate of 25 ml/min. Periodically, samples were taken from the reservoir and analyzed for cell counts. The counts decreased over a period of time due to attachment of cells over the membrane as seen in (Figure 4.5). This was also evident from a gradual increase in pressure drop across the reactor.
A total of 10 g pellets were added to the reactor (36 ft long, 0.3 mm spacing) in batches of 1 g and 2 g with a subsequent increase in pressure from 10 to 25 psig (see Figure 4.6) at a flow rate of 25 ml/min. In the module with higher spacing (1.2 mm), and 12 ft length of membrane, 6 g pellets were added. The initial biomass loading on the 36 ft long, 0.3 inch spacing module was 1.27 mg dry biomass/inch² of membrane, and that in the 12 ft long, 1.2 mm spacing module was 2.28 mg dry biomass/inch² of membrane.

The reservoir was then injected with phenol to make the reservoir concentration 100 ppm, and 85 mg ammonium sulfate was added as a fixed nitrogen source. The stream was recirculated, and the initially attached biomass was then allowed to grow for two days. This was done in order to have an actively attached biofilm in addition to the initial passive biofilm. Due to this there was a further increase in pressure drop, as seen from the pressure vs flow relationship in Figure 4.7.

4.6 ANALYTICAL METHODS

Liquid samples were taken and analyzed for phenol, dissolved oxygen (DO), carbon dioxide, and cell counts. Phenol concentration was measured on a Varian (3300) gas chromatograph equipped with an FID. A stainless steel
column (6' x 1/8'') packed with 10% SP-2100, 100/120 suppelcoport (Supelco) was used at an oven temperature of 140°C.

DO was monitored using an Orion (97-08 model) oxygen electrode in experiments with the membrane reactor. A Clark-type DO electrode connected to a signal conditioner and a chart recorder assembly was used to measure and control DO levels in alginate bio-bead reactor.

Carbon dioxide dissolved in the aqueous phase was detected on a Shimadzu (model GC8AIT) gas chromatograph equipped with a TCD. A stainless steel column (6' x 1/8'') packed with Porapak, 100/120 mesh (Alltech) was used at 50°C temperature.

Cell counts were done on a Coulter multisizer. A 100 ul liquid sample was diluted in 100 ml Isoton™ and analyzed on the counter for cell counts.

4.6.1 Computer Control of Dissolved Oxygen

A Clark-type dissolved oxygen probe was used to monitor levels of DO, and also as a sensor to control the DO levels. The microprocessor based controller was obtained from Omega Engineering, Stamford, CT. The DO levels were maintained using a set point control logic with a minimum dead band. The controller has capabilities to perform A/D
conversion, do real time graphing of input variables, and to do data logging.

Prior to this oxygen supply was controlled on a time basis, and since the reactor was operated in batch mode the oxygen consumption and requirements changed with changes in phenol concentration. Consequently the typical DO level was never constant, but always varied. It decreased slowly, and passed through a minimum before rising back to original level as seen in Figure 4.8 (curve A).

Later using the computer with set point control, better control over the supply of DO was achieved (see Figure 4.8 (curve B). This was necessary to determine the consumption patterns for phenol by keeping the DO level essentially constant.

Dissolved oxygen sources used were filtered air, and hydrogen peroxide.

4.7 BATCH RECIRCULATION EXPERIMENTS IN THE MEMBRANE REACTOR

Batch recirculation experiments were done at various flow rates of the recycle stream in individual reactors to determine the optimum flow rate in the range of 10 ml/min to 100 ml/min. Phenol at a starting concentration of 50 ppm was used, and the reservoir was maintained at 8 ppm of DO. The reactions were conducted at 25 +/- 2°C.
Experiments were also conducted at various concentrations of phenol (25 to 250 ppm) in the higher spacing module to study the effect of phenol concentration on biodegradation rates. The recycle stream flow rate was 100 ml/min, and the reservoir was maintained at 8 ppm DO by sparging filtered air.

The effect of DO on biodegradation rates of phenol in the membrane reactor was also investigated. Hydrogen peroxide (3% w/v) was used as a reactant to generate oxygen within the reactor. DO levels were maintained between 20 to 25 ppm by periodically injecting 1 ml of 3% hydrogen peroxide solution in the reservoir. Phenol concentration was varied from 25 to 1000 ppm, and the recirculation flow was maintained at 100 ml/min. These experiments were conducted in the higher spacing module.

The effect of spacing (cross sectional area for flow) on the attachment of microorganisms, pressure vs flow relationship, and biodegradation rates were investigated.

4.8 BATCH RECIRCULATION EXPERIMENTS WITH 2-CHLOROPHENOL AS A SUBSTRATE IN THE MEMBRANE REACTOR

To determine the applicability of the membrane reactor over a wide range of contaminants, a few experiments were
conducted with 2-chlorophenol as the substrate. Due to its recalcitrant nature, 2-chlorophenol is not easily biodegraded as is phenol. The objective here was to evaluate the performance of the membrane reactor under more stressful conditions of operation.

Experiments were done at 25 and 50 mg/l starting concentrations of 2-chlorophenol in batch recirculation mode. Recirculation flow rate was studied at 25, 50, and 100 ml/min. All experiments were done with the reservoir maintained at 7.5 +/- 0.5 ppm of DO by sparging filtered air.

4.9 CONTINUOUS FLOW EXPERIMENTS IN THE MEMBRANE REACTOR

In continuous flow experiments, an inlet concentration of 50 ppm was used, and three different flow rates (3, 10, and 18 ml/min) were studied with the 0.013 inch spacing module. In the higher spacing module, flow rate was varied from 10 ml/min to 100 ml/min, at a feed concentration of 50 ppm phenol and 8 ppm DO.

The effluent from the reactor in all experiments was analyzed for phenol and DO, and the consumption rates were followed until a steady state was reached. The steady state effluent concentrations of phenol, along with DO, were the
key parameters to be monitored in continuous flow experiments.

4.10 BATCH RECIRCULATION EXPERIMENTS IN THE CALCIUM ALGINATE BIO-BEAD REACTOR

In batch recirculation mode the parameters studied were concentration of phenol, concentration of dissolved oxygen, and the recirculation rate. The biomass loading in the reactor (600 g beads/2.5 l of medium) was selected based on previous studies in the lab.

A good recirculation rate is required to operate under a regime not limited by solid/liquid mass transfer resistance of the rate limiting compound, and also to maintain physical integrity of the beads.

Phenol concentration was varied from 25 to 1000 ppm, and two levels of DO (5 to 8 ppm, and 25 to 30 ppm) were studied.

4.11 CONTINUOUS FLOW EXPERIMENTS IN THE CALCIUM ALGINATE BIO-BEAD REACTOR.

In continuous flow mode the steady state percent degradation of phenol was studied by varying influent
concentrations of phenol (50 to 1000 ppm), the DO levels (3 to 25 ppm), and the residence time (0.5 to 7 hours). The objective was to determine the operating windows rate limited by either phenol or DO in single pass configuration.
### TABLE 4.1: PHYSICAL/CHEMICAL PROPERTIES OF THE MEMBRANES

<table>
<thead>
<tr>
<th>PROPERTY</th>
<th>MEMBRANES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FMCW</td>
</tr>
<tr>
<td>POROSITY</td>
<td>75-80%</td>
</tr>
<tr>
<td>PORE SIZE</td>
<td>1.2-1.5 µ</td>
</tr>
<tr>
<td>WETTING CHARACTERISTICS</td>
<td>LESS</td>
</tr>
<tr>
<td>COMPOSITION</td>
<td>55% SILICA</td>
</tr>
<tr>
<td></td>
<td>45% PVC</td>
</tr>
<tr>
<td></td>
<td>CARBON (TRACE)</td>
</tr>
</tbody>
</table>
FIG. 4.1 EXPERIMENTAL SETUP OF MICROASSAY REACTOR
FIG. 4.2 EXPERIMENTAL SETUP OF CALCIUM ALGINATE BIOREACTOR (BATCH RECIRCULATION MODE)
FIG. 4.3 EXPERIMENTAL SETUP OF CALCIUM ALGINATE BIOREACTOR (CONTINUOUS FLOW MODE)
FIG. 4.4 EXPERIMENTAL SETUP OF MEMBRANE BIOREACTOR
FIG. 4.5 BIOMASS LOADING IN THE MEMBRANE BIOREACTOR: VARIATIONS IN CELL COUNTS AND PRESSURE
FIG. 4.6 EFFECT OF BIOMASS LOADING ON PRESSURE DROP ACROSS THE MEMBRANE BIOREACTOR

Flow rate: 25 ml/min
FIG. 4.7 EFFECT OF BIOMASS LOADING ON PRESSURE–FLOW RELATIONSHIP IN THE MEMBRANE BIOREACTOR

- OOOOO without biomass
- □□□□ with dry biomass (563 mg)
- △△△△ with growth (2 weeks)
FIG. 4-8 CONTROL OF DISSOLVED OXYGEN

A: TIME BASED
B: SET POINT BASED
CHAPTER V

RESULTS AND DISCUSSION

5.1 PRELIMINARY STUDIES AND SELECTION OF MEMBRANE

The objective of this preliminary study was to investigate the possibility of immobilizing microorganisms from activated sludge on the two biosupport membranes. The parameters studied were DO consumption in the microassay reactor by the membrane discs, change in weight of the discs, consumption of phenol, and finally microscopic observations.

5.1.1 DO Consumption in the Microassay Reactor

After three days of incubation in the shaker, the discs were removed and washed under tap water for 5 minutes. Activity (endogeneous respiration) of the microorganisms on the discs was then determined in the microassay reactor.

Figure 5.1 illustrates the change in dissolved oxygen (DO) levels with addition of FMCG discs to the microassay reactor. A comparison of the untreated discs (control) with the treated discs indicates that there is significant attachment of the microorganisms on the FMCG discs. Increase in oxygen consumption rates are observed with addition of
discs which shows that the attachment is on all the discs. It is also observed, that the rate of oxygen consumption does not increase proportionally with addition of discs. This is because the last discs were added at a lower oxygen concentration. The extent of attachment also probably varies from disc to disc at this point. As mentioned in the experimental section, four sets of experiments were done with each membrane, and the oxygen consumption rates could be duplicated to within +/- 7% variation.

Unlike the FMCG membrane, the FMCW membrane shows lower oxygen consumption rates (see Figure 5.2), and hence less affinity to attach the microorganisms on its surface. The oxygen consumption rates were on an average 37% higher with the FMCG membrane discs. Attachment was observed on all the discs, but again it was not uniform as was with the FMCG membrane. In this case the variation in oxygen consumption rates was +/- 4%.

The discs were further incubated for six days after which oxygen consumption tests were performed. Figure 5.3 shows variation in oxygen consumption rates for FMCW as well as FMCG membranes with the number of discs added. It is seen that the rates for FMCG membrane are again much higher than the rates for FMCW membrane. Another observation is that the rates increased almost linearly with increasing number of
discs. This suggests that an incubation period of six days is probably required for the attachment to be uniform.

It can be said that over the period of three days the attachment is mainly passive (weak physical forces), and some microorganisms may have been removed while washing under the tap water. Consequently it was observed that the attachment was not uniform. After six days the attachment is more active (strong chemical or electrochemical forces), and the microorganisms are firmly held to the surface, and were not removed during washing.

5.1.2 Consumption of Phenol

All four Erlenmeyer flasks (one control, and one with biomass for both white and gray membranes) were spiked with 100 ppm phenol. Over a period of 10 hours the change in concentration of phenol was monitored in all the flasks. The flasks were kept in the shaker at room temperature and shaken at 120 rpm. The spikes of 100 ppm were given daily for eight days. The consumption of phenol was 77 to 83 % higher in the flasks containing the biomass attached discs than in the flasks containing untreated membrane (no biomass, control). Consumption of phenol was almost the same in both FMCW and FMCG membrane flasks which suggests that biomass activity was same. However this activity is a total
of both free and immobilized microorganisms which were the same to begin with in both the cases, and so based on phenol consumption experiments no conclusions could be drawn, but there was sufficient evidence to show that the microorganisms were active and biodegrading phenol when attached on the membranes.

The discs from both flasks containing the inoculum were next separated, washed under tap water, and their phenol consumption was monitored with 25 ppm spikes of phenol for 1 week. Table 5.1 shows the consumption of phenol by both FMCG and FMCW membranes with and without attached biomass. The removal of phenol was 69 to 83% higher in the flasks containing membranes with attached biomass when compared to control. The removal rates with FMCG membranes were also marginally higher than those with FMCW (about 26%), indicating that the FMCG membrane was again better in removing the organics.

5.1.3 Change in Weight and Microscopic Observation of the Discs

There was very little change in weight of the discs over the total duration of experiments (10 days) to derive any conclusions. As far as physical appearance is concerned, upon drying at room temperature, the treated material
appeared slightly darker with probable colony spots on the surface. When viewed under the microscope, large, dense, and distinct microbial colonies were seen on the surface of treated discs, while the control discs showed few small colonies, probably from contamination.

5.1.4 Comparison Between the Two Membranes

Based on the physical and chemical properties of the membranes, and the results obtained from the preliminary experiments some reasoning can be given for the better performance of the FMCG membrane in attaching the microorganisms over the FMCW membrane.

Looking at the hydrophylic/hydrophobic properties of the two membranes it can be said that the aqueous medium carrying the free cells has a better chance to make contact with a hydrophylic surface than to a hydrophobic surface. Consequently a hydrophylic membrane will attach more microorganisms in the same time than the hydrophobic surface. Since FMCG is more hydrophylic than the FMCW it attached more microorganisms than the relatively less hydrophylic FMCW membrane.

The FMCG membrane also has charcoal as one of its constituents. Charcoal or activated carbon is known to have good adsorption capabilities. Hence it is possible that the
charcoal in FMCG membrane helps in attaching more microorganisms by affecting a change in surface charge as described by Loosdrecht [11]. Consequently it was decided to use the FMCG membrane in a reactor configuration for more detailed studies.

5.2 IMMOBILIZING MICROORGANISMS ON THE MEMBRANE MODULE

As described in the materials and experimental methods section, the membrane was wound in a spiral configuration and used as a reactor. Two parameters important to this configuration are the spacing between adjacent layers (cross sectional area for flow), and the membrane length, both of which were investigated.

The cross sectional area for flow influences the pressure drop, the attachment of microorganisms and the linear velocity in the channel. Two different spacings of 0.3, and 1.2 mm were studied.

As seen in Figure 5.4, a fourfold increase in nominal cross sectional area decreased the pressure drop across the channel by six times. Along with a lower pressure drop, the linear velocity in the channel was also reduced. Although no biomass was attached during this experiment, this is critical because the system may run under the limitation of
external mass transfer across the solid/liquid interface when the biomass is attached. Also, by increasing the spacing, a microbial cell in the bulk stream has less chance or will take more time to attach itself on the surface of the membrane. As seen in Figure 5.5, this increase in spacing increased the time to attach one gram of microbial pellets by two times over the same length of membrane.

The other parameter of concern is the membrane length which also influences the pressure drop, and is a critical design parameter parallel to bed height in packed bed reactors. Other than its influence on pressure drop, of more importance is whether the entire membrane length is being used effectively or not. From the experiments with the 36 ft long membrane it was observed that no attachment took place on the last 5 ft of the membrane. This was concluded after a 5 ft piece of membrane from the outside periphery was peeled off and examined at the end of the experiments. Although the membrane was not unrolled beyond 5 ft, it is possible that much more of the membrane was vacant without any attached biomass. In the next set of experiments, the membrane length was reduced to 12 ft. In this set of experiments the entire membrane was being used effectively. This was evident from visual observation of the excess biomass which washed out from the system and attached itself on the outermost layer of the membrane.
The width of the membrane was 1 inch in all the modules, and was one parameter which was not varied. It is however important to note that if additional membrane area is needed for scale-up, the ideal and efficient way to do so is by increasing the width and not the length. This will allow more input of the feed without increasing the pressure drop.

5.3 BATCH RECIRCULATION EXPERIMENTS IN THE MEMBRANE REACTOR WITH PHENOL

5.3.1 Control

Control experiments were done to account for physical losses of phenol by adsorption on the membrane and by stripping from the reservoir in the absence of biomass. Figure 5.6 shows that biodegradation is the primary removal mechanism for phenol. Starting with a 50 ppm concentration, phenol was completely removed in 6 hours in the presence of biomass, when compared to only 5% removal in the absence of biomass during the same time period in the small spacing module.
5.3.2 Batch Recirculation Experiments in Small Spacing Module

Biodegradation experiments were conducted at four different flow rates of the recycle stream (2, 8, 20, and 30 ml/min). A starting concentration of 45 ppm was used. Concentration of phenol was monitored in the reservoir, while dissolved oxygen concentration was monitored at the exit of the reactor. The reservoir was maintained at saturation levels of DO (7.5 ppm) by continuous sparging of air.

Figure 5.7 shows biodegradation of phenol at different flow rates. An increase in flow rate shows a decreasing trend in biodegradation time for the same starting concentration of phenol, and this can be due to two reasons. First, by increasing the flow rate, mass transfer resistance between the biofilm and the liquid stream is reduced, resulting in an increase in reaction rate. Secondly, by increasing the flow rate, the rate of transfer of dissolved oxygen from the reservoir to the inside of the reactor is increased. If availability of DO is a limiting factor, then increasing flow rates will increase biodegradation rates. Also, at low flow rates it is possible that only the initial part of the membrane is being used. At higher flow rates, a larger fraction is being used which results in higher degradation rates.
Figure 5.8 shows the variation in DO level at the exit of the reactor at different flow rates. At a flow rate of 2 ml/min, the minimum DO level at the exit is 4 ppm, and at a higher flow rate of 30 ml/min, the exit DO level falls to 2 ppm. A drop in exit DO level indicates that the reaction was limited at lower flow rates more due to film diffusion.

The above experiments were performed under a constant biomass concentration inside the reactor. This was confirmed from a profile of pressure vs flow rate taken before and after the experiments. The profile remained essentially the same indicating that there was no significant change in biomass concentration during that period.

The oxygen consumption trend is seen to follow closely the trend in phenol consumption. The DO level decreases initially because oxygen is consumed for biodegrading phenol. Later, when the phenol concentration becomes low, the DO level rises again on its own as seen in Figure 5.9. This shows that the oxygen consumption rates were enhanced in the presence of phenol.

5.3.3 Batch Recirculation Experiments in Higher Spacing Module
5.3.3 (a) Effect of recirculation flow rate on biodegradation of phenol

Starting with a concentration of 50 ppm phenol, the flow rate of the recycle stream was varied from 10 ml/min to 100 ml/min. Flow rates in excess of 100 ml/min could not be studied because at 150 ml/min the linear velocity was very high and resulted in washout of the biomass.

Figure 5.10 shows the change in relative concentration of phenol with time at different flow rates. A comparison with control (without biomass) at 100 ml/min flow indicates that 96% of total phenol removed was being biodegraded. The remaining 4% is lost by entrainment from the reservoir and other abiotic means.

Similar to that observed in the small spacing module, the reaction rates increased as the flow was increased, and a ten fold increase in flow (from 10 to 100 ml/min) increased the reaction rate 5.25 times as seen in Figure 5.10.

Dissolved oxygen concentration was monitored at the exit of the reactor (before the stream entered into the reservoir). Figure 5.11 shows the variation in exit DO levels at different flow rates. At 100 ml/min flow the DO level at the exit falls rapidly and much lower than that at 10 ml/min, indicating that reaction rates are high at higher
flow velocities. A similar trend is observed at all flow rates wherein the DO level falls initially (when spiked with phenol), and later when the phenol concentration is low, it rises again.

5.3.3 (b) Effect of phenol concentration on biodegradation rates

Experiments were conducted at starting concentrations of phenol in the range of 25 to 250 ppm. Above 250 ppm phenol, and at 100 ml/min flow the exit DO level fell below 2 ppm (almost anaerobic conditions), and consequently experiments above 250 ppm phenol were not conducted in the membrane reactor with air as a medium for DO.

The reaction rates varied from 4.2 to 12.12 mg phenol/hr*gm dry biomass in the range from 25 to 250 ppm phenol. The maximum reaction rate was observed at 100 ppm of phenol (see Table 5.2). The decrease in reaction rate above 100 ppm phenol may be due to the limitation of DO, biomass or substrate inhibition, and this was determined from experiments done at higher DO levels using hydrogen peroxide as a source of dissolved oxygen.
5.3.3 (c) Effect of DO on biodegradation of phenol in membrane reactor

An interesting trend was observed in the DO profiles at the exit of the reactor, and in the reservoir when hydrogen peroxide was injected. Unlike the experiments with air where the DO was high in the reservoir and low at the exit, a totally opposite trend was observed with hydrogen peroxide. As seen in Figure 5.12, the DO at the exit is higher than that at the entrance (reservoir) of the reactor after hydrogen peroxide has been injected at point "A". At first this seemed to be contradictory, but after a close examination it was found to be obvious because the reaction sites where hydrogen peroxide decomposes into oxygen are inside the reactor. Consequently the exit stream is richer in DO than the entering stream because catalase is continuously making oxygen across the length of the reactor, and also because the production rate of DO from hydrogen peroxide is higher than its rate of consumption.

For Figures 5.12 and 5.13, the starting phenol concentrations were zero, and the recirculation rate was 25 ml/min.

As the reaction of hydrogen peroxide with catalase proceeds, the DO level in the reservoir also rises (due to addition of high DO containing exit stream), but is always
lower than the DO in the exit stream. At point "B", essentially all the hydrogen peroxide is converted to oxygen, and the concentration of DO begins to fall due to the consumption by microbes and due to dilution. At point "C" the trend reverses again, and this time the exit DO concentration falls below the inlet DO concentration. At this time the consumption of DO is faster than the supply, and its time for the next injection of hydrogen peroxide.

From the area between the two curves in Figure 5.12, the average DO concentration in the system over a time frame can be computed. A higher and lower DO level can be maintained by either injecting higher or lower concentrations/volumes of hydrogen peroxide as seen in Figure 5.13.

Table 5.2 summarizes the results obtained from the experiments done in batch recirculation mode in the membrane reactor. At higher DO levels the reaction rates do not increase as the concentration of phenol is increased beyond 150 ppm, which indicates that at higher phenol concentrations DO is not a limiting factor. This would imply that either the microbes are inhibited or the biomass is rate limiting.

After examining the DO profiles (see Figure 5.14) it can be said that a decrease in degradation rate is more due
to the inhibition effect. If biomass were rate limiting, the DO profile at the exit should remain essentially the same at all concentrations of phenol. Contradictory to this the DO consumption rates at the exit show a decreasing trend with increase in concentration of phenol. Hence it can be said that the oxygen consumption rates decreased due to substrate inhibition at phenol concentrations above 150 ppm.

Also, in the lower phenol concentration range (25-125 ppm), no significant increase in biodegradation rates are observed at high DO levels (20-25 ppm), when compared to the rates at 5-8 ppm DO level (see Table 5.2). This is obvious as was with the calcium alginate reactor that at lower phenol concentrations the reaction rates are not limited by DO.

5.4 CONTINUOUS FLOW EXPERIMENTS (SINGLE PASS) IN THE MEMBRANE REACTOR

5.4.1 Continuous Flow Experiments in the Small Spacing Module

In continuous flow experiments, an inlet concentration of 50 ppm was used, and three different flow rates were studied (3, 10, and 18 ml/min). The effluent from the
reactor was analyzed for phenol and DO, and their consumption rates were followed till a steady state was reached.

Figure 5.15 shows transient variation in effluent phenol concentration at different feed rates in continuous flow configuration before steady state is reached. The steady state phenol concentration in the effluent increases with the feed rate. At a feed rate of 3 ml/min or residence time of 1.4 hours, 50 % conversion was obtained at steady state.

Figure 5.16 shows variations in effluent DO for the same experiments. At feed rates of 3 and 10 ml/min, the steady state DO in the effluent is far below 2 ppm which suggests that the reaction may be limited due to the availability of oxygen at those feed rates. It is also seen that initially the steady state effluent DO level decreases when the flow rate is increased from 3 to 10 ml/min, and this indicates a regime of mass transfer limitation below a flow rate of 10 ml/min.

When the flow rate was increased to 18 ml/min, the effluent DO level increased because dissolved oxygen was being transported across the reactor at a faster rate. Although a high DO level was observed at the exit, the oxygen consumption rate per unit flow was higher than that at a feed rate of 10 ml/min (Figure 5.17).
Similar to the batch recirculation experiments, a profile of pressure drop vs flow rate was taken before and after the experiments to account for changes in the biomass concentration. The profile indicated no significant change in biomass concentration during the course of experiments.

5.4.2 Continuous Flow Experiments in the Higher Spacing Module

5.4.2 (a) Effect of flow rate on biodegradation rate of phenol at equilibrium

In the higher spacing module, feed rate was varied from 10 ml/min to 100 ml/min, and the feed concentration of phenol was maintained around 50 ppm, and the D.O. in the feed was maintained around 8 ppm by sparging air. As seen in the right side of Figure 5.18, at lower feed rates (high residence times) the percent degradation at equilibrium is low, and this is due to low DO levels as is evident from DO measurements at the exit. As the flow rate is increased, the equilibrium degradation value increases because DO is being carried across at a faster rate. This unique behavior is because both the linear velocity and the space velocity are changed when the flow rate is varied in this configuration. At higher feed rates an optimum value is attained at which
point the combination of linear velocity and contact time is the best. At this point the feed rate is 50 ml/min and 27% degradation is obtained.

Beyond this point further increases in feed rate reduce the contact time and consequently the steady state degradation values decrease.

Since degradation rates were probably oxygen limited at high residence times, the same set of experiments were repeated at higher DO levels using hydrogen peroxide. Figure 5.19 shows the results obtained from these experiments. At lower residence times, the percent degradation of phenol was not limited by DO, but by the contact time, and hence no significant changes were observed. As the residence time is increased, the percent degradation in the reactor operated with air begins to fall while that in the reactor operated with hydrogen peroxide increase a little before leveling off. At a residence time of 0.42 hours the percent degradation is 4.5 times larger at higher DO (15-25 ppm).

5.4.2 (b) Effect of DO and feed concentration of phenol.

From the results obtained in batch mode it was evident that any concentration of phenol above 150 ppm was inhibitory irrespective of the DO level, consequently experiments at higher concentrations of DO and phenol were
not conducted in continuous flow mode for the membrane bioreactors.

5.5 COMPARISON BETWEEN THE HIGHER AND LOWER SPACING MODULES

Table 5.3 summarizes the reaction rates for both the modules biodegrading 50 ppm phenol in batch recirculation mode. The higher spacing module was started with 2.2 times less biomass per unit reactor volume being treated, and almost the same ratio of membrane area to the reactor volume being treated as the lower spacing module. But, the reaction rates are higher in the wider spacing module than those in the lower spacing module when compared at the same cross sectional velocities.

The explanation for such an observation is that, the two times higher biomass in the narrow spacing module does not have sufficient space to grow, and develop a thick biofilm when exposed to fixed nitrogen source and phenol in the beginning. The reactor cross section in the wider spacing module is four times that in the narrow spacing module which allows the biofilm to grow thicker. This increases the biomass concentration in the wider spacing module, which results in higher reaction rates.

Table 5.4 shows equilibrium degradation rates for the higher as well as the lower spacing modules treating 50 ppm
phenol in single pass configuration. A comparison based on residence time is difficult because at those residence times the linear velocities in the two channels are different. The important thing however is that higher flow rates along with higher degradation rates are obtained in the wider spacing module.

It should however be noted that beyond a certain point, additional increases in spacing may not increase the reaction rates significantly because the biofilm cannot grow indefinitely due to the limitation of inherent kinetics.

The reactor volume depends on the length, width and the height of the spiral channel. By changing the length and the height of the channel, the membrane area also changes. Without changing the membrane area, the biomass concentration in an elemental reactor volume can only be changed by changing the spacing (keeping the surface area of membrane constant, and increasing the biofilm thickness), and hence this volume is a function of only the spacing between the adjacent layers.

A wider spacing between two adjacent layers of membranes is therefore required to increase the reaction rates (by increasing the biofilm thickness) and to allow more throughput per unit time through the reactor. An optimum spacing is one which will allow the entire biofilm
to grow (before it starts sloughing off), and in addition provide sufficient channel space for the medium to flow.

5.6 BATCH RECIRCULATION EXPERIMENTS WITH 2-CHLOROPHENOL AS A SUBSTRATE IN THE MEMBRANE REACTOR

5.6.1 Effect of Recirculation Flow on Biodegradation Rate

To study the performance of the membrane reactor over a broader range of organic compounds, a few experiments were done with 2-chlorophenol as a substrate in batch recirculation mode.

Starting with 25 ppm concentration of 2-chlorophenol, flow rates of 25, 50 and 100 ml/min were investigated in the wider spacing module. Similar to what was seen with phenol, and increase in flow from 25 to 50 ml/min increased the reaction rate on an average by 1.5 times (see Figure 5.20). A further increase to 100 ml/min decreased the rate by 1.25 times from that observed at 50 ml/min flow.

Since washout occurred only at 150 ml/min flow, this decrease in reaction rate is probably due to a high concentration of 2-chlorophenol at the biofilm/liquid interface, resulting in substrate inhibition.

Figure 5.21 shows variation in exit DO for a typical run in the above set of experiments. A drop in DO by
increasing the flow from 25 to 50 ml/min justifies the observed increase in reaction rate. On the other hand a rise in DO level in going from 50 to 100 ml/min flow justifies the decrease in reaction rate.

5.6.2 Effect of 2-Chlorophenol Concentration on Biodegradation Rates

The reactor response was studied at 25 and 50 ppm starting concentrations of 2-chlorophenol. The reaction rate decreased on an average from 12.4 to 7.44 mg phenol/hr*gm dry cells in going from 25 to 50 ppm starting concentration. Unlike that in the case of phenol where the reaction rate increased, a decrease in the case of 2-chlorophenol indicates an inhibitory effect.

When compared to control (without biomass), at least 82% of the total 2-chlorophenol removed could be accounted for as being due to biological removal as seen in Figure 5.22.

Although indepth studies were not done with 2-chlorophenol, it is important to know that the applicability of the membrane reactor can be broadened over other chemicals. When compared to the biodegradation rates of 2-chlorophenol in calcium alginate beads from earlier studies [9], the degradation rates (expressed as
mg 2-chlorophenol/hr * gm dry cells) in the membrane reactor were almost the same at 25 ppm 2-chlorophenol concentration, but were approximately three times less at 50 ppm concentration of 2-chlorophenol. These results once again show that the membrane reactor is not capable of treating higher concentrations of inhibitory compounds.

5.7 BATCH RECIRCULATION EXPERIMENTS IN THE CALCIUM ALGINATE BIOBEAD REACTOR

The calcium alginate bio-bead reactor showed excellent removal of phenol, especially at higher concentrations. Figure 5.23 shows variations in phenol, carbon dioxide, and pH during a typical experiment. When compared to control (without biomass), starting with a concentration of 1000 ppm, 89% phenol was removed biologically. Phenol concentration is reduced rapidly in the beginning, but below 100 ppm, the fall in concentration is slower.

Control experiments were also performed with hydrogen peroxide (10% v/v) and phenol (1000 ppm) in the absence of biomass to investigate the possibility of chemical oxidation of phenol in the presence of hydrogen peroxide. Over a period of 36 hours, phenol concentration decreased by 11%. While this by itself is significant, it becomes less so for
the biodegradation experiments during which the average phenol concentration is far less. Furthermore, the half life of hydrogen peroxide in presence of catalase is only a few minutes, and hence the possibility of significant chemical oxidation of phenol is ruled out.

The reason for using hydrogen peroxide was to operate the reactor at higher DO levels. While treating higher concentrations of phenol (above 250 ppm) it was observed that after sometime, the oxidation rates were very high. The time based control of providing air was not able to meet the oxygen requirements, and as a result of high reaction rates, the DO levels kept falling. After the phenol concentration fell below 10 ppm, the DO level would rise up on its own (see Figure 5.24) when the reaction neared completion. From this it seemed that the system was operating at times under oxygen limiting conditions, and higher biodegradation rates could be attained by operating at a higher DO level.

A mass balance on oxygen consumed (based on complete conversion of hydrogen peroxide) shows that only +/- 8% excess hydrogen peroxide was consumed from what was required theoretically (see Table 5.5). However a balance on carbon dioxide showed that only 49% of carbon dioxide produced from theoretical calculations was detected (see Table 5.5). This
was because only liquid samples were analyzed for carbon dioxide, and the head space was not. Consequently the detection of carbon dioxide was limited by its solubility in water. Because the reservoir was well stirred, the remainder of carbon dioxide presumably escaped into the gas phase. The intention at this point was only to confirm the presence of carbon dioxide.

The parameters studied were the recirculation rate, the concentration of phenol, and the DO concentration.

5.7.1 Effect of Recirculation Rate

The recirculation rate is an important parameter for two reasons. At first it determines what flow rates are needed to minimize the external mass transfer resistance across the solid/liquid interface, and second it determines the rate at which DO is carried from the reservoir to the reaction sites in the reactor. Since phenol is added only in the beginning during biodegradation experiments, what controls the biodegradation process is the rate of transfer of oxygen from the reservoir to the reactor. This transfer rate is a function of recirculation rate, and the aeration rate in the reservoir as seen in Figure 5.25. The drop in DO level across the bed is high at lower cross sectional velocities, and at low aeration rates in the reservoir. The
maximum drop of 40% was observed at an aeration rate of 200 ml/min, and a cross sectional velocity of 0.14 cm/sec.

The above values were obtained in the absence of any phenol in the reactor (endogeneous respiration), and hence the drop in DO levels will be much higher in the presence of phenol. Since the recirculation rate cannot be increased indefinitely, additional DO requirements can be met by maintaining higher DO levels in the reservoir by using pure oxygen or $\text{H}_2\text{O}_2$.

5.7.2 Effect of Phenol Concentration

Effect of phenol concentration on the reactor performance was studied by following the initial bio-oxidation rates (rate of oxygen consumption on addition of phenol), and the biodegradation rates of phenol. At high phenol concentrations, DO may be a rate limiting factor, and hence initial bio-oxidation rates were measured at different spiking concentrations of phenol.

From Figure 5.26 it is observed that initial increases in phenol concentration increase the bio-oxidation rates as expected but, above a concentration of 500 ppm phenol, the initial bio-oxidation rates do not change significantly. Hence it may be indirectly concluded that above 500 ppm phenol, the degradation rates are limited by the
availability of DO at given conditions of air flow and other system parameters.

Figure 5.26 also shows the biodegradation (phenol disappearance) rates at different starting concentrations of phenol. The trend is similar to that of initial bio-oxidation rates at those concentrations. The biodegradation rates for alginate beads in this range (25 to 1000 ppm) vary from 0.74 to 5.25 mg phenol/hr*gm dry cells. The maximum rate of 5.25 mg phenol/hr*gm dry cells is obtained at a starting concentration of 500 ppm phenol.

Initially an increase in phenol concentration increases the reaction (oxygen consumption) rates, which means that other conditions are favorable and phenol is rate limiting in this region. Above 500 ppm the phenol degradation rates begin to fall, and the oxygen consumption rates do not increase significantly. This indicates that either phenol is inhibiting at this high concentration, or the reaction rates are limited by the availability of DO. In the above experiments DO levels have been maintained in the range of 5 to 7 ppm.

5.7.3 Effect of DO Level on the Rate of Biodegradation

In another set of experiments (see Table 5.2), phenol concentrations were again studied in the range of 25 to
1000 ppm feed concentration of phenol, but the DO levels were maintained between 20 to 25 ppm using hydrogen peroxide. A higher DO level had no significant effect on biodegradation rates in the range of 25 to 250 ppm phenol. Above 250 ppm phenol, the biodegradation rates were twice as high when compared to the rates at 5 to 8 ppm DO level. This clearly indicates that DO was rate limiting when phenol concentration was above 250 ppm. Further, a higher maximum rate was reached.

This again indicates that models describing the kinetics with the substrate as a rate limiting parameter are not adequate. The rate limiting parameter is also dependent on the operating window which itself is a function of biomass concentration, DO level, and substrate concentration.

5.7.4 Evidence of Partial Oxidation and Formation of Intermediates

In all the experiments a most interesting observation was that of an unusual trend in oxygen consumption rates. As seen in Figure 5.24, the oxidation rates are slow when the alginate bioreactor is spiked with 1 g phenol. As the phenol concentration decreases with time, the oxidation rates start increasing. This is an anomalous behavior because the
oxidation rates should be high when the phenol concentration is high.

This observation leads to a new hypothesis about partial oxidation. It is possible that phenol is not completely oxidized to carbon dioxide and water, and hence the oxygen requirements are not high in the beginning. Later when the concentration of phenol drops, the intermediates from partial oxidation are completely mineralised to carbon dioxide and water for which the requirements of oxygen are high.

By identifying the intermediates, the metabolic pathway can be determined.

5.7.5 Operational Stability and Bead Life

On an average the bead life is estimated to be from six to eight weeks. A "1 kilo" bead reactor (actually 900 g beads, 25 g dry biomass) was operated continuously for 2 months, and it treated by weight 32 g of solid phenol. The activity of this reactor was followed by determining its oxygen consumption rates every day in the absence of phenol for about 10 minutes, and when challenged with phenol as shown in Figure 5.27. Over a period of 41 days the baseline oxygen consumption rates (in absence of phenol) ranged from 1.2 to 3.5 nmoles O₂/min*ml, while the oxidation rates in
the presence of phenol ranged from 4.2 to 14 nmoles 
O_2/min*ml depending upon the concentration of phenol 
injected.

Higher oxidation rates in presence of phenol when 
compared to the background oxidation rates show substrate 
dependent oxygen uptake, and this is a measure of positive 
response of the system.

Of more importance is the fact that microbial activity 
changes from day to day, and consequently the experimental 
data should account for these changes. Controlled 
experiments were performed in the laboratory to eliminate 
this effect from interfering with the data.

However, the primary objective from a practical point 
of view was to observe the long term operation and stability 
of such a process.

5.8 CONTINUOUS FLOW (SINGLE PASS) EXPERIMENTS IN THE CALCIUM 
ALGINATE REACTOR

Continuous flow experiments were done at different 
flow rates, input concentrations of phenol, and DO levels. 
The aim here was to determine percent degradation of phenol 
at various residence times.
5.8.1 Effect of Residence Time on the Steady State Degradation Rates of Phenol

The residence time was varied from 0.56 to 6.94 hours at a feed concentration of 100 ppm phenol. The degradation of phenol at equilibrium varied from 55% to 96% in the above range as seen in Figure 5.28. All experiments to study the effect of phenol concentration and DO level were consequently conducted at a residence time of 6.94 hours.

5.8.2 Effect of Phenol Concentration and DO Level on the Steady State Degradation Rates of Phenol

Figure 5.29 shows the percent degradation of phenol at steady state at feed concentrations between 25 to 1000 ppm, and at DO levels in the range of 3 to 25 ppm. It can be seen that the conversion is a function of both phenol concentration and DO level for the given biomass concentration. Consequently the rate limiting compound keeps changing, and so do the reaction rates.

At a lower feed concentration of phenol (up to 25 ppm) the percent degradation is almost constant and is not changed by any increase in DO level. As the feed concentration of phenol is increased (above 50 ppm) the percent degradation begins to drop because DO demands are high, and this indicates a DO limited reaction zone.
By increasing the DO levels at higher feed concentrations of phenol, the percent degradation of phenol can be increased. At 1000 ppm feed concentration of phenol, the percent degradation increased from 28% to 83% by increasing the DO from 3 ppm to 25 ppm.

5.9 COMPARISON BETWEEN CALCIUM ALGINATE BIO-BEAD AND POLYMERIC MEMBRANE REACTORS

Figure 5.30 illustrates the flow profiles, and the transport processes occurring within the alginate, and the membrane bioreactors. Although biomass concentration is high in the bead reactor, the interaction of beads with the liquid stream is poor. Moreover, the reaction rates may also be limited due to diffusional resistance. This flow pattern and configuration is bad for treating low bulk concentration of substrates, but is of great advantage in treating exceedingly high substrate concentrations.

In contrast, the membrane reactor has a low biomass density, but a much better flow pattern as well as interaction with the liquid stream. This configuration enhances the biodegradation rates significantly in the lower bulk concentration regime. The disadvantage is that at higher bulk concentration the biomass concentration becomes
limiting or the substrate concentration becomes inhibitory, and decreases the reaction rates.

5.9.1 Comparison in Batch Recirculation Mode

Experimental evidence presented here shows that the polymeric membrane reactor (immobilization by attachment on external support) is best suited for treating low concentrations of phenol. Below a concentration of 100 ppm phenol, the reaction rates in the membrane reactor are almost 10 times the corresponding rates in the bio-bead reactor in the range of 5 to 8 ppm of DO (see Figure 5.31). At 250 ppm phenol, the rates are almost equal.

Above 250 ppm phenol, biodegradation rates for the alginate bio-bead reactor increase marginally. The biodegradation rates in the membrane reactor show a decreasing trend from a phenol concentration of 100 ppm and above. This shows the inability of the membrane reactor system to compete with the bio-bead reactor above this concentration in the range of 5 to 8 ppm of DO.

At higher DO levels (20 to 25 ppm), and higher concentrations of phenol the degradation rates in the alginate reactor increase significantly (in the range of 3 to 4 times) over those at 5 to 8 ppm of DO, but the degradation rates on the membrane decrease due to substrate inhibition.
Figure 5.31 shows how the two reactor systems can be combined to reduce the overall reaction time in treating a stream contaminated with phenol when operated in batch mode. The curve ABCD gives the best operating regime to treat a waste stream of phenol in the range of 25 to 1000 ppm concentration.

Although this operating line is optimum from the point of view of reaction rates, the optimum combination from the point of view of cost may not be the same. Depending on the material and operating costs of the two systems the desired operating combination will deviate from the curve "ABCD".

It should also be noted that the cost per unit reactor volume, and per unit dry biomass immobilized is higher for the membrane reactor than that for the alginate reactor, but one installation of membrane will also last as long as several batches of alginate beads. Hence economically the membrane reactor can be cheaper in treating low concentrations of phenol.

5.9.2 Comparison in Continuous Flow Mode

Since high phenol concentrations were inhibitory in the membrane reactor (as explained earlier), a comparison can only be done in the range of 25 to 150 ppm feed concentrations of phenol. At 50 ppm feed concentration of
phenol, the calcium alginate reactor requires 5.5 times more reactor volume than the membrane bioreactor for close to 90% degradation based on residence time calculations from Figures 5.19 & 5.29.

At feed concentrations of phenol above 150 ppm, the volume of membrane reactor will start increasing because the stream will have to be diluted before treatment. Consequently, the fixed and operating cost for the membrane reactor will increase beyond that for the alginate reactor.

5.9.3 Feasibility of a combined membrane and calcium alginate bioreactors system

Both reactors were individually operated in batch recirculation mode and single pass mode during this study. The alginate bio-bead reactor takes more time in the lower concentration region, and hence becomes costly. The membrane reactor is slow at higher concentrations, and to compete with the alginate reactor in this region the cost of membrane will make it uneconomical.

In this study, it is primarily the inhibition effect of phenol and chlorophenol which is shown to limit degradation rates at higher concentrations in the membrane reactor. However, the same trend may also be observed in the case of non-inhibitory compounds. High substrate
concentration can be treated effectively only in the presence of high biomass concentration. In the membrane reactor, additional increase in biomass concentration (after a threshold level) requires extra membrane area, and hence the cost will be high.

The local biomass density within the alginate gel can be made much higher, and with relatively less cost. Consequently, high degradation rates at higher substrate concentration can be obtained at a much lower cost.

The calcium alginate bio-bead reactor can be operated in either a batch recirculation or a CSTR type configuration. Since the spiral membrane reactor is limited from operations at high linear velocities due to washout, and is also suitable at lower substrate concentrations, it will perform better when operated in a close to plug flow regime.

From a reactor design consideration when the substrate concentration is much lower than \( k_M \), a CSTR requires much more enzyme / residence time for higher conversions than a PFR. The analogy is also true in this case which means that the membrane reactor operated in a PFR configuration will need less biocatalyst/residence time to achieve high equilibrium degradation rates.

Three important parameters which will decide an optimum as well as a cost effective combination for a given
duty are the reaction rates, the cost per unit rector volume, and the operational stability for the two systems.

### TABLE 5.1: CONSUMPTION OF PHENOL BY ACTIVATED SLUDGE IMMOBILIZED ON FMCG AND FMCW BIOSUPPORT MEMBRANES

<table>
<thead>
<tr>
<th>TIME (days)</th>
<th>PHENOL CONCENTRATION AFTER 24 HRS (ppm)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL FMCG</td>
</tr>
<tr>
<td>1</td>
<td>19.0</td>
</tr>
<tr>
<td>2</td>
<td>19.5</td>
</tr>
<tr>
<td>3</td>
<td>19.0</td>
</tr>
<tr>
<td>4</td>
<td>22.0</td>
</tr>
<tr>
<td>5</td>
<td>21.5</td>
</tr>
<tr>
<td>6</td>
<td>22.0</td>
</tr>
<tr>
<td>7</td>
<td>20.5</td>
</tr>
</tbody>
</table>

"\(^a\)" every day the biosupport discs were spiked with approximately 25 ppm phenol solution.

"\(^b\)" concentration values reported are average values of two sets of experiments.
TABLE 5.2: EFFECT OF PHENOL AND DISSOLVED OXYGEN CONCENTRATIONS ON BIODEGRADATION OF PHENOL IN BATCH RECIRCULATION MODE BIOREACTORS

<table>
<thead>
<tr>
<th>PHENOL (ppm)</th>
<th>BIODEGRADATION RATES (mg phenol/hr*gm dry biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CALCIUM ALGINATE</td>
</tr>
<tr>
<td></td>
<td>DO 5-8 ppm</td>
</tr>
<tr>
<td>25</td>
<td>0.74</td>
</tr>
<tr>
<td>50</td>
<td>0.99</td>
</tr>
<tr>
<td>100</td>
<td>1.32</td>
</tr>
<tr>
<td>125</td>
<td>1.85</td>
</tr>
<tr>
<td>150</td>
<td>2.71</td>
</tr>
<tr>
<td>250</td>
<td>4.55</td>
</tr>
<tr>
<td>500</td>
<td>5.25</td>
</tr>
<tr>
<td>750</td>
<td>3.97</td>
</tr>
<tr>
<td>1000</td>
<td>3.01</td>
</tr>
</tbody>
</table>

All rates reported are average values of three sets of experiments.
### TABLE 5.3: COMPARISON OF BIODEGRADATION RATES IN HIGHER AND LOWER SPACING MEMBRANE MODULES (BATCH RECIRCULATION MODE)

<table>
<thead>
<tr>
<th>LINEAR VELOCITY (cm/min)</th>
<th>RATE (mg phenol/hr* gm dry cells)</th>
<th>0.3 mm spacing</th>
<th>1.2 mm spacing</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>1.05 (58.94%)</td>
<td></td>
<td>2.19 (57.10%)</td>
</tr>
<tr>
<td>82</td>
<td>---</td>
<td></td>
<td>2.99 (59.53%)</td>
</tr>
<tr>
<td>105</td>
<td>1.19 (62.94%)</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>164</td>
<td>---</td>
<td></td>
<td>24.69 (68.10%)</td>
</tr>
<tr>
<td>275</td>
<td>2.73 (71.93%)</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>328</td>
<td>---</td>
<td></td>
<td>11.97 (77.22%)</td>
</tr>
<tr>
<td>395</td>
<td>2.65 (77.44%)</td>
<td></td>
<td>---</td>
</tr>
</tbody>
</table>

Numbers in parantheses represent maximum DO drop across the reactor.
<table>
<thead>
<tr>
<th>FLOW RATE (ml/min)</th>
<th>PHENOL CONSUMPTION AT EQUILIBRIUM (ug/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIGH SPACING 1.2 mm</td>
</tr>
<tr>
<td>3</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>90</td>
</tr>
<tr>
<td>18</td>
<td>NA</td>
</tr>
<tr>
<td>25</td>
<td>225</td>
</tr>
<tr>
<td>35</td>
<td>385</td>
</tr>
<tr>
<td>50</td>
<td>600</td>
</tr>
<tr>
<td>75</td>
<td>775</td>
</tr>
<tr>
<td>100</td>
<td>900</td>
</tr>
<tr>
<td>150</td>
<td>WASHOUT</td>
</tr>
</tbody>
</table>
### Table 5.5: Material Balance on Calcium Alginate Bio-Bead Reactor (Batch Recirculation Mode)

<table>
<thead>
<tr>
<th>DO Level (ppm)</th>
<th>Phenol (gm)</th>
<th>Oxygen Balance</th>
<th>CO₂ Balance</th>
<th>Rate (mg phenol/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 to 8</td>
<td>1</td>
<td>+18%</td>
<td>-45%</td>
<td>40.36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+08%</td>
<td>-49%</td>
<td>30.00</td>
</tr>
<tr>
<td>20 to 30</td>
<td>1</td>
<td>-10%</td>
<td>-38%</td>
<td>75.27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-09%</td>
<td>-28%</td>
<td>43.09</td>
</tr>
</tbody>
</table>
FIG. 5.1 CONSUMPTION OF DISSOLVED OXYGEN BY BIOACTIVE FMCG MEMBRANE

- treated
- untreated

Growth Period: 3 days
Temperature of operation: 25–27°C

A: Added 2nd disc
B: Added 3rd disc
C: Added 4th disc
FIG. 5.2 CONSUMPTION OF DISSOLVED OXYGEN BY BIOACTIVE FMCW MEMBRANE

Growth period: 6 days
Temperature of operation: 25–27°C

- OOOOO treated
- △△△△ untreated

A: Added 2nd disc
B: Added 3rd disc
C: Added 4th disc
FIG. 5.3 VARIATION IN OXYGEN CONSUMPTION
CONSUMPTION RATES OF FMCG AND FMCW
MEMBRANE DISCS ATTACHED WITH BIOMASS

Temperature of operation: 25–27°C
Growth period:
FMCG: 5 days
FMCW: 6 days

RATE OF OXYGEN CONSUMPTION (nmoles/h)

NUMBER OF DISCS
FIG. 5.4 EFFECT OF CROSS SECTIONAL AREA ON PRESSURE–FLOW RELATIONSHIP IN THE MEMBRANE BIOREACTOR

![Graph showing the effect of cross sectional area on pressure-flow relationship in the membrane bioreactor. The graph plots pressure (psig) against flow (ml/min) for two different spacings: 1.2 mm and 0.3 mm. The graph shows a linear relationship between pressure and flow for both spacings.]
FIG. 5.5 BIOMASS LOADING: EFFECT OF MEMBRANE SPACING ON ATTACHMENT TIME IN THE MEMBRANE BIOREACTOR

RELATIVE COUNTS

0.0 0.2 0.4 0.6 0.8 1.0 1.2

0 20 40 60 80 100 120 140 160 180 200

TIME (min)

0.3 mm spacing

1.2 mm spacing

○○○○○ 0.3 mm spacing

△△△△△ 1.2 mm spacing
FIG. 5.6 BIODEGRADATION OF PHENOL IN MEMBRANE BIOREACTOR (0.3 mm spacing module)
FIG. 5.7 EFFECT OF FLOW RATE ON BIODEGRADATION OF PHENOL IN MEMBRANE BIOREACTOR (batch mode)

0.3 mm spacing module

- ◆◆◆◆◆ 2 ml/min
- ○○○○○ 8 ml/min
- ▲▲▲▲▲ 20 ml/min
- ***** 30 ml/min

TIME (hr)

PHENOL (ppm)
FIG. 5.8 VARIATION IN EXIT DO WITH FLOW RATE IN MEMBRANE REACTOR (batch mode)

0.3 mm spacing module

D.O. (ppm)

TIME (hr)
FIG. 5.9 VARIATION OF PHENOL AND DISSOLVED OXYGEN IN THE MEMBRANE BIOREACTOR (batch mode)

flow rate: 30 ml/min

- exit D.O.
- phenol

0.3 mm spacing module
FIG. 5.10 VARIATION IN CONCENTRATION OF PHENOL WITH RECIRCULATION FLOW RATE IN THE MEMBRANE BIOREACTOR (batch mode, 1.2 mm spacing module)

Relative concentration: 1 = 50 ppm phenol

- --- --- 10 ml/min
- --- --- 25 ml/min
- --- --- 50 ml/min
- --- --- 100 ml/min
- --- --- control
FIG. 5.11 VARIATION IN EXIT DISSOLVED OXYGEN WITH FLOW RATE IN THE MEMBRANE BIOREACTOR (batch mode, 1.2 mm spacing)

Relative concentration: 1 = 8 ppm D.O.
FIG. 5.12 RELEASE OF OXYGEN IN THE MEMBRANE BIOREACTOR ON ADDITION OF HYDROGEN PEROXIDE (batch mode, 1.2 mm spacing module)

RECIRCULATION FLOW = 25 ml/min
H₂O₂ INJECTION = 1 ml (3 x V/V)
PHENOL CONCENTRATION IS ZERO

relative concentration : 1 = 8 ppm D.O.

TIME (min)
FIG: 5.13 PROFILES OF DISSOLVED OXYGEN AT EXIT IN THE MEMBRANE BIOREACTOR WHEN INDUCED WITH HYDROGEN PEROXIDE

RELATIVE CONCENTRATION: 1 = 8 ppm D.O.

PHENOL CONCENTRATION IS ZERO
RECRYCULATION FLOW = 25 ml/min

- O O O O O O 1 ml H₂O₂ (3%)
- △△△△△△ 2 ml H₂O₂ (3%)
FIG. 5.14 DISSOLVED OXYGEN PROFILES IN THE MEMBRANE BIOREACTOR
AS A FUNCTION OF PHENOL CONCENTRATION IN BATCH MODE
(in 1.2 mm spacing module)
FIG. 5.15 EFFECT OF FLOW RATE ON EXIT CONCENTRATION OF PHENOL IN THE MEMBRANE BIOREACTOR (single pass configuration)

INLET PHENOL = 50 ppm
SPACING = 1.2 mm

Exit Phenol (ppm)

Time (hr)
FIG. 5.16 EFFECT OF FLOW RATE ON EXIT CONCENTRATION OF DISSOLVED OXYGEN IN THE MEMBRANE BIOREACTOR (single pass configuration)
FIG. 5.17 EFFECT OF FLOW RATE ON OXYGEN CONSUMPTION RATE IN THE MEMBRANE BIOREACTOR (single pass configuration)
FIG. 5.18 DEGRADATION OF PHENOL AT EQUILIBRIUM IN THE MEMBRANE BIOREACTOR: EFFECT OF RESIDENCE TIME IN SINGLE PASS CONFIGURATION

PERCENT DEGRADATION AT EQUILIBRIUM

0.3 mm spacing module

Δ Δ Δ Δ Δ EXIT DO
ΟΟΟΟΟ DEGRADATION

PHENOL IN FEED: 50 ppm
DO IN FEED: 8 ppm

EXIT D.O. (ppm)

PERCENT DEGRADATION AT EQUILIBRIUM

PHENOL IN FEED: 50 ppm

RESIDENCE TIME (hr)

0.00 0.10 0.20 0.30 0.40 0.50
FIG. 5.19 EFFECT OF DISSOLVED OXYGEN ON DEGRADATION OF PHENOL AT EQUILIBRIUM IN THE MEMBRANE BIOREACTOR (single pass configuration)

FEED = 50 ppm PHENOL
1.2 mm spacing module

PERCENT DEGRADATION AT EQUILIBRIUM

RESIDENCE TIME (hr)

D.O. SOURCE: AIR (2–7 ppm)
D.O. SOURCE: H₂O₂ (15–20 ppm)
FIG. 5.20 EFFECT OF FLOW RATE ON BIODEGRADATION OF 2-CHLOROPHENOL IN THE MEMBRANE BIOREACTOR (batch mode, 1.2 mm spacing module)

RELATIVE CONCENTRATION: 1 = 25 ppm 2-CHLOROPHENOL

- Flow 25 ml/min
- Flow 50 ml/min
- Flow 100 ml/min

TIME (hr)
FIG. 5.21 EFFECT OF RECIRCULATION FLOW ON EXIT CONCENTRATION OF DISSOLVED OXYGEN IN THE MEMBRANE BIOREACTOR (batch mode, 1.2 mm spacing module)

STARTING 2-CHLOROPHENOL CONCENTRATION = 25 ppm

RELATIVE CONCENTRATION: 1=8 ppm D.O.

FLOW 25 ml/min
50 ml/min
100 ml/min

RELATIVE CONCENTRATION

TIME (hr)
Fig. 5.22 Biodegradation of 2-Chlorophenol in the Membrane Bioreactor (batch mode, 1.2 mm spacing module).

Recirculation flow = 25 ml/min

- 25 ppm
- 50 ppm
- Control (50 ppm)
**FIG. 5.23 BIODEGRADATION OF PHENOL IN CALCIUM ALGINATE BIO-BEAD REACTOR**

- **PHENOL (ppm)**
  - 113

- **TIME (hrs)**
  - 0 4 8 12 16 20 24 28 32 36 40

- **CARBON DIOXIDE (mg/l)**
  - 0 250 500 750 1000 1250

- **pH**
  - 4.5 5.0 5.5 6.0 6.5 7.0 7.5

**Key Symbols**:
- ★★★★★ BIODEGRADATION
- ▲▲▲▲▲ CONTROL
- ++++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ CARBON DIOXIDE
- ◆◆◆◆◆ pH
FIG. 5.25 DROP IN D.O. CONCENTRATION ACROSS THE BED AS A FUNCTION OF LINEAR VELOCITY AND AERATION RATE IN THE ALGINATE BIO-BEAD REACTOR

** Percent Drop in D.O. vs Linear Velocity **

- **AERATION**
  - 200 ml/min
  - 400 ml/min
  - 1200 ml/min
  - 2000 ml/min

---

**Legend:**
- ** ***** ** 200 ml/min
- ** OOOOO ** 400 ml/min
- ** AAAAA ** 1200 ml/min
- ** +++++ ** 2000 ml/min

**Axes:**
- **Y-axis:** Percent Drop in D.O.
- **X-axis:** Linear Velocity (cm/sec)
FIG. 5.26 EFFECT OF PHENOL CONCENTRATION ON INITIAL BIO-OXIDATION RATES AND BIODEGRADATION RATES OF PHENOL IN THE ALGINATE BIOREACTOR (batch mode)

INITIAL BIO-OXIDATION RATE (nmoles O2/ml/min)

BIODEGRADATION RATE (mg phenol/hr)

STARTING D.O. LEVEL = 8 ppm

STARTING PHENOL CONCENTRATION (ppm)
FIG. 5.27 ACTIVITY PROFILE OF CALCIUM ALGINATE REACTOR BIODEGRADING PHENOL

RATE (nmoles O2/min/ml)

TIME (days)

- Baseline
- On addition of phenol
FIG. 5.28 EFFECT OF RESIDENCE TIME ON DEGRADATION OF PHENOL AT EQUILIBRIUM IN ALGINATE BIOREACTOR

PHENOL IN FEED = 100 ppm
FIG. 5.29 EFFECT OF D.O. LEVEL AND FEED CONCENTRATION OF PHENOL ON DEGRADATION OF PHENOL AT EQUILIBRIUM IN THE BIO-BEAD REACTOR (SINGLE PASS CONFIGURATION)
FIG. 5.30 FLOW PATTERNS AND TRANSPORT EFFECTS IN THE BEAD AND THE MEMBRANE BIOREACTORS

REACTOR CONCEPTS

BEAD

DIFFUSION IN
ENZYME INSIDE BEAD
DIFFUSION OUT

POWDER—PELLET HANDLING

MEMBRANE

.020"

.0004" PORES

.020"

NO DIFFUSION—ENZYME OUTSIDE

MODULAR
FIG. 5.31 COMPARISON OF BIODEGRADATION RATES IN ALGINATE AND MEMBRANE BIOREACTORS IN BATCH RECIRCULATION MODE

- 

<table>
<thead>
<tr>
<th>PHENOL (ppm)</th>
<th>RATE (mg phenol/hr*g dry biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-200</td>
<td></td>
</tr>
<tr>
<td>200-400</td>
<td></td>
</tr>
<tr>
<td>400-600</td>
<td></td>
</tr>
<tr>
<td>600-800</td>
<td></td>
</tr>
<tr>
<td>800-1000</td>
<td></td>
</tr>
<tr>
<td>1000-1200</td>
<td></td>
</tr>
</tbody>
</table>

Legend:
- 

- Calcium Alginate
- Membrane (3-8 ppm D.O.)
- Membrane (20-25 ppm D.O.)
CHAPTER VI

RATE EQUATIONS

Based on the literature review of previous work done in this field, kinetic models for the two systems are being proposed. These models have been selected based on the results of experimental work done in the above study. These models describe the apparent kinetics for the two systems, and along with appropriate flow equations for any reactor configuration, their validity can be tested.

6.1 CALCIUM ALGINATE BIOREACTOR

Following are the model requirements for the alginate bioreactor.

1. Use two substrate-limiting kinetic expression, because both phenol as well as oxygen were found to be rate limiting in various regimes of operation.

2. Use Haldene type expression for an inhibitory compound such as phenol [2, 30].

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3. Use Monod kinetics for oxygen.

4. Account for the simultaneous diffusion and reaction of both substrates within the beads (alginate gel, and biofilm).

A mass balance over a thin shell of biofilm leads to the following equations describing the concentration profiles of phenol and oxygen.

\[
\frac{u}{S^2/k_i + S + K_S} \frac{C}{C + K_C} = \\
\frac{1}{r} \frac{d}{dr} \left( \frac{r^2}{D_s} \frac{dS}{dr} \right) + \frac{x}{Y_{x/s}} \frac{u_m}{S^2/k_i + S + K_S} \frac{C}{C + K_C} \quad \text{(2)}
\]

\[
\frac{1}{r} \frac{d}{dr} \left( \frac{r^2}{D_c} \frac{dC}{dr} \right) + \frac{x}{Y_{x/c}} \frac{u_m}{S^2/k_i + S + K_S} \frac{C}{C + K_C} \quad \text{(3)}
\]

The corresponding boundary conditions for these equations are:

\[
\frac{dS}{dr} = \frac{dC}{dr} = 0, \quad \text{at } r = 0 \quad \text{.................................(4)}
\]

\[
S = S_s, \quad \text{at } r = R \quad \text{..................................................(5)}
\]

\[
C = C_s, \quad \text{at } r = R \quad \text{..................................................(6)}
\]

where:

- \(S\) = organic substrate concentration (mg/l)
- \(C\) = concentration of dissolved oxygen (mg/l)
- \(t\) = time (hr)
\( u = \) specific growth rate of biomass (h\(^{-1}\))
\( u_m = \) maximum specific growth rate of biomass (h\(^{-1}\))
\( k_i = \) inhibition constant for phenol (mg/l)
\( K_S = \) monod constant for phenol (mg/l)
\( K_C = \) monod constant for oxygen (mg/l)
\( x = \) biomass density (mg/l)
\( Y_{x/S} = \) yield coefficient (mg biomass/mg phenol)
\( Y_{x/C} = \) yield coefficient (mg biomass/mg oxygen)
\( r = \) radius at any point within the bead (cm)
\( R = \) radius of the bead (cm)
\( D_S = \) diffusion coefficient of phenol in gel and biofilm (cm\(^2\)/s)
\( D_C = \) diffusion coefficient of oxygen in gel and biofilm (cm\(^2\)/s)
\( S_S = \) concentration of phenol at the surface of the bead (mg/l)
\( C_S = \) concentration of oxygen at the surface of the bead (mg/l)

**Assumptions**

1. There exists only a radial concentration gradient within the beads.
2. There is no convective transport within the beads.
3. The ratio of diffusivities of phenol in water to gel is same as that of oxygen in water to gel.
4. Immobilization of the cells into the biofilm does not change the kinetic parameters describing the growth.

**Solution**

The proposed equation for alginate bioreactor is a complex, second order non-linear ordinary differential equation. To fit the experimental data, the technique of orthogonal collocation or Runge Kutta will be required to solve the differential equations as described by Livingston and Chase [22].
6.2 MEMBRANE BIOREACTOR

Numerous models for substrate utilization by biofilms have been proposed [31-35]. These steady state models incorporate diffusional resistances within the biofilm. These models are applicable in reactors like rotating biological contactor, and trickling bed filters where the biofilm thickness is significant. The maximum spacing studied in the membrane bioreactor was only 1.2 mm, which means that the biofilm thickness was at least two orders of magnitude less than the spacing. From the above arguments, the model requirements for the membrane bioreactor are as follows:

1. It has been shown that most biofilms are active for only small depths, and most biological reactions are relatively slow at low substrate concentrations. For these reasons it should not be expected that diffusion within the biofilm is a limiting factor. Experimental results in this study have also shown that biomass was a major rate limiting factor.

2. The membrane bioreactor operates best at low substrate concentrations, and in this range the biodegradation rates are essentially independent of oxygen concentration.
Consequently an organic substrate limiting model can be used to describe the reaction kinetics. A Haldene type substrate inhibitory model is selected because experimental results also showed inhibitory effects.

\[
\frac{dS}{dt} = \frac{u_m \cdot S}{S^2/k_i + K_S + S} \cdot \frac{x}{Y_{x/s}}
\]

Assumptions

1. The biomass forms an even coating on the surface of the membrane.

2. The free cells sloughed off the membrane make only a negligible contribution to the overall degradation rate. This is reasonable in view of the relative holdup of immobilized biomass in the reactor.

3. Growth limiting nutrient is only phenol, and other nutrients are present in excess.

4. The membrane remains totally inert, and does not adsorb any phenol.
Solution

Equation 7 for the membrane reactor was fit to the experimental data using a least square linear regression method. As seen in Figure 6.1, the experimental data shows a close fit to the predicted equation with the following values of rate constants: $k_1 = 352$ ppm, $K_S = 9.7$ ppm, and $u_m = 0.62 \text{ h}^{-1}$. The yield coefficient value ($Y_{X/S} = 0.585$) reported by Yang and Humphrey (1975) [36], was used. The rate constants are in good agreement with those reported in the literature [2, 22, 30, 36].
FIG. 6.1 HALDENE KINETICS PREDICTION OF DEGRADATION RATES OF PHENOL IN MEMBRANE BIOREACTOR.

R A T E (mg phenol/hr*gm biomass)

P H E N O L (ppm)
CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

The following are some of the most important conclusions that can be drawn from this study.

1. A new and more effective combined reactor system has been developed and evaluated on the laboratory scale to treat organic wastes.

2. The combined system of a high biomass density reactor (calcium alginate bio-beads) followed by a low biomass density polishing reactor is shown to require less reactor volume, less reactor time and hence less treatment cost over the two systems operating individually.

3. The spiral reactor configuration significantly enhances the performance of a bioactive membrane from an engineering point of view by offering flexibility, ease of scale up, and above all better substrate degradation rates in the low concentration regimes.
4. The importance of defining operating windows for any bioreactor with respect to the rate limiting parameter which changes with time and position within the reactor is demonstrated. This helps in structuring better mathematical models to describe the reaction kinetics.

5. The calcium alginate system exhibits dual-substrate-limiting kinetics (phenol and oxygen) which explains the apparently different findings of other workers regarding effects of dissolved oxygen. Moreover, there exists a critical ratio between phenol and DO above which there is no return in the volumetric reaction rates by investing further in aeration/oxygen.

5. Inability of the membrane reactor to treat high concentrations of organic wastes is also due to the fact that it cannot sustain high biomass concentration because of biofilm sloughing, as well as inhibition.

7.2 RECOMMENDATIONS

1. The experimental data were analyzed from an engineering point of view, and by accounting for transport effects, but
it is possible that there might be a more fundamental reason for such a behavior. Factors like changes in metabolic pathway due to different immobilization methods may be the reason behind such observations, and this needs to be investigated.

2. Using the operating windows, and the rate limiting parameters from this study, mathematical models should be set-up, and the data from these experiments should be verified to fit the models. These models are useful for scaling and designing new systems.

3. Experiments should be done with a single microbial species to eliminate any difference that would occur due to the quality of microbial cultures existing in the two reactors. This will highlight the role of metabolic pathway in the observed results.

4. Although phenol and 2-chlorophenol were the only compounds studied separately, it needs to be seen how the system responds to a stream of mixed organic compounds.

5. Further investigations are needed to determine the lowest treated concentration that can be obtained in the membrane reactor (in the ppb range).
6. The process developed in this study is non-conventional, and will not replace the existing wastewater treatment plants. Alternatively, this process in combination with the conventional treatment plants will help to debottleneck and/or upgrade the existing plants.

7. The process is more suitable when applied to small, site specific, and short term problems where the conventional processes will be costly as well as inapplicable.
BIBLIOGRAPHY


