BIOLOGICAL TREATMENT OF INDUSTRIAL WASTEWATER USING BACTERIA IMMOBILIZED IN ALGINATE POLYMER MATRIX

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I dedicate my thesis to my wife, Neena and son, Rohan whom I denied company during the two years of my graduate studies in the United States and who, even half a world away in India, were the motivation for me and the reason for all my efforts. I also dedicate this thesis to my parents Raghava Chaubey and Sumati Chaubey, my in-laws and my brother Rajesh Chaubey and his family for everything and for who they are. I am deeply indebted to my brother-in-law Mr. Anand Ojha and his family who sponsored my graduate studies - not only for the vital financial support he provided me but also for his guidance and help.

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CHAPTER I

INTRODUCTION

Immobilized Cells - Definition

Immobilized cells are physically confined or localized in a certain defined region of space with the retention of their catalytic activity and - if possible, their viability (Klein and Wagner, 1983). These (immobilized) cells are intended to be capable of repeated and continuous use. Immobilization involves formation of a macroscopic catalytically active solid phase dispersed in or in contact with a liquid reactant medium. This discrete compartmentalization of the cells, most often in bead form, results in high cell densities in the solid phase.

The process of viable cell immobilization allows a very large number of microbes to be present and active per unit volume of the reactor. This situation is unlike that of non-immobilized systems where the biomass content reaches a maximum value (that is much lower compared to what can be achieved by immobilized cell systems) limited by, among other things, the volume of the reactor (Mattiason, 1983). Also, completely mixed suspended growth systems are susceptible to cell washout at low Hydraulic Retention Times (HRT's). The use of immobilized microbes opens up the possibility of building reactors which would be more compact in size and hydraulically stabler than the conventional activated sludge systems. Also, the immobilized microbes are more tolerant to shock loads of toxics as they are protected, especially in case of entrapped cells (Rozich and Gaudy, 1985), by the immobilizing matrix.

Types of Immobilization

To achieve the objective of cell immobilization for obtaining long-life whole-cell catalysts, the following four types of immobilization methods can be used (Mattiason 1983):

1. Covalent coupling, including crosslinking;
2. Adsorption;
3. Entrapment in three dimensional polymer network;
4. Physical entrapment in porous materials (eg. membranes).

Considering the state of the immobilized cell independent of the method of manufacture, the following four categories can be identified:

1. Carrier-free immobilization;
2. Immobilization of biomass onto a pre-formed carrier;
3. Immobilization of a given biomass in the course of carrier preparation;
4. Immobilization by the growth of immobilized cells.

In the carrier-free immobilization, a compartmentalization of cells by adsorptive or covalent crosslinking of cells (with one another) occurs. This method yields clusters of microbes stuck together by covalent bonds. A direct chemical crosslinking of cells with bivalent coupling reagents like glutaraldehyde can also be used.

Immobilization onto pre-formed carriers is achieved by adsorption. In this process, the carrier matrix can be prepared even under harsh chemical or physical conditions, if required, because the cells attach to them after they are made. The flexibility, with regards to the method used in the preparation of carrier materials, is thus very high. The matrix must contain pores larger than the cell diameters to allow for cell penetration to the inner surfaces of the carrier. Usually porous particulate carriers are used and immobilization is achieved by immersing the matrix particles in a cell suspension. Growth of cells within the pores of the biocatalysts is achieved by incubating them under suitable growth conditions.

.Immobilization by growth of immobilized cells is carried out on cells already immobilized by one of the other methods by incubating them in conditions encouraging microbial growth.

Immobilization in the course of carrier preparation is by far the most common approach in whole cell immobilization (Mattiason, 1983). This can be achieved by entrapment. In the process of entrapment, a porous network which guarantees a high degree of cell retention is prepared. Also, transport processes for substrates and products are sufficiently fast to obtain a high efficiency of catalytic activity (Bordelius and Vandamme, 1988). Several synthetic and natural polymers have been used as immobilizing matrices. Acrylamide and polyurethane are examples of the former while collagen, agar, alginate, carragenan and chitosan are examples of the latter.

In the present work, biomass, that had been concentrated by centrifuging, was entrapped in a three dimensional polymer network of calcium alginate. Alginate is a naturally occuring plant derivative. The biocatalysts were in form of beads of approximately 4 mm diameter. Alginate yields a porous hydrated gel with good substrate diffusion and mechanical properties (Sofer *et al* , 1990). The method of manufacture of alginate beads (to be discussed later) does not, at any stage, involve a process or use a reagent which could adversely affect the viability of the cells being immobilized. These alginate biocatalysts were used to treat a simulated complex industrial waste.

Protocols of the Investigation

In the rest of this text, the term 'immobilized cells' will refer to cells entrapped in the alginate matrix in the course of carrier preparation unless otherwise qualified. The term biocatalysts or beads will refer to the calcium alginate polymer biocatalysts in the form of spheres.

Alginate has been investigated by Sofer *et al.* (1990) for the degradation of 2 chlorophenol. However, the investigation of a synthetic complex waste stream with 2,4-Diaminotoluene (2,4-DAT) as the hazardous waste component makes this study unique. In this study, a chemostat containing calcium alginate biocatalyst was compared with a conventional bench scale activated sludge reactor with internal sludge recycle. Both reactors had the Continuously Stirred Tank Reactor (CSTR) configuration and were operated at the same hydraulic retention times (HRT's).

In the first of the two phases of the investigation (phase I), the Immobilized Cell Reactor (ICR) and the Activated Sludge Reactor (ASR) were examined simultaneously under conditions of varying concentrations of the toxic compound, 2,4-Diaminotoluene (2,4-DAT), at a constant hydraulic retention time. In the second part of the study (phase II), the hydraulic retention times were varied while keeping the concentration of the toxic compound at a level at which the reactors had been seen to run smoothly in phase I of the study. The experimental strategy has been presented in Chapter 3.

In a conventional ASR, operation at low HRT's can result in a very poor performance due to cell washout. The purpose of this study was to entrap the cells into a heavier carrier bead and obtain both a high biomass concentration as well as heavier (compared to free biomass) biologically active treatment media.

Research Objectives

The objectives of the study were as follows:

1. To gain a better understanding of the general behavior of immobilized cell systems and the problems encountered in operating such systems; and,

2. To study the behavior of the immobilized cell system under (a) gradually increasing (stepwise) compound concentrations at a constant Hydraulic Retention Time

(HRT); and (b) under conditions of gradually decreasing (stepwise) HRT's at a constant concentration of the test compound 2,4-DAT.

CHAPTER II

LITERATURE REVIEW

The Scope of the Review

This chapter is intended to acquaint the reader with the research in the area of immobilized cells and with its potential in the field of wastewater treatment. Immobilized cell techniques have been used in the pharmaceutical and cosmetic industries for many years and have only recently been considered for investigation in the area of hazardous waste treatment (Bordelius and Vandamme, 1988). This technique is not yet fully understood (Sofer *et al.*, 1990). Ongoing investigations are focussing primarily on studying various operational characteristics of such systems and on increasing the stability of the polymer matrices that contain the microbes. The findings of various studies discussed here cover the following areas :

1. The substrate utilization of bacteria using complex wastes;
2. The properties of the target compound 2,4-DAT , and its amenability to degradation;
3. Reactor configurations and their performance characteristics;
4. The properties of the calcium alginate bead matrix and its stability in simulated situations that would be encountered during actual waste treatment, and its comparison with other bead matrices;
5. Analytical techniques used for 2,4-DAT detection;

6. Experimental strategies used to investigate the performance of immobilized cell systems.

Immobilized Microbial Cells vs. Immobilized Enzymes

Several advantages of whole cell immobilization have been pointed out in literature. Mattiason (1983) compared whole cell immobilization with enzyme immobilization and listed the following advantages in favor of the immobilized cells:

1. Expensive and troublesome purification of enzymes is avoided;
2. Enzymes are more potent in the presence of intracellular matter (even in case of autolysed cells when compared to pure enzymes.);
3. Viable cells maintain catalytic activities of certain labile enzymes which can not be easily immobilized directly; and
4. Viable cells are useful in reactions where several enzymes and coenzymes are involved.

The following disadvantage has, however, also been noted:

Proliferation of cells inside the matrix causes problems of cell leakage into the medium.

Acclimation and Substrate Utilization by Microbial Populations

In a study on substrate utilization and acclimation of bacterial cultures, Rozich and Colvin (1986) observed that even after extensive acclimation, heterogeneous populations of microbes demonstrated inhibitory growth kinetics. This was characterized by an increase in specific growth rate with increasing substrate concentration to a peak growth rate after which growth declined with further increases in substrate concentration. The presence of a preferred carbon source (glucose) inhibited the utilization of a less preferred substrate to

which a culture had been acclimated. However, the uptake rate of the preferred carbon source remained unaffected by the presence of the less preferred substrate. If a culture acclimated to a less preferred substrate like phenol, was subjected to a feed containing glucose, the preference of the microbes gradually shifted from phenol to glucose.

In an investigation of enzyme inhibition and repression, and their effects on substrate utilization by heterogeneous bacterial communities, Stumm-Zollinger (1966) observed that under conditions where several substrates were being given to a heterogeneous population of microbes, repression of enzyme production (caused by catabolites or reaction products) or enzyme inhibition (production of an enzyme which remains inactive in that environment) could result. The presence of glucose as a substrate, in many cases, inhibited the utilization of other substrates. Inhibition and repression by metabolites was very often caused by the presence of carbohydrates.

Keweloh et. al.(1989) studied the effects of immobilization on the growth kinetics of certain species of microbes fed with phenol. In this study, the phenol tolerance of *Pseudomonas putida* was studied. *Eschrischia coli* and *Staphylococcus aureus* (in free and immobilized states) were similarly studied. Calcium alginate was used as the immobilizing matrix. The researchers used batch cultures and a glucose / sodium succinate based feed containing the essential salts. Phenol was dosed to the cultures at a fixed time after the beginning of the growth phase. Growth rates after the addition of phenol were calculated from the exponential phase of reduced growth. The growth rates were compared to that of a control culture that had not been exposed to phenol. Immobilized cells tolerated higher phenol concentrations than free cells. This finding agreed with an earlier study by Bettmann and Rehm (1984). This effect was observed to be independent of culture conditions and growth rates. It was also observed that the phenol degrading strain *P putida P8* was more sensitive to phenol when compared to the other bacteria tested. This was thought to have resulted from a chance mutation in the strain of the bacteria. Significant protection against phenol was obtained only when cell colonies developed in the gel matrix.

The size of the micro-colonies appeared to determine the extent of phenol tolerance. The morphology of cells within a colony differed and the Scanning Electron Microscopy (SEM) revealed that extra-cellular material was deposited by *P putida*. Under conditions of salt limitation, microbes of the *Pseudomonas sp*. synthesize alginate (Brierly et. al.,1985). It was not conclusively proved by this study that this extra-cellular matter played any part in the phenol tolerance of the cells. The authors concluded that close cell to cell contact influenced the dynamics of the cell membrane and was a pre-requisite to a change in cell physiology. This change was seen to play an important part in enhancing the cells' tolerance and defence against toxic agents. A switch from glucose to sodium succinate did not alter the increased tolerance of immobilized cells to phenol.

O'Reilly and Crawford (1989) studied the degradation of p- cresol by microbes of *Pseudomonas sp*. immobilized by entrapment in both alginate and a polyurethane media. The microbes for the study were obtained from soil contaminated with p-cresol (PCR). A correlation between PCR concentration and PCR transformation was established in batch and continuous flow reactors. The microbes used in this study utilized PCR as their sole supply of carbon. Oxygen limitation (for the cells entrapped in alginate) was suspected to be taking place. The degradation rates of PCR increased when pure oxygen was supplied to the reactors. However, the oxygen limitation was not seen in case of the polyurethane immobilized cells. The authors thought that this resulted from the structure of the matrices and the differences in cell densities between the two matrices. Although the number of cells added initially to the two types of the matrices were the same, a low rate of transformation of PCR in the case of the polyurethane foam pointed toward a loss of cells from the large pores of the polyurethane matrix.

Shock loading and reacclimation.

A study by Arbuckle and Kennedy (1989), which focussed on the response of activated sludge to shock loads of parachlorophenol, throws light on the behavior of cell systems to shock loads of toxics. In this study, activated sludge reactors were fed synthetic waste. The sludge was subjected to transient parachlorophenol loading. The original dose of 50 mg/L was tapered to zero and maintained for 7 days. In this period, the cells lost their acclimation. The system was once more subjected to the original loading of parachlorophenol, and the reactor reacclimated within two days. Once acclimated, the cells developed the capacity to reacclimate faster if they lost their acclimation in absence of exposure to the compound.

Immobilized Cell Systems

Protective Properties of the Matrix.

In an experiment set up to study the degradation of 2- chlorophenol using alginate immobilized activated sludge, Sofer *et al.* (1990) observed that : (a) immobilized cell systems can tolerate higher flow rates (as compared to activated sludge systems) as washout of the bacteria is prevented; (b) the microbes in such systems are more resistant to shock loads of toxic compounds; and (c) extremely high density of cells, as compared to free cells, can be achieved. Increased resistance to shock loads of toxic compounds was also reported by Bettmann and Rehm, (1984) using a *Pseudomonas sp.* isolated from soil contaminated with phenol. These microbes were immobilized both in alginate as well as polyacrylamide (PAAH). The cultures were housed in air lift fermenters (operated in non-continuous mode). Immobilized cells were found to degrade phenol at an initial concentration of 2 g/L with a degradation rate greater than 1 g/L*d. Free cells did not grow at this concentration. The carriers acted as a protective cover against phenol toxicity.

However, PAAH was observed to be more effective in protecting the cells against toxicity than alginate. A close correlation was seen between change of pH values and the phenol concentration. Initiation of reduction of the phenol content was accompanied by a decrease of pH. The initial pH of the culture containing 2 g/L of phenol was 6.5. As phenol degradation progressed, the pH dropped. At the time the pH reached its minimal value of 4.5 in two days, more than 90 % of the phenol was degraded. Also, by establishing a nitrogen limiting condition in the feed, the growth of cells in the liquid medium could be controlled. Cell growth was seen to be concentrated in the outer layer of the bead matrix. The reason for this was deduced to be the limitation of oxygen and phenol, and / or the rapid consumption of phenol and oxygen by the cells in this active layer.

Substrate and Oxygen Diffusion in Alginate Biocatalysts.

Bordelius and Vandamme (1988) point out that alginate immobilized cells have been known to convert the substrate into product faster than the rate at which the substrate can be supplied through the bead surface. In such cases, a gradual decrease in conversion rate towards the center of the particle can be observed. This is due to the decreasing substrate concentration towards the center of the bead. At high cell loadings no conversion at all might take place in this region (See Figure 1.). Figure 1 shows the effect of cell loading (in a bead) on the substrate concentration plotted against distance from the center of the bead. Maximum utilization of oxygen in such immobilized cell systems takes place at a temperature of 37^{o} Celsius. The oxygen uptake of the beads increases with an increase in the biomass content in the beads. The sodium alginate (Na- Alg) concentration in the beads has little impact on oxygen uptake. Oxygen uptake decreases only marginally (from 7.5 to 6 nmoles / min) by increasing the sodium alginate concentration from 0.75 to 1.5 % w/w. Beyond a concentration of 1.5%, the solution becomes too viscous for extrusion.

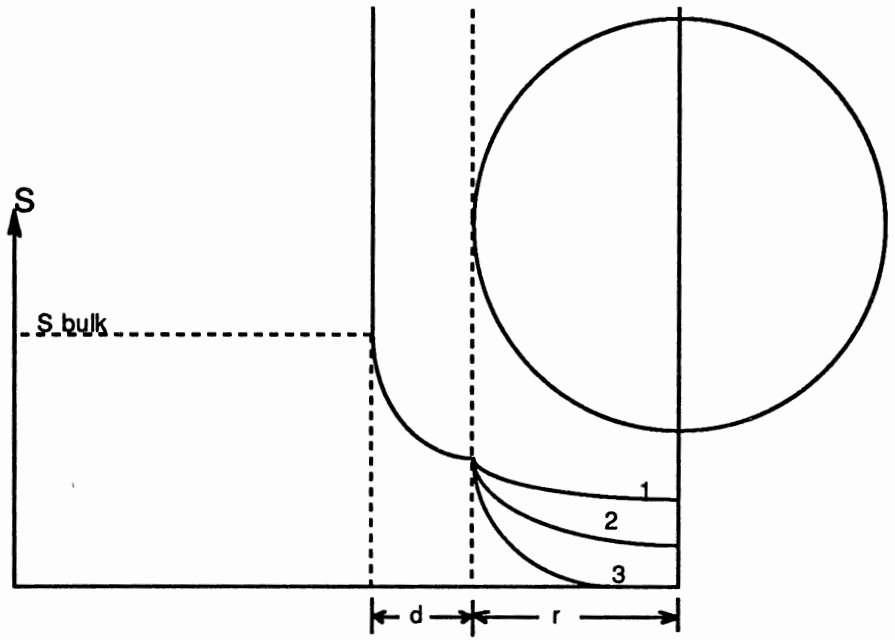


Figure 1. Schematic Diagram of Substrate Concentration Profile within a bead
(1) Low (2) Intermediate and (3) High Cell Loadings
S bulk : Concentration of substrate in the external medium
d : Thickness of stagnant layer around the bead
r : Radius of the bead

(Ref: Bordelius and Vandamme, 1988)

In an effort to compare the characteristics of the immobilized cell system with those of a free cell system, Westmeier and Rehm (1987) targeted 4-chlorophenol degradation by *Alcaligenes sp* A 7-2 cells in both immobilized and free states. This study indicated that increases in the concentration of 4-chlorophenol were better tolerated and degraded faster by the immobilized organisms than by the free cells. High frequency feeding of small amounts of the target compound was more favorable to the removal of 4-chlorophenol than low frequency feeding of larger amounts. At low concentrations, the degradation rates for both systems were almost the same. The authors concluded that calcium alginate protected the cells against high concentrations of 4-chlorophenol and promoted more rapid degradation. This was not believed to be due to shielding of the cells by the calcium alginate because the molecules of 4-chlorophenol are small enough to diffuse freely through the matrix. The authors speculated that a coating effect which gave rise to some kind of membrane stabilization (not elaborated upon) was responsible for cell protection and better removal rates.

The Calcium Alginate Polymer Matrix

Chemical and Physical Properties of Alginate.

Alginate is derived from a sea weed called Kelp. It is available commercially as a sodium salt in form of a powder. It dissolves in water to give a viscous solution. A calcium alginate matrix can be prepared by adding an aqueous solution (7.5 to 1.5 % w/w) of sodium alginate to a well stirred solution of calcium chloride (1.1% w/w). The formation of calcium alginate takes place due to the substitution of calcium ions for sodium ions in sodium alginate polymer chains. Calcium alginate is a mechanically strong and a durable polymer formed by the crosslinking of linear polymers. The process of Ionotropic gelation of the alginate results in a living cell biocatalyst. Ionotropic gelation may be

defined as network formation by ion exchange due to ionic crosslinking of poly-ionic prepolymer chains with multi-valent counterions (Mattiason, 1983)

Bordelius and Vandamme (1988) have characterized the mode of immobilization in alginate as entrapment. This is milder (in terms of chemical and physical conditions) than many other methods, and gel formation can be carried out under conditions resulting in viable cell preparations. The entrapment procedure minimizes the detachment of cells from the immobilized particles as compared to adsorption or chemical immobilization methods.

Manufacturing Aspects of Alginate Biocatalysts.

In a study of the biodegradation of 2-chlorophenol using immobilized activated sludge, Sofer *et al.* (1990) used calcium alginate as the immobilizing matrix. In this study, the concentration of the calcium chloride solution used for the manufacture of the calcium alginate beads did not appear to have a bearing on the life or characteristics of the beads. Upon varying the sodium alginate concentration from 0.75 to 1.5 % w/w. during the manufacture of the beads, the gain in mechanical strength of the beads was significant. Beyond a concentration of 1.5%, the solution became too viscous for extrusion. Beads were tested for stability by subjecting them to vigorous mechanical agitation. At a sodium alginate concentration of 0.75 %, bead stability was only 8 hours, whereas at a concentration of 1.5 %, the bead stability increased to 71 hours.

Degradation of phenol by a coimmobilized entrapped mixed culture was studied by Zache and Rehm (1989). *Pseudomonas putida* P8 and *Cryptococcus elinovi* H1 were used as a mixed culture. Calcium alginate and chitosan-alginate were used for the entrapment of the cells. Under continuous use, the calcium alginate beads became unstable as a result of replacement of calcium ions by other cations (low molecular ions) present in the medium.

The integrity of calcium alginate beads has been shown to be adversely affected by chelating agents with a greater affinity for calcium ions than L-guluronic acid. Thus alginate is unsuitable for use when phosphate is an important constituent of either the growth medium or the substrate stream. The calcium alginate polymer matrix has been shown to be unstable in the presence of phosphate ions (Sofer *et al.*.,1990) which cause the matrix to dissolve and the beads to break up. This is a limitation of the alginate matrix, as phosphates are an essential bacterial nutrient. The effects of a small amount of calcium chelators can be overcome by including calcium ions in the process stream. The effects of calcium chelation have been avoided either by substituting strontium ions for calcium ions as a cross linking agent or by further cross-linking the alginate chains by using poly-ethylenimines. The use of poly-ethylenimines has, however, been observed to have an adverse effect on the viability of the cells At low concentrations of phosphate (less than 10 mg / L), the calcium alginate beads have been used for about one month (Sofer *et al.*. ,1990). Kuu and Polack (1983) observed that calcium alginate gel is unstable in the presence of phosphate buffer and certain cations such as Mg^{++} or K^{+}. The gel was seen to rupture completely in 0.1 M K_2HPO_4 / KH_2PO_4 solution in 15 minutes.

Cheetham and Bucke (1984) pointed out that during the manufacture of the calcium alginate beads, the user has very little control over the size of the beads. This is determined principally by the viscosity and the surface tension of the alginate cell suspension. It is possible to form alginate into sheets and use it in a rolled up fashion. Alginate gel was weakened by large concentrations of cells and deformation of the beads. This resulted in compaction and if columns were used, blockage eventually took place. The growth media fed to the immobilized cells caused 'secondary growth' within the beads resulting in a loss of gel strength especially at the outer layer of the beads, which is the predominant site for cell proliferation. It was observed that immobilized cells retained their viability for longer periods as compared to cells in the liquid media and that the activity of the immobilized cells could be increased after a period of use by feeding a complete growth medium to allow cell

growth inside the beads. When immobilized cells were supplied with a substrate that did not encourage growth, no cells were released from the beads after the first 24 to 36 hours. If a rich medium is supplied, the cells first reproduce and fill up all the available spaces in the matrix and then spill out into the liquid phase so that the immobilized cell preparation acts as a large inoculum. Calcium alginate beads keep good mechanical strength due to their inherent gel integrity even after the emergence of the cells and the formation of carbon dioxide.

Substrate Diffusion in Alginate Matrix.

Tanaka *et al.* (1984) discovered that molecules with a molecular weight up to 20,000 could diffuse freely into and out of the alginate bead matrix. Glucose diffused into the gel with ease, whereas albumin (Mol. Wt. of Bovine albumin = 66,000) could not diffuse in freely. With an increase in the molecular weight of the substrate, an increase in the time lag of diffusion was seen. No appreciable effect was seen on the diffusion rate by alteration of the concentration of calcium chloride solution used in the process of bead manufacture. It was thought that the structure of the substrate molecule could also have a bearing on the diffusion rates. The diffusion characteristics were not seen to change due to an increase in concentration of the sodium alginate solution used in the manufacture of the beads. The pore size of the calcium alginate gel was found to be larger than that of the polyacrylamide gel (which could also be used for enzyme immobilization) and therefore could be used only for the entrapment of whole cells (Tanaka *et al.*, 1984). The characteristics of calcium alginate beads made from different batches of sodium alginate were seen to differ. This could have resulted from a possible variation in the characteristics (eg. age, part of the sea weed, extracting process etc.) of the alginate.

High Performance Liquid Chromatography Techniques

High Performance Liquid Chromatography (HPLC) using normal phase packing and acetonitrile saturated chloroform has been used to detect the presence and quantify the levels of 2,4-DAT in aqueous samples (Unger and Friedman, 1979). This method has been used by several researchers and the results have been accurate and reproducible. Unger and Friedman (1979) used HPLC to detect 2,6 and 2,4-DAT levels in rat urine and plasma samples. The retention times were highly reproducible. Linearity was also observed between the peak heights and the amounts injected. Over-all linearity, reproducibility, sensitivity and precision parameters in the measurement of 2,4-DAT using the HPLC method were very good.

In a separate work (Snyder and Breder, 1982), HPLC was used to determine the levels of 2,6 and 2,4-DAT in the aqueous extracts obtained from boil-in food bags and retortable pouches. In this method, methylene chloride was used to extract 2,4-DAT and concentrate it to a 50 fold higher concentration. The lower limit of quantification of the compound was approximately 40 ng/L in unconcentrated aqueous solutions. Basic solutes such as 2,4-DAT , upon being used on reversed phase columns, undergo adsorptive interactions which contribute to chromatographic peak tailing and causes loss of resolution and sensitivity. To circumvent this negative aspect, ion-pairing and ion suppression methods can be used. In ion suppression, buffers are added to the mobile phase to attain a pH where the ionization of the solute is minimal. In ion pairing, the pH is adjusted to maximize the ionization and a counter ion (alkyl sulfonic acid) is added to yield a non ionic pair. Both methods give neutral properties to the solute molecules.

Waste Characteristics

Base Mix.

Several toxic and non toxic compound can be found in industrial wastewaters. The presence of certain substrates which are easily beiodegradable could result either in diauxic substrate utilization or cometabolism. In diauxic phenomenon, the various substrates are consumed sequentially depending on the ease of its biodegradability and the preference of the microbe species responsible for degradation. In this type of phenomenon, the toxic substrate is utilized only after the more preferred substrate is totally degraded. In the period following the exhaustion of the more preferred substrate, the microbes switch their enzyme systems and prepare to utilize the toxic component. In cometabolism, the substrates are degraded simultaneously, even though at different rates. Conventionally, the parameters of measuring treatment of wastewaters were BOD_5, COD, TOC etc. It is likely that in future, limits of specific compounds will be enforced on the effluent quality of wastewater treatment facilities. For this study, a 'base mix' consisting of ethylene glycol, ethyl alcohol, glucose, glutamic acid, phenol and nutrient salts was used. Originally proposed by Stover and Kincannon (1981), these compounds closely represent the composition of certain industrial (petrochemical, plastics, chemicals) waste effluent streams. The composition of this mix was designed so as to provide the capability of supporting a broad range of microbial species, reasonably good kinetic rates and effluent quality (Kincannon and Stover, 1981). Specific toxics could be added to this base mix to simulate a particular industrial wastewater.

The Compound 2,4-Diaminotoluene.

A chemical that is produced in large quantities, 2,4-diaminotoluene (See Appendix B) is formed as an intermediate in the manufacture of polyurethane, industrial dyes, toluene isocyanates, hydraulic fluids, impact resins, antioxidants and fungicide stabilizers (Unger and Friedman, 1979; Howard, 1989). This chemical has been reported to be mutagenic and carcinogenic to rodents (Unger and Friedman,1979). In an article in Newsweek (April 19, 1991) 2,4-DAT, which is a breakdown product of silicon breast implants, has been identified as a cancer causing agent. With a high water solubility of 7,470 mg/L calculated from its log octanol /water partition coefficient of 0.337, 2,4-DAT has a very low Henry's law constant and vapor pressure (Howard, 1989). Due to this nature, it is not expected to volatilize significantly from soil and water. It is also not expected to hydrolyze significantly. However, the amine groups in the compound may be oxidized by certain radicals produced due to sunlight. It is for this reason that 2,4-DAT is, to some extent, subject to photooxidation in water. This compound biodegrades but high removals by biodegradation have not been seen (Matsui *et al.*, 1975).

Treatability of 2,4-Diaminotoluene.

Very little research has been done in the area of biodegradation of 2,4-DAT. A very limited amount of information was available regarding the characteristics exhibited by this compound as a toxic material. Although a suspected carcinogen (Howard 1989), 2,4-DAT has not been studied adequately yet. It is not believed to be acutely toxic. Little is known about the biochemical pathways of its biodegradation and the identity of its breakdown products.

In an experiment aimed at studying the biodegradation of 35 organic substances using acclimated activated sludge , Matsui et. al.(1975), simulated the waste coming into

the Fukashiba Wastewater Treatment Plant in Japan. They used a fill and draw apparatus with two aeration cylinders. TOC and COD were used as parameters to measure removal. Although 2,4-DAT yielded a higher MLVSS than several other compounds, it exhibited only a 45 % TOC removal and 11% COD removal over a period of 4 hours. The high MLVSS indicated that this compound did not exhibit acute toxicity towards the activated sludge organisms and thus did not seriously inhibit microbial growth. Among the seven substances containing the toluene structure, 2,4-DAT exhibited lower COD as well as TOC removal when compared to the rest of the compounds. It was not clearly documented if 2,4-DAT was the sole carbon source. This made a precise estimate of 2,4-DAT removal difficult.

Miscellaneous Aspects

As reported in a study by Khan et. al (1981), and referenced by Yang and Wang (1989), no simple method exists to actually measure the amount of cells immobilized in the carrier, especially when the carriers used were insoluble. In one of their original works, Yang and Wang (1989) observed that immobilization of cells could effectively prevent washout of cells.

In a study on the effect of food to microbe ratio (F:M) and SRT on the operation and control of an activated sludge system, El Gamal (1980) encountered problems when operating an activated sludge system with a SRT of 6 days at a F:M ratio of 0.5. At this HRT the mixed liquor started to wash out from the system. The color of the mixed liquor changed from brown to milky white. The clarifier could not handle the dispersed non-flocculated solids, and the effluent suspended solids increased. Suspecting this to be caused by a shortage of nitrogen in the feed, the nitrogen in the feed was doubled but still, the washout of the solids continued and nothing attempted could correct it.

Anselmo et. al.(1985) used cells of a fungus *Fusarium flocciferum* in an immobilized state in various matrices (alginate being one) to study their effect on phenol degradation. This fungus occurs naturally in phenol laden industrial discharge. All types of matrix systems gave similar phenol removal efficiencies. An abrupt initial drop was observed in the concentration of phenol in the case of the alginate, agar and carragenan matrices. It was concluded that these matrices possessed the capability to adsorb phenol. Polyurethane foam was seen to be the most mechanically stable when compared to the other matrices.

Nillson and Ohlson (1981) used *Pseudomonas denitrificans* immobilized in a calcium alginate matrix to denitrify drinking water under anaerobic conditions. They concluded that this method can be effectively used for denitrification of water and provides a useful alternative to other established methods.

Lewandowski et. al. (1990) studied reactor designs for the treatment of 2-chlorophenol using white rot fungus *Phanerochaete chrysoporium* immobilized both in an alginate matrix (in a well mixed reactor) as well as attached to a silica based porous support (in a packed bed reactor). A synthetic feed was used in the study. *P chrysosporium* immobilized within alginate beads could degrade 2-chlorophenol at concentrations of 520 ppm. Calcium alginate beads were made and 2000 of these were placed in a 400 cm^3 upflow reactor. The feed containing 520 ppm of 2-chlorophenol was injected at the bottom of the reactor. The aeration rate was 0.3 L/min and was sufficient to keep the beads in suspension and maintain well mixed conditions. The alginate beads remained intact for about 3 weeks before the alginate began to deteriorate under attack of the fungus. The fungus was observed to perform very well in an immobilized state. In a suspended growth reactor, it was less active.

In a separate study Lewandowski et. al. (1987) examined alginate entrapped nitrifying organisms *Nitrosomonas* and *Nitrobacter* and autotrophic denitrifying bacteria. The denitrifying microbe was *Thiobacillus denitificans*.. The addition of calcium

carbonate to the calcium alginate gel as a stabilizing agent in the nitrifying experiment makes this study very interesting. The reactions involved are as follows:

$$NH_4^+ + 1.5\ O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 2H^+ + H_20$$

$$NO_2^- + 0.5\ O_2 \xrightarrow{\text{Nitrobacter}} NO_3^-$$

Protons released during the first stage of the nitrification process react with calcium carbonate as follows :

$$CaCO_3 + 2H^+ \longrightarrow H_2CO_3 + Ca^{+2}$$

Immobilization of calcium carbonate with the nitrifiers within the calcium alginate gel was designed to act as an internal source of calcium ions to stabilize the calcium alginate gel. The relevant finding of this study was that calcium alginate gel could be stabilized by immobilization of calcium carbonate along with the microorganisms when protons are released as a metabolic product.

CHAPTER - III

MATERIALS AND METHODS

Experimental Design

Scope of the Design.

The investigation of a new treatment process involves evaluating its response under different operating conditions. Establishing the desired range of operating conditions can be done by two approaches. Each approach focuses on one of the two parameters (feed concentration and HRT) that determine the volumetric loading rate. The first approach involved increasing the toxic compound concentration in the system, while keeping the HRT and the concentrations of other substrate compounds at a fixed value. This approach was used for the first phase of this study. In the second approach, the target compound concentration was kept at a mid range level (found while investigating the reactor in the first phase of the study) and the HRT decreased from the above fixed value in a stepwise fashion. This approach was used for the second phase of this study. It was possible through this process to define the limits of the system and simultaneously procure information regarding different factors that affect the overall performance of the system.

Two reactors were used in the investigation. A chemostat (complete mix without internal recycle) was used as the ICR while the second unit was a bench scale ASR with internal recycle. This reactor, like the ICR, had the configuration

of a completely stirred tank reactor. Both reactors were operated at the same HRT's for the purpose of comparison. The choice of the activated sludge system was based on the following considerations:

1. Activated sludge systems are widely used as a suspended growth system;
2. Its behavior and system characteristics have been extensively studied and are well understood;
3. It is easy to operate and maintain; and,
4. With internal recycle and a built in clarifier the ASR was inherently resistant to biomass washout.

Selection of the Immobilization Matrix.

Alginate was selected for use in this study because it offered many advantages over other materials currently in use in the area of microbe immobilization. A derivative of sea weed, alginate is easily and economically available. The alginate beads were made under non-toxic conditions at room temperatures and tolerated high cell loadings. Alginate gives a strong matrix (Sofer et al 1990). Alginate yields a porous hydrated gel through which substrate and oxygen readily reach the entrapped cells. In addition, alginate entrapped cells have been recently used (Sofer et al., 1990) in the treatment of toxic compounds.

Experimental Design.

For this investigation, steady state was operationally defined as the period when the soluble COD of the effluent of either unit stabilized for over five days and the deviation of any of the values was not more than 10 % of the mean value.

In the first phase of the experiment, both the ICR and the ASR were run at an HRT of approximately 10 hours. The feed flow rate at the 10 hr HRT was 2.65 L/ day for the immobilized cell reactor and 10.94 L / day for the activated sludge reactor. Both the reactors were supplied with feed containing a base mix (See Tables I and II) and 50 mg/L of 2,4-DAT. Once the systems reached a steady state in terms of 2,4-DAT and SCOD removals, the 2,4-DAT concentration was increased in steps of 50 mg/L while keeping the base mix concentration at a steady value. In this phase of the investigation, the influent 2,4-DAT concentration was raised to 250 mg/L.

In the second phase of the experiment, the influent 2,4-DAT dose was held steady at a target level of 150 mg/L and the HRT was varied. Initially an HRT of 8 hr was established and the reactors were run until steady state was reached. After this, the HRT was dropped to 5 hr and data were collected. In the final stage of the second phase of the investigation, the HRT was lowered to 3 hr. The parameters measured during both phases of the investigation are shown in Figure 2. Samples from the effluent from both the ICR as well as the ASR were tested for TCOD, SCOD, solids, pH and the compound 2,4-DAT. Influent samples were also tested for TCOD, SCOD, PH and 2,4-DAT concentration. High Performance Chromatography was used for the measurement of the 2,4-DAT concentration in the samples.

Base-Mix, Target Compound and Dilution Regime for the Feed

The feed to the reactors was a synthetic wastewater designed to mimic the effluent from the chemicals / plastics industry. It was composed of a readily biodegradable base mix combined with the desired concentration of 2,4-DAT. The base mix was the same as the one used by Kincannon et al. (1981).

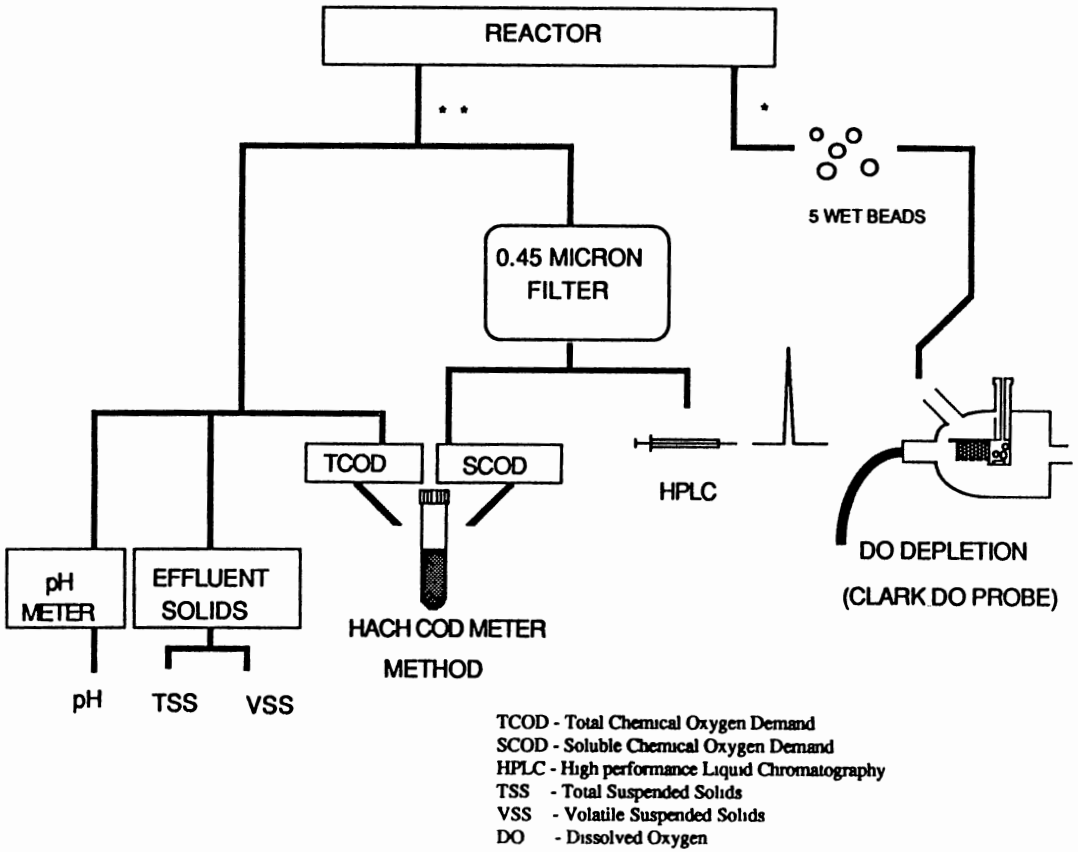


Figure 2. The Measured Parameters of the Inverstigation

* For the ICR only
** For both ICR and ASR

Two stock solutions, one supplying the carbon sources and another supplying the salts, were prepared and stored at 4 ^{o} C. Desired quantities of the two stock solutions were mixed only before preparing feed for the reactors everyday. For making one liter of the base mix, 4.5 ml of carbon stock and 3.0 ml salt stock were diluted using tap water. The required amount of 2,4-DAT was then dissolved in this base mix to yield the synthetic wastewater. The constituents and their concentrations in the stock solutions and their final concentrations (excluding 2,4-DAT) are given in Tables I and II. This feed simulates the typical effluent waste stream of a plastics / chemical industry. The pH of the influent feed solution was in the range of 7.8 to 8.0 throughout the study. The TCOD of the feed solution was 540 mg/L. The theoretical TCOD of a 100mg / L solution of 2,4-DAT was calculated and found to be 164 mg / L.

Start up Procedure

Establishment of the Stock Culture.

The microbes that were immobilized were taken from an 8 liter stock culture maintained in the laboratory. The initial inoculums for this culture were obtained from the return activated sludge line of the secondary clarifier at the Ponca City , (Oklahoma) Wastewater Treatment Plant and from the activated sludge unit at the Conoco refinery at Ponca City. Inoculum was drawn from these two mixed liquors to make a larger number of species of microbes available, thus providing a wide range of microbial population in the initial culture.

The biomass was acclimated to the complex wastewater by dosing it with 36 ml of the carbon source stock solution and 24 ml of the nutrient stock solution, in 8 liters of sludge every day for a period of 10 days, with continuous aeration at

TABLE I

CONCENTRATION OF COMPOUNDS IN THE CARBON STOCK AND FEED

Carbon Sources	Stock Concentration (g/L)	Feed Concentration (mg/L)
Ethylene glycol	24.5	110.4
Ethyl alcohol	17.5	78.6
Dextrose	22.1	99.7
Glutamic acid	8.4	37.9
Acetic acid	30.4	136.6
Phenol	3.5	15.8

TABLE II

CONCENTRATION OF SALTS IN THE STOCK AND FEED

Salts	Stock Concentration (g/L)	Feed Concentration (mg/L)
Ammonium chloride	17.82	53.5
Phosphoric acid	3.11	9.3
Calcium chloride	0.0014	4.2×10^{-3}
Manganese sulphate	0.0012	3.6×10^{-3}
Ferric chloride	0.066	0.2
Magnesium sulphate	6.64	19.9

room temperature. During this phase, the culture was spiked with 50 mg/L of 2,4-DAT (99% purity, Aldrich Chemical Co., Milwaukee Wis) every alternate day to maintain and / or induce the desired enzymatic properties of the bacterial population. Each day, one sixth of the mixed liquor was discarded, and the remaining fluid was allowed to stand for 1 hour in order to settle the biomass. About half of the remaining supernatant was removed, and the system was brought to the original volume by the addition of tap water and concentrated feed stock solutions, as mentioned earlier. This procedure was done as a precaution to remove breakdown compounds arising out of the degradation of 2,4-DAT, which could cause product inhibition. After the initial acclimation, during which the microbial population stabilized, the frequency of addition of 2,4-DAT to the stock culture was decreased to once every three days. Dosing at this frequency was continued throughout the period of the research.

Use of the Stock Culture.

The ASR was started with an inoculum drawn from the acclimated 8 liter stock culture. Whenever required, biomass was harvested from the stock culture for immobilization in the bead matrix by centrifuging 50 ml aliquots of the sludge at 2,800 rpm for 20 minutes using an IEC Centra-7 centrifuge (Damon Inc., MA.). Typically 20 gms of centrifuged wet cells were immobilized in 400 ml of the alginate solution.

The Immobilized Cell Reactor

The immobilized cell reactor set up consisted of a 1000 ml sidearm Kimax Erlenmeyer flask. Figure 3 shows the schematic drawing of the experimental

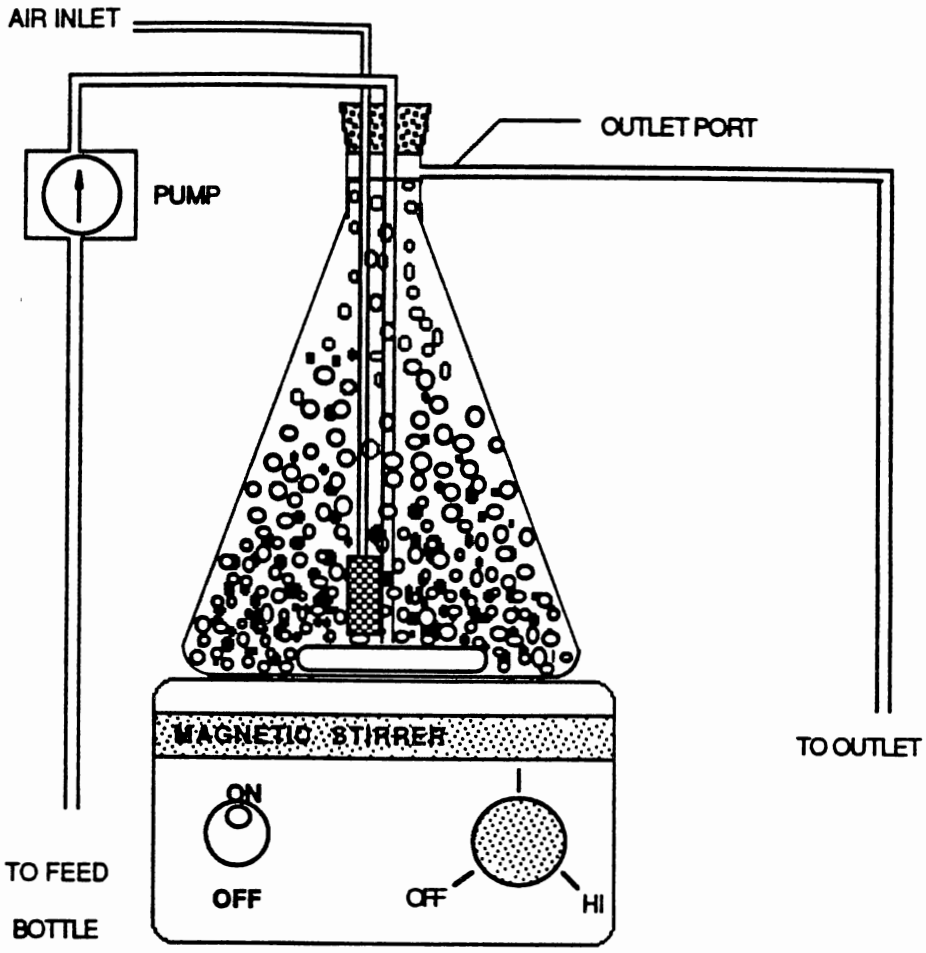


Figure 3. Schematic Diagram for the Immobilized Cell Reactor

immobilized cell reactor. The opening at the top was closed by a two holed rubber stopper through which the influent tube and the aerator tube, ending in the diffuser stone inside the reactor, were passed. The side arm served as the effluent port. Tygon tubing was used to carry the effluent from this port to a 26 liter glass effluent collection bottle. The ICR was not equipped with any type of effluent clarifier. The Erlenmyer flask was placed on a magnetic stirrer, which had to be employed only during the start-up procedure when the beads were freshly made. Air supply from the laboratory pressurized air system was maintained at a constant rate of 1 L / min using an air flow control meter.

The feed was pumped 12 cm below the free liquid surface in the reactor. The inlet was very close to the aerator stone, thus ensuring good mixing of the feed. Delivery of the feed was accomplished using a Cole Parmer positive displacement pump (Model No Cat. # 7553-30) fitted with Masterflex tubing to a standard pump head (model # 7014 - 20) that had an external solid state Master Flex control device (Model No 1326). Tygon tubing was used in the rest of the system.

Manufacture of Alginate Biocatalysts.

Alginate beads were manufactured (See Figure 4.) following the method used by Sofer et al (1990). A 1.5 % w/w solution of sodium alginate (Sigma Chemical Co.) was made by heating sodium alginate powder in water (1.5 gms. of sodium alginate powder per 100 ml. of water) in a boiling water bath to yield 400 mls of a pale yellow viscous liquid. This solution was allowed to cool to room temperature. Twenty grams of centrifuged cells were added to this solution and were mixed thoroughly in a blender. This liquid was then added drop-wise to a 1.1 % w/v solution of calcium chloride that was kept lightly stirred using a magnetic stirrer. The droplets hardened on contact with the solution to form beads about 3 to 4 mm in diameter. These beads were then allowed to remain in the calcium

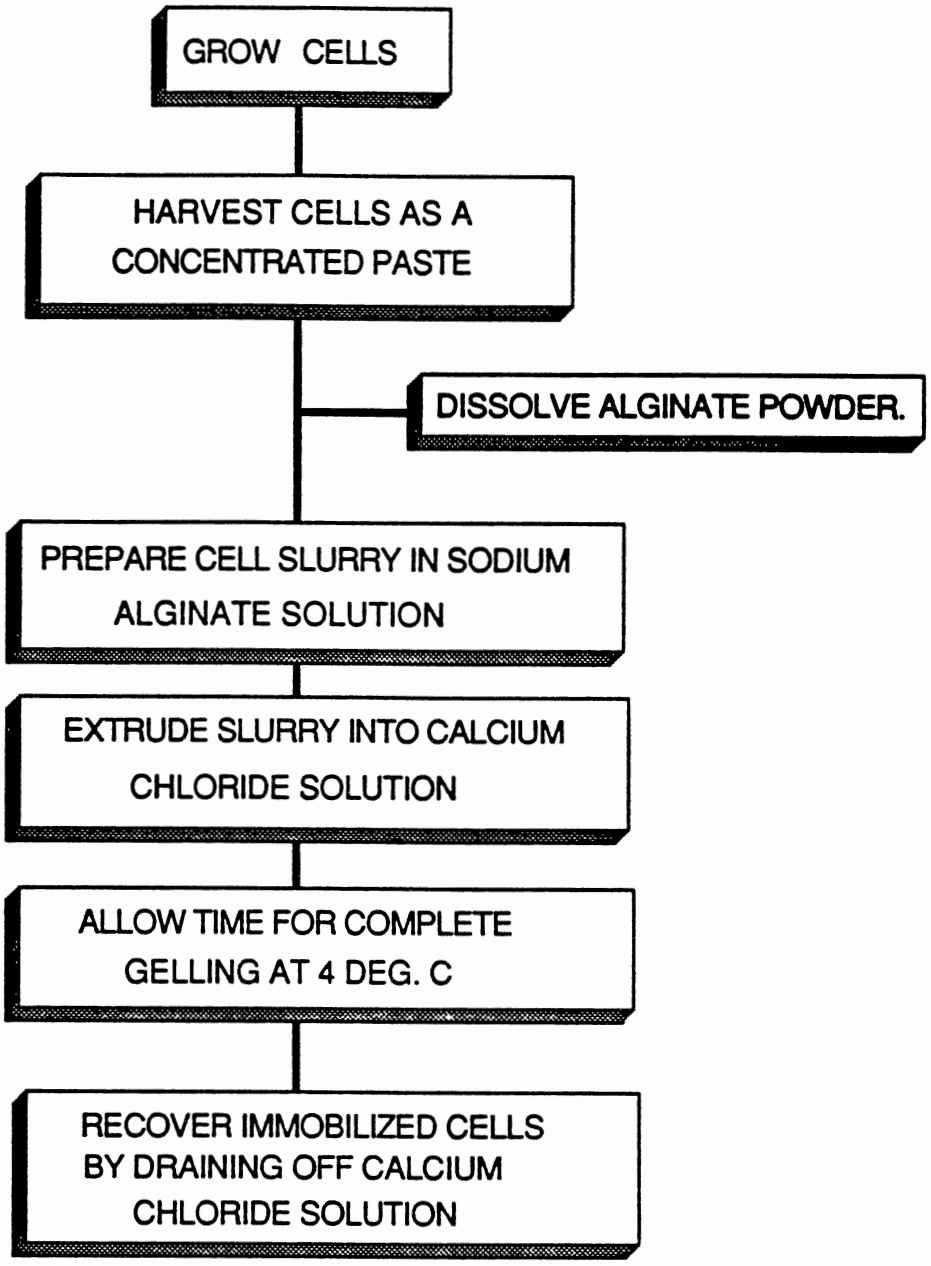


Figure 4. Flow Diagram for Immobilization of Cells in Alginate

chloride solution at 4 ^{o} C overnight for curing (complete crosslinking of polymer chains) before use. A small amount of base mix was added to the curing solution to serve as a substrate for the entrapped cells. The alginate beads had good mechanical strength and were in the form of a hydrated gel. A total volume of 400 ml of sodium alginate and 20 grams of concentrated biomass were used in a single batch of beads for the ICR.

The Activated Sludge Reactor (ASR)

A bench-scale ASR equipped with an internal clarifier and a provision for internal solids recycle was used as a control reactor. It was made of plexiglass and was transparent (See Figures 5 and 6). The total volume of the unit was 4.5 liters, of which the aerated section occupied 2.94 liters. The remaining volume was taken up by the clarifier (1.56 L). The feed was pumped into the aerated section using a standard piston driven pump (Milroyal, model DB-2-117R, Milton Roy Co., Penn.). The clarifier had an effluent port at the top edge of the quiescent zone which was connected by Tygon tube to a 26 liter glass effluent collection bottle.

Start up and Control of the ASR.

The continuous flow activated sludge reactor was started by transferring 4.5 liters of mixed liquor from the acclimatized stock culture into the reactor vessel. Aeration as well as complete mixing was achieved by supplying 1.5 L / min of air into the reactor by means of an aerator stone. The partition wall between the aerator and the clarifier sections was adjusted (0.63 to 0.95 cm. opening) to facilitate proper and adequate recycling from the settler into the main reactor, while ensuring that the settler functioned properly. The aerator stone was placed in such a fashion that a circular motion of the activated sludge was established within the aerated reactor chamber. The ASR was run as per the guidelines

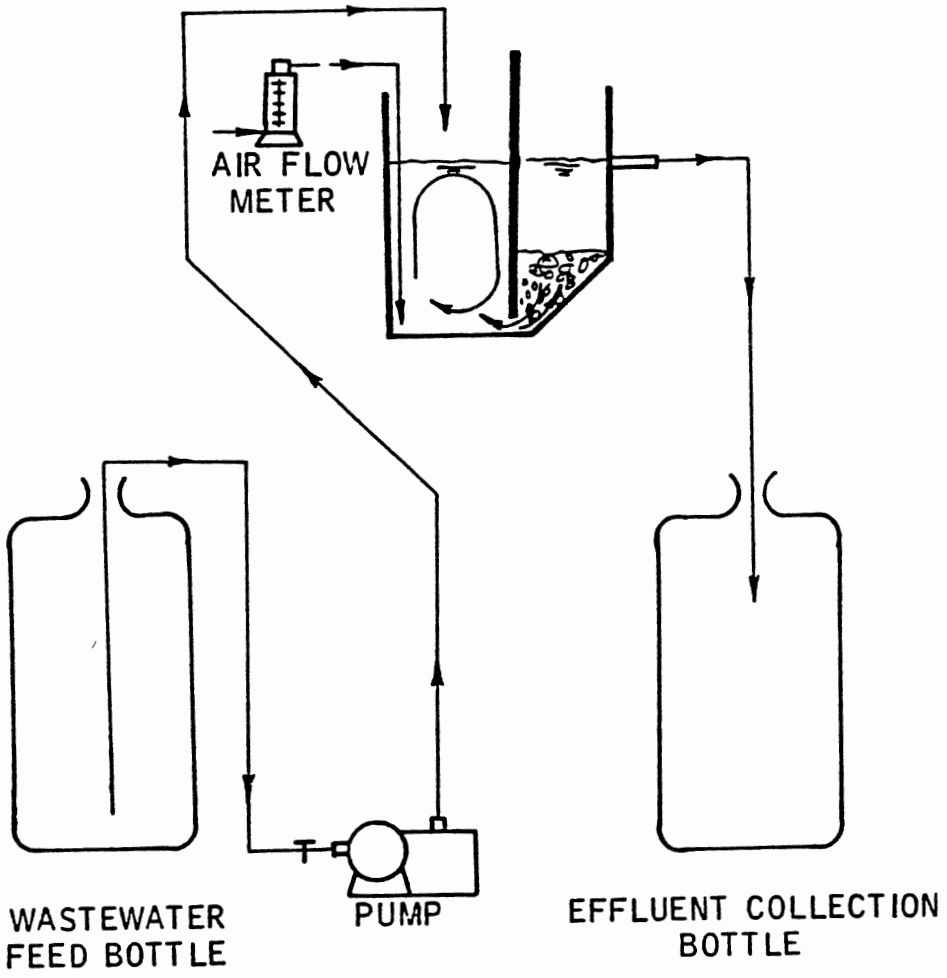
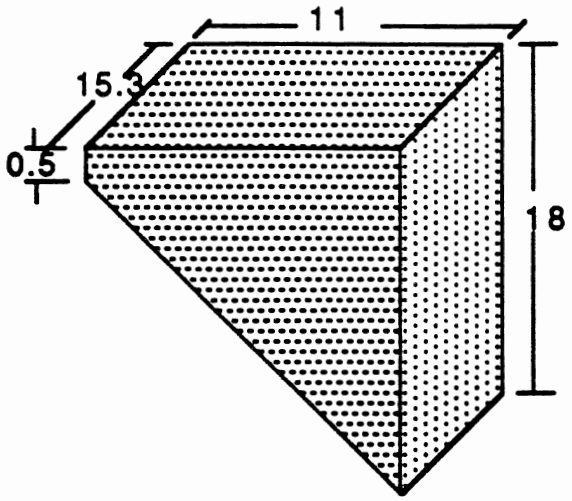


Figure 5. Experimental Activated Sludge Reactor



Dimensions of the Settler of the ASR unit

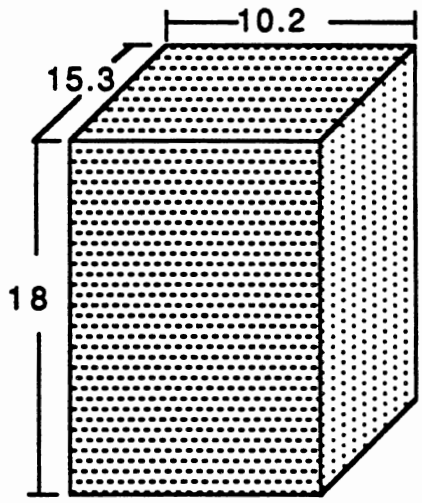


Figure 6. Dimensions and Set Up of the ASR Unit

NOT TO SCALE
ALL DIMENTIONS IN Cm.

proposed by Reynolds (1982) (See Chapter II). The biomass concentration within the reactor was monitored by determining the MLVSS (Mixed Liquor Volatile Suspended Solids) concentrations once every two to three days. This value along with the effluent VSS (Volatile Suspended Solids) data was used in determining the sludge wastage rate. From the very beginning of the investigation, a solids residence time (SRT) of six days was maintained by wasting a calculated amount of the sludge every day. The SRT of six days was chosen as it is the value at which most of the real life wastewater treatment plants operate. The baffle wall was removed and the contents of the reactors were thoroughly mixed up before sludge was taken out for wasting. This volume was calculated using the model equation based on Monod kinetics that was devised by Lawrence and McCarty (1967). The sludge wastage rate was calculated using the following relationship :

$$F_W = [(V^*X/SRT) - F^*X_e] / (X - X_e)$$

Where:

F_W	=	Sludge Wastage Rate (L/day)
V	=	Total Reactor Volume (L)
X	=	Mixed Liquor Volatile Suspended Solids (mg/L)
X_e	=	Solids Concentration in the effluent (mg/L)
F	=	Feed Flow Rate (L/day)
SRT	=	Sludge Retention Time or Sludge Age (days)

Analytical Techniques

Analysis of 2,4-DAT using High Performance Liquid Chromatography (HPLC)

The concentration of 2,4-DAT in the influent and effluent of the reactors was measured using High Performance Liquid Chromatography. All glassware used for the experiments were washed with tap water using phosphate free detergent. This was followed by washing them with distilled water. Finally, they were washed with methanol and dried. This procedure was followed to remove any organic or inorganic matter contaminating the glassware which could either adsorb 2,4-DAT or could get dissolved in the methanol used as the mobil phase for the HPLC. The method and procedures adopted were those established by Snyder and Breder (1981) in their determination of 2,4 and 2,6 Diaminotoluene in aqueous extracts. A reversed phase Beckman C-18 column (250 X 4.6mm I.D) having 5 μm pore size was used for the detection of 2,4-DAT in the sample. A 20: 80 HPLC grade methanol, water mixture was buffered using mono and dibasic sodium phosphates at a pH of 7.4 and used as the mobile phase. This mixture was filtered through a 0.2 mm Nylon -66 Rainin filter (Rainin Instrument Co. Inc. Woburn Mass.) prior to use in order to remove any particulate matter that could block the column. Helium gas was bubbled through the mobile phase at the beginning of each run to expel any dissolved air. The mobile phase was pumped through the column using an electronically controlled Beckman pump (Model 110A) at a flow rate of 1 ml / min. Samples for the HPLC analyses were taken from the influent and the effluent of both reactors. The samples were filtered through 0.45 μm Nylon-66 Rainin filters. Injection of the samples was manually done into an Altex 210 injection port attached to the system. Samples were stored for a maximum of one week at 4^{o} C in the dark in order to minimize any possible amine oxidation (Unger and Friedman, 1979) and photodegradation . The quantity of each sample injected was 50 μL throughout the investigation. The detector

(Waters Associates 44 D) had a fixed wavelength UV detection capability (254 nm) and was operated at an aufs (absorbance units full scale) of 0.5. The signal from the detector was fed into an integrator (Hewlett Packard, Model # 3380 A) to obtain the peak traces and areas. The elution time of 2, 4- DAT was observed to be about 7 minutes.

The HPLC system was operated in strict adherence to recommended procedures (Dolan and Snyder, 1989). At the end of each day's use, the column was flushed with 25 to 30 column volumes of the mobile phase to dislodge any remaining compound. Distilled water (25 column volumes) was then used to flush the column before shutting down the pump to prevent bacterial growth and prevent salt deposition inside the column.

Using known concentrations of 2,4-DAT, a standard curve was prepared by plotting concentrations (independent variable) ranging from 50 to 300 mg/L versus peak areas (dependent variable). Linear regression was performed on these values. It yielded a coefficient of correlation of 0.9993. This curve was used for obtaining concentration values from the peak areas. Before running each batch of samples on the HPLC, a known concentration of 2,4-DAT was injected to verify the validity of the standard curve. This was done to discount any possibility of changes in the column characteristics due to aging, temperature, mobile phase characteristics etc. New standard curves were made after every 20 runs of the HPLC using the same 2,4-DAT concentrations as originally used. All the subsequent standard curves had a coefficient of correlation higher than 0.99. No significant deterioration of the column was seen during the period of the research.

Determination of Biocatalyst Activity by the D.O Uptake Method.

The activity of the biocatalyst beads was measured by the Dissolved Oxygen (D.O) depletion method (Sofer et al 1990) using a Clark electrode, a Gilson water jacket and a strip chart recorder. The uptake of oxygen by immobilized cells is a simple method to determine the number of viable cells inside the matrix. Using this method becomes

imperative as it is non destructive. This investigation was carried out at room temperature. The Clark electrode had a Teflon membrane tip with 5N KCl solution trapped inside. The membrane was secured in place by a rubber ring. The highly sensitive Clark electrode is designed to fit the water jacket with its sensor tip reaching up to a 1.6 ml sealable cavity. A magnetic micro-stirrer was placed inside the cavity and was used to agitate the samples during the test.

The Clark electrode (YSI Model # 5775) was connected to the strip chart recorder (Omniscribe Model # 135116-4) by an electronic amplifying circuit driven by a 3V DC power source. The recorder was calibrated to zero by exposing the electrode to an aqueous solution to which sodium sulfite had been added to deplete the D.O. Regular calibration of the chart recorder was carried out using water samples with varying D.O contents, as determined by a D.O electrode (Orion Research Model # 97-08-00) connected to an analog pH meter (Orion Research Model #301). This was done by the step-wise addition of a sodium sulfite solution to a sample of water which had earlier been saturated with oxygen using pure oxygen. The calibration curve was used to convert strip chart readings to D.O values.

The D.O depletion studies on the biocatalysts were carried out by placing 5 beads with an aliquot of well aerated feed solution in the Gilson jacket. A capillary holed stopper was used to seal the opening and allow excess water to escape without entrapment of air. The D.O values were recorded continuously for a sufficiently long interval (typically ten minutes) during which the falling D.O inside the cavity of the water jacket was recorded. D.O values were recorded against time once steady state conditions had been reached. The D.O uptake was calculated by reducing the D.O. depletion to a per minute value.

Total and Soluble Chemical Oxygen Demand

Influent and effluent samples from the ICR and the ASR were tested for Chemical Oxygen Demand (COD) (HACH 1992). This was done using 'HACH' high range (0 - 1500 mg/L) COD tubes and a 'HACH' spectrophotometer (Model # DR/3). For the test, 2 ml of sample was added to the COD tube containing the reagents. The sample was digested at 150 degrees C for two hours in a 'HACH' COD oven (Model # 16500-10). A 'blank' tube containing 2 ml of distilled water was also prepared and digested. Once cooled, the tubes were placed in an adapter and inserted into the 'HACH' spectrophotometer (Model DR/3). A 'High range' COD template was used as a dial during the measurement. The wavelength of light used was 620 nm. The 'blank' was used to calibrate the meter to zero.

Soluble COD tests (SCOD) were carried out on the samples after filtering them through a 0.45 μm Rainin filter. The TCOD tests were carried out on unfiltered samples.

pH Measurement.

pH was measured routinely using an analog pH meter (Accumet Model # 900, Fisher Scientific) with a Fisher electrode. The electrode was calibrated using Fisher standard pH solution of pH 7 at the beginning of each set of tests as most pH values recorded varied in the range of 7.2 to 8.6.

Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS).

The TSS and VSS were measured as per the HACH method nos.8158 and 8164, respectively (HACH ,1991) as described in HACH's Handbook of Waste Analysis, 1991. These methods differ from the solids measurement in Standard Methods (APHA, 1982) in that the HACH method allows the use of aluminium dishes for the experiment whereas

Standard Methods (APHA, 1982) requires the use of porcelain crucibles. For these analyses, 100 ml samples were taken directly from the effluent ports of the reactors and were filtered using a vacuum filtration unit fitted with 47 mm dia. glass microfiber filters (Whatman, #934 - AH). A Fischer Scientific XA analytical balance was used for weighing. TSS and VSS of effluent samples of both the reactors were monitored every alternate day. Solids in the mixed liquor of the ASR were monitored about every 3 days by taking grab samples.

Scanning Electron Microscopy of Alginate Beads

Scanning Electron Microscopy was done on both plain beads and beads containing entrapped biomass with the help of OSU Veterinary Medicine Dept. personnel (Pennington, 1991). The electron microscope used for this purpose was a JEOL 35 U. The purpose of this investigation was to examine the growth pattern and distribution of microbes inside the polymer matrix. Plain beads were freshly made for this investigation. For the beads containing biomass, beads were taken out of the working reactor twenty days after their manufacture.

The beads were put in distilled water after washing them thoroughly to prevent interference from attached biomass. The beads were placed in a fixative (3% glutaraldehyde and 3% sucrose) for three hours. The beads were then washed thoroughly with a 0.2 M solution of sodium cacodylate buffer (pH 7.4) and were left in the refrigerator overnight. The beads were then dehydrated in a graded series (50,70,90,95,100%) of ethanol going up to 100% concentration and frozen with liquid nitrogen. The beads were then fractured with a razor blade. The pieces were then put back in 100 % ethanol and dried to the critical point required for electron microscopy. The acceleration voltage used for the electron microscopy was 25 KV. Considerable shrinkage was observed in the

calcium alginate beads as these are hydrated gels and in the process of drying they lost most of the water contained in them.

Identification of the Bacterial Strains

In order to identify the viable strains of the bacteria which resulted from the activated sludge seed after acclimation to 2,4-DAT, microbiological assays were conducted with the help of Dr. Grula (1991) of the Microbiology Department of Oklahoma State University (See Appendix C). Based on the type of cells typically present in a complex synthetic wastewater like that from the plastic industry, it was initially suspected that the bacterial culture would consist primarily of *Pseudomonas* sp. Specific tests for confirmation of the strains were done in addition to the more general ones that could identify a genus.

A sample from the acclimation culture was used to make several dilutions in the ranges from 10^3 to 10^7 viable cells per ml using distilled water. Three pour plates of each dilution were made with nutrient agar media containing beef liver extract. Two of these were incubated at 30 ^{o} C and the remaining one at room temperature. Three distinct colonies based on color and morphology in most of the plates were observed. These were labelled as White Smooth (WS), Yellow Smooth (YS) and Yellow Ridged (YR). A sample from each of the colonies was streaked onto fresh nutrient agar slants and by successive streakings, essentially pure, homogeneous cultures were obtained. The agar slants were made and stored at 4^{o} C. Tests conducted on these cultures are listed in Appendix C.

Growth of Microbes in the Alginate Beads

Attempts were made to determine if there was growth of the microbial population inside the alginate beads. Since alginate dissolves in sodium hexametaphosphate, thus

releasing all the entrapped microbes, this method was used. This method was used by Keweloh et. al. (1989).

A batch of 40 freshly made beads was separated from the liquid medium of the reactor and washed with 0.05 M potassium phosphate buffer, pH 7.0 for 5 minutes. They were then dissolved by shaking in a 0.05 M sodium hexametaphosphate solution for 15 minutes. The resulting liquid was filtered using a 0.45μM glass microfiber filter. The TSS and VSS of the retained matter were then determined. Another batch of 40 beads from the original lot was taken out of the reactor after 35 days of operation. These beads were then dissolved (as above) and the solids were determined. The difference in the VSS values of the old and the fresh beads were used as an estimate of cell growth inside the beads.

CHAPTER IV

RESULTS AND DISCUSSION

The Objective of the Investigation

The aim of the investigation was to compare a bench scale ICR with an ASR in order to gain a better understanding of immobilized cell systems and to assess the response of both units under different operating conditions (increasing toxic compound concentrations and decreasing HRT's). This investigation also addressed the desired range of operating conditions and the removal of TCOD, SCOD and the target compound 2,4-DAT. These objectives were pursued by conducting the investigation in two phases.

The first phase of the investigation was conducted by increasing the concentration of the toxic compound 2,4-DAT from 50 to 250 mg/L (in increments of 50mg/L) in the influent to the two reactors while maintaining the hydraulic retention time constant at 10 hours for both. This phase of the investigation addressed the maximum influent 2,4-DAT concentration that could be treated and the maximum removal of TCOD, SCOD and 2,4-DAT at steady state conditions.

The second phase of the investigation was conducted at a 2,4-DAT concentration of 150 mg/L. This represented an optimal reactor performance as found in the first phase of the study - characterized by very steady removals in terms of COD's and 2,4-DAT by both reactors. The hydraulic loading rates were then increased by lowering the HRT in steps (8, 5 and 3 hours). This was achieved by increasing the feed flow rates to the reactors. The HRT's for both reactors were kept the same at any given time during the

investigation (to ensure similar volumetric loading rates) for the purpose of comparison. This part of the study addressed both the highest loading and removal rates of the TCOD, SCOD and 2,4-DAT before either treatment efficiency dropped significantly or hydraulic instability occurred in one of the reactor systems.

During both phases of the investigation, samples collected from the units were analyzed for the same parameters, except for DO uptake measurements which were carried out only on the biocatalysts of the ICR. The data collection was continuous throughout the period of the research. This enabled not only the final treatment efficiencies to be examined but also the time it took to reach steady state and other responses exhibited by the reactor systems. The HRT's of each of the two reactors were calculated on the basis of their total liquid reactor volumes. The desired feed flow rates required to achieve these conditions were then calculated. Total volume of the ICR was considered because the amount of alginate used to make the beads for one batch was negligible compared to the volume of the reactor vessel. Also, since alginate formed a hydrated gel, a large fraction of the volume of the matrix was taken up by water.

The results of the research have been presented in both graphical and tabular forms. The graphical presentation includes all data collected. The tabular presentation focuses on the steady state performance of the reactor systems and shows the average values at steady state conditions. Observations on the general behavior of both treatment systems have also been presented at relevant places in this chapter.

Trial Reactor Configuration

Prior to selecting a continuously stirred tank configuration for the immobilized cell reactor, an expanded bed reactor with an upflow configuration was tested. It consisted of a tubular glass reactor with a perforated base plate through which the liquid was recirculated (See Appendix A). Aeration was done externally in a glass aerator. The liquid phase

exited the reactor for recirculation at the top of the reactor, and passed through the aerator before going to the recirculation pump. The feed to the system was injected at the base of the reactor using a separate feed pump. The effluent from the system was taken out by overflow from the top of the reactor. This reactor was operated over many weeks but it suffered from several operational problems. The main disruption in the operation of this reactor was caused by an apparent generation of large quantities of solids in the liquid phase. It was not established whether the solids were cell debris, biomass or extra-cellular solids produced by the microbes. The slimy white growth plugged the column in about three days after the commencement of operation. Several attempts were made to rectify the situation including periodic agitation of the bead bed by allowing small air bubbles to pass through the bed. In spite of all these efforts the solids growing outside the beads choked the recirculation system and caused the system to fail. The upflow expanded bed was finally abandoned in favor of a continuously stirred tank configuration.

The Compound Dose Increase Study

In this part of the study (phase 1), the 2,4-DAT concentration was increased from 50 mg/L to 250 mg/L in steps of 50 mg/L (at a constant HRT of 10 hr) in both the ICR and the ASR. During the course of this phase of the experiment, the TCOD and the SCOD loading rates varied from 1.48 to 2.21 g/L*d and 1.42 to 2.15 g/L*d, respectively in the ICR and from 1.5 - 2.24 g/L*d and 1.44 - 2.17 g/L*d, respectively in the ASR (See Table III). The change in the COD loadings was solely due to the increasing concentrations of 2,4-DAT in the influent as no change in the base mix COD concentration was made when increasing the influent 2,4-DAT concentration. At each influent 2,4-DAT concentration (i.e. 50, 100, 150, 200 and 250 mg / L), the reactors were run until removal of the main parameters stabilized and a steady state condition was observed. A minimum of three readings was taken at each steady state condition before moving on to the next

influent 2,4-DAT concentration. The operating parameters of the reactors for this part of the study, including the average steady state TCOD and SCOD values at each compound concentration level, are shown in Table III. A constant amount of biomass was immobilized in the beads during the experiments. The value of 20 grams of wet biomass (used for immobilization at the time of bead manufacture) was chosen since this represented a very high value of biomass which was more than what could normally exist on a per volume basis in an ASR. Similar large amounts of biomass have been used by another researcher (Sofer et al , 1990). In addition, the round figure of 20 grams was selected based on the ease of harvesting the cells from the 8 L culture stock.

Attempts were made to maintain a Solids Retention Time (SRT) in the ASR of 6 days by wasting solids from the ASR on a daily basis. The amount of solids wasted was calculated based on the latest determination of the MLVSS in the reactor and the effluent (as per the equation given in Chapter III). Maintaining a stable SRT proved difficult because the sludge continually underwent sudden physical changes. In spite of this, the SRT was maintained at approximately 6 days. It was not possible to determine the SRT in the ICR as the rate of cell growth inside the beads could not be determined on a day to day basis. For both the ASR as well as the ICR, the HRT was computed using the total reactor volume. For the ASR this was done according to guidelines set out by Reynolds (1982) (See Chapter II).

TCOD and SCOD removal.

The average steady state influent TCOD's and SCOD's varied from 625 - 935 mg/L and from 600 - 907 mg/L, respectively (Table IV) during the course of phase I of investigation. The TCOD and SCOD values of the ICR effluent varied in the ranges from 155 - 472 mg/L and 82 - 222 mg/L, respectively. In the case of the ASR, the effluent TCOD and SCOD varied in the ranges from 185 - 707 mg/L and 118 - 635 mg/L,

TABLE III

OPERATING PARAMETERS DURING THE COMPOUND DOSE INCREASE STUDY

Reactor type and related parameters	DAT Dose (mg/L)	Av. Influent COD (mg/L)		COD Loading (g/L.d)		S R T (days)	Immobilized* biomass / MLVSS** (gm)
		TCOD	SCOD	TCOD	SCOD		
Immobilized Cell reactor (ICR)	50	625	600	1.48	1.42	— —	20
Volume = 1120 ml	100	788	768	1.93	1.88	— —	20
H R T = 10.14 hr	150	876	857	2.09	2.04	— —	20
Flow rate = 2.65 L/d	200	927	907	2.18	2.13	— —	20
	250	936	913	2.21	2.15	— —	20
Activated Sludge Reactor (ASR)	50	625	600	1.50	1.44	6.0	3 7
	100	788	768	1.95	1.91	6.0	3.8
Volume = 4556 ml	150	876	857	2.12	2.07	5.8	7.5
HRT = 9.99 hr	200	927	907	2.21	2.16	6.0	5.6
Flow rate = 10.94 L/d	250	936	913	2.24	2.17	6.1	5.3

* mass of biomass immobilized ** for ASR only

TABLE IV

COD REMOVAL DURING THE COMPOUND DOSE INCREASE STUDY

2,4 DAT Dose (mg/L)	Days of operation	Influent COD		Immobilized Cell Reactor (ICR)		Activated Sludge Reactor (ASR)	
		TCOD (mg/L)	SCOD (mg/L)	Eff. TCOD (mg/L)	Eff. SCOD (mg/L)	Eff. TCOD (mg/L)	Eff. SCOD (mg/L)
50	1 - 13	625	600	155	82	185	118
100	15 - 29	815	795	182	105	227	115
150	31 - 48	883	863	290	148	315	243
200	51 - 75	923	903	363	172	485	448
250	85 - 107	935	907	472	222	707	635

respectively. The TCOD and SCOD removals are given in Tables V (for the ICR) and VI (for the ASR). The maximum removal (in terms of percentage of loading rate) of both TCOD and SCOD for the ICR and ASR took place at the 2,4-DAT dose of 100 mg/L and steadily decreased at the higher concentrations of 2,4-DAT (See Figures 7 and 8 and Tables V and VI). The highest TCOD removal rates for the ICR and the ASR in this phase of the study were 1.5 and 1.4 gm/L*day, respectively. For the ICR this rate corresponded to 77.7 % removal (Table V), while for the ASR the maximum rates occurred twice, and corresponded to 72.3 and 64.6 % removals. In terms of SCOD, the highest removal rates obtained for the ICR was 1.72 gm/L*day and corresponded to a removal of 80.8 %. For the ASR, the maximum removal rate was 1.64 gm/L*day and corresponded to 85.9 % removal. The TCOD and SCOD removal rates observed in the ICR were equal to or greater than (within a range considered significant) those seen in the ASR under all operating conditions tested in this phase of the study. Since the ICR and ASR produced different quantities of solids in the liquid phase, SCOD removal was considered to be a more representative and accurate measure for comparing the performance of the ICR with the ASR as opposed to TCOD values.

Referring to the TCOD and SCOD plots (Figures 9 and 10) and to Tables V and VI, it can be seen that both the ICR and ASR exhibited similar removals up to an influent concentration of 150 mg/L of 2,4-DAT. However, at an influent concentration of 200 mg/L, the plots for the effluent TCOD and SCOD's (Figures 9 and 10) of the ASR and the ICR started to separate and the plots diverged and stabilized only when steady state was reached about two weeks after the start of this loading. At an influent concentration of 250 mg/L, the plots diverged still further and the ASR's performance was much poorer as compared to the ICR. Figures 9 and 10 show a sharp increase in the effluent TCOD and SCOD of the ASR at this influent concentration. The considerable divergence in the TCOD as well as the SCOD plots (when influent 2,4-DAT dose was raised to 250 mg/L) towards the end of the run clearly indicates that the ASR was stressed due to the high

TABLE V

COD REMOVAL DURING THE COMPOUND DOSE INCREASE STUDY

2,4 DAT Dose (mg/L)	Days of operation	Influent COD Loading Rates (g/L * day)		Immobilized Cell Reactor (ICR)			
				Removal Rates (g/L * day)		% Removal	
		TCOD	SCOD	TCOD	SCOD	TCOD	SCOD
50	1 - 13	1.48	1.42	1.1	1.23	74.3	86.6
100	15 - 29	1.93	1.88	1.5	1.63	77.7	86.7
150	31 - 48	2.09	2.04	1.4	1.69	67.0	82.9
200	51 - 75	2.18	2.13	1.3	1.72	61.0	80.8
250	85 - 107	2.21	2.146	1.1	1.62	49.8	75.7

TABLE VI

COD REMOVAL DURING THE COMPOUND DOSE INCREASE STUDY

2,4 DAT Dose (mg/L)	Days of operation	Influent COD Loading Rates (g/L * day)		Activated Sludge Reactor (ASR)			
				Removal Rates (g/L * day)		% Removal	
		TCOD	SCOD	TCOD	SCOD	TCOD	SCOD
50	1 - 13	1.50	1.44	1.1	1.16	70.7	80.6
100	15 - 29	1.95	1.91	1.4	1.64	72.3	85.9
150	31 - 48	2.12	2.07	1.4	1.49	64.6	72.0
200	51 - 75	2.21	2.16	1.1	1.09	47.5	50.5
250	85 - 107	2.24	2.17	0.5	0.65	24.6	30.0

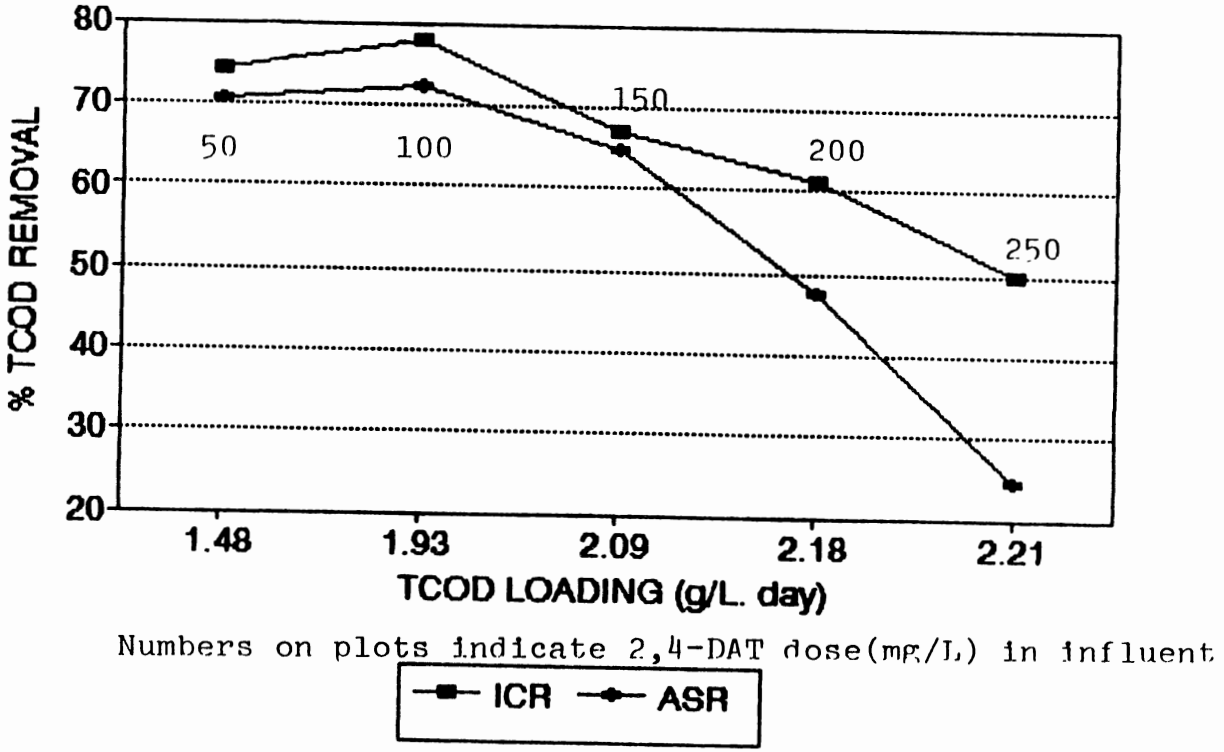


Figure 7. TCOD Removal vs. Loading Rates (Compd.Dose Increase Study)

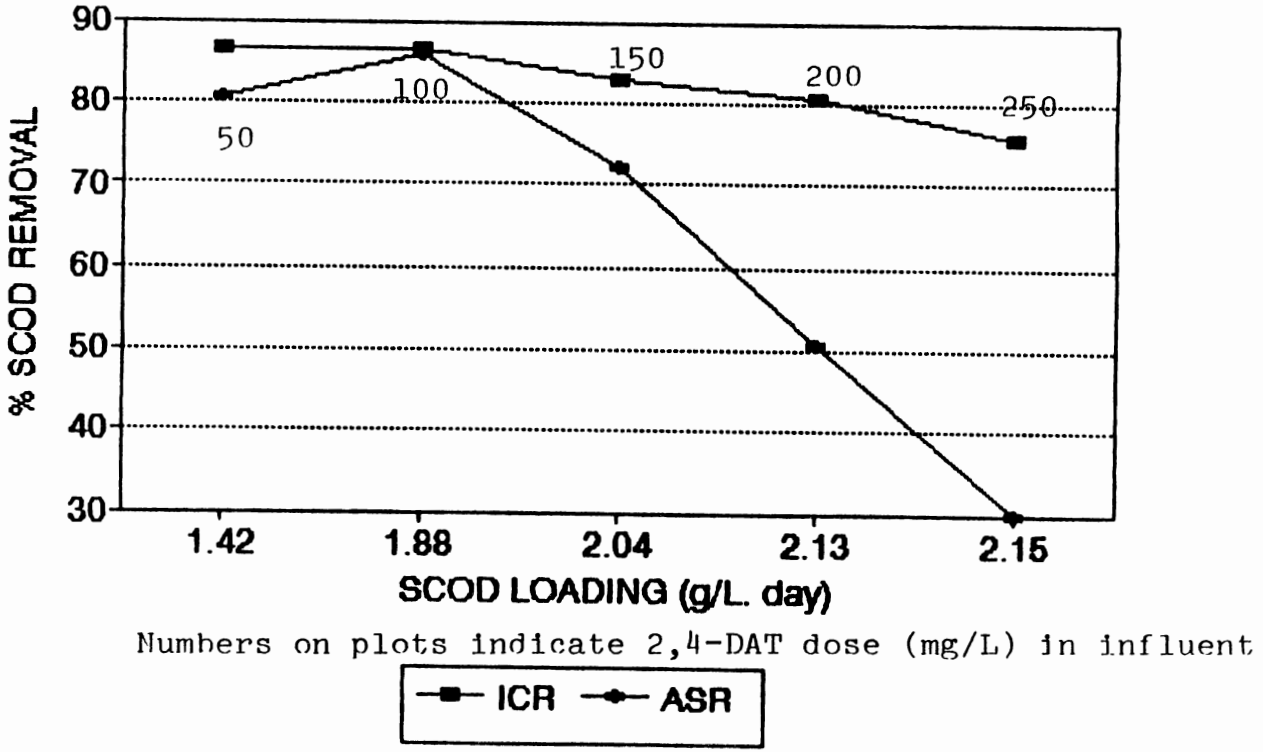


Figure 8. SCOD Removal vs. Loading Rates

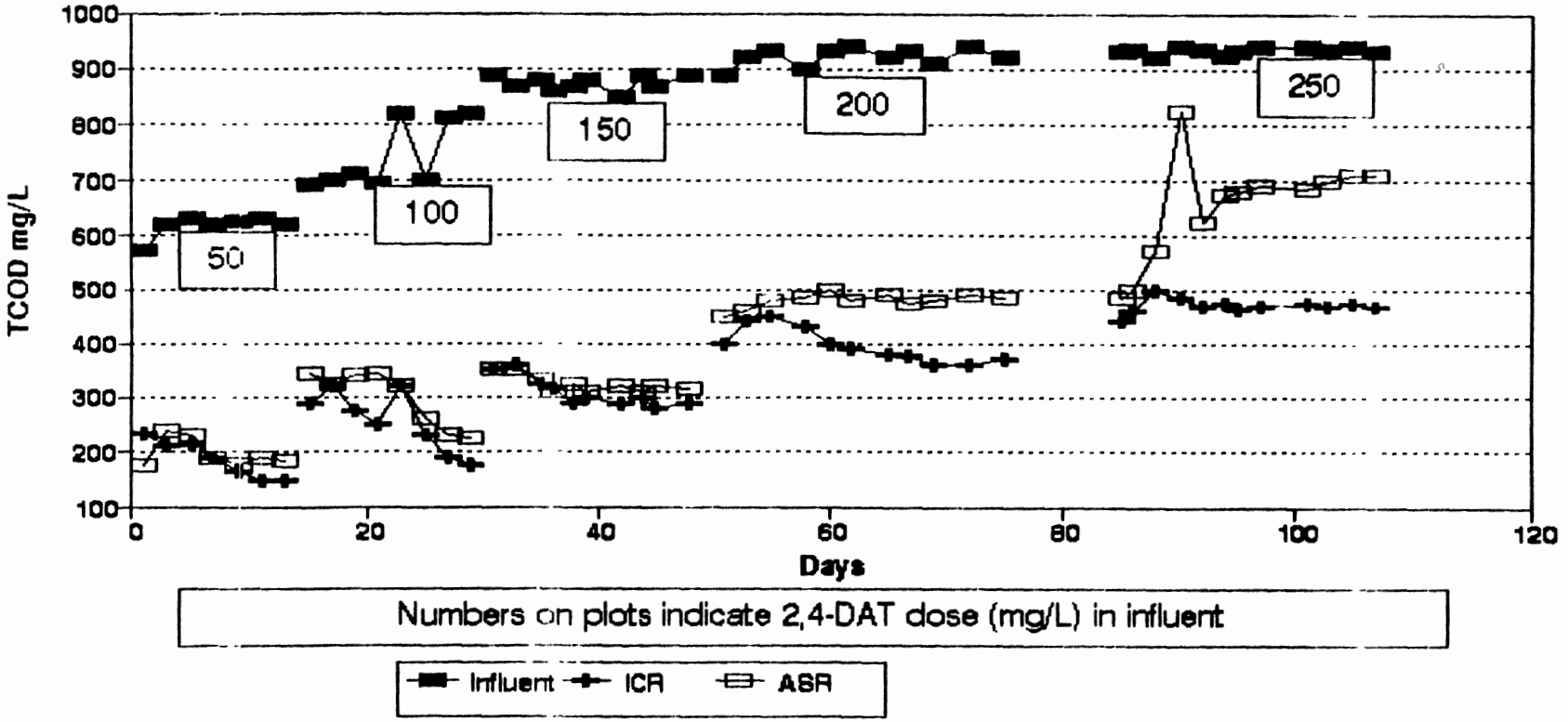


Figure 9. Effluent TCOD Concentrations of ICR and ASR

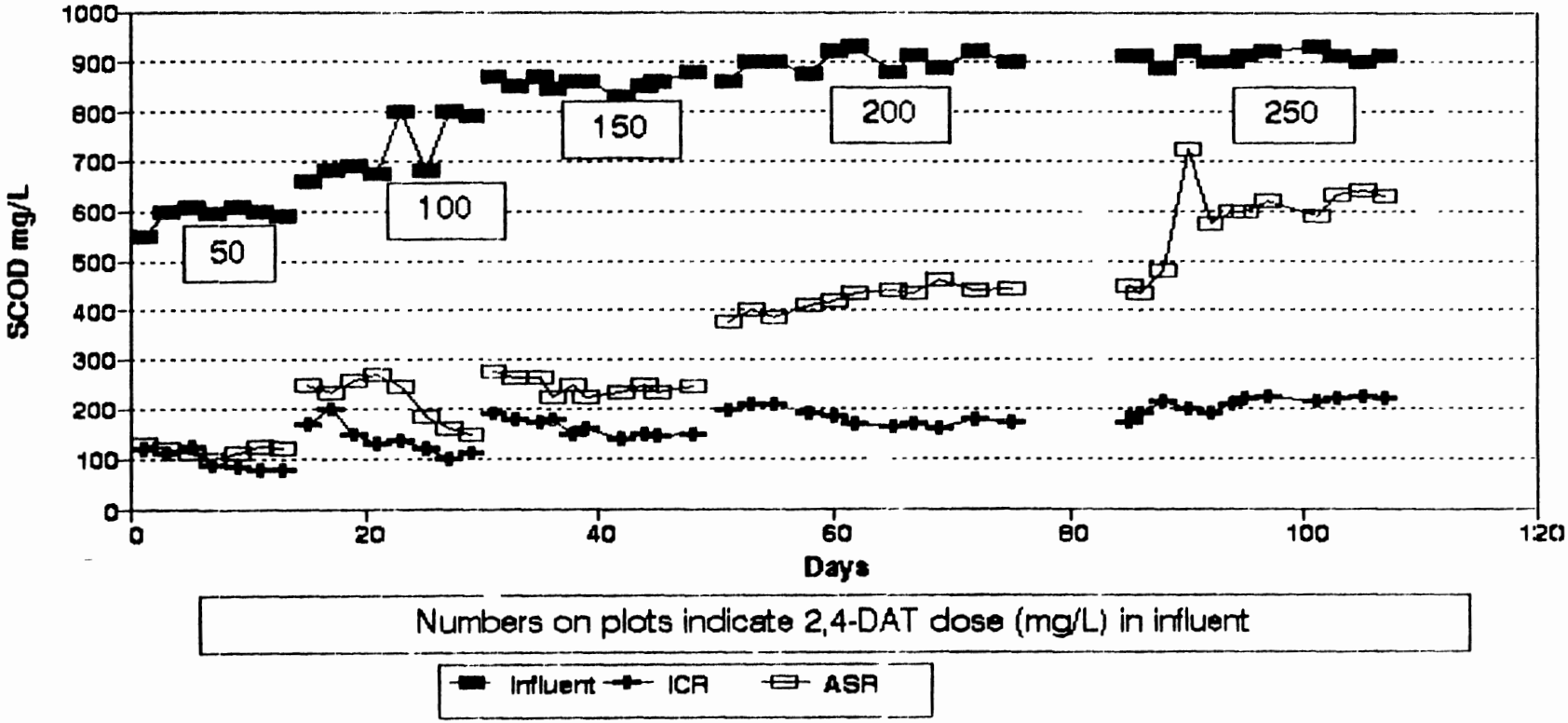


Figure 10. Effluent SCOD Concentrations of ICR and ASR

concentrations of 2,4-DAT, while the ICR was not as stressed. A possible reason for this could be that the entrapment of the microbes inside the alginate matrix shielded them from the toxic effect of high concentrations of 2,4-DAT present outside the beads in the liquid phase. Because of this shielding effect, immobilized cell systems tolerate higher concentrations of target pollutants better than activated sludge systems. The ability of immobilized cell systems to withstand higher pollutant concentrations as compared to free cell systems has been observed in other studies (Keweloh et al 1989) and is supported by this investigation.

2,4-DAT Removal.

The concentration of 2,4-DAT in the influent was increased from 50 mg/l to 250 mg/l in steps of 50 mg/L. A summary of the influent and effluent concentrations for both reactors is shown in Table VII. Tables VIII and IX show the percentage removals of 2,4-DAT from the ICR and ASR under all five 2,4-DAT influent concentrations to which the systems were subjected during this phase of the investigation. The effluent concentration for the ICR varied from 34.1 to 224.4 mg/L, thus representing a variation in removal (Table VIII) ranging from 33.3 to 10.2 %. In case of the ASR, the effluent 2,4-DAT concentration varied from 34.0 to 247.9 mg/L for the lowest and the highest values of the influent 2,4-DAT concentrations, respectively. This represented a variation in removal (Table IX) in the range of 38.5 to 1.7 %. The percentage removal for both reactors fell as the influent 2,4-DAT loading increased. Still, the removal for the ICR was better compared to the ASR, except at an influent 2,4-DAT concentration of 50mg/L when the ASR gave better removal compared to the ICR (38.5% vs. 33.3%). At the next higher concentration of 100 mg/L, the performance of the ICR improved over that of the ASR and as the influent 2,4-DAT concentration was increased, this difference in percentage removal between the ICR and the ASR also increased. At an influent concentration of 250 mg/L,

TABLE VII

2,4 DAT REMOVAL DURING THE COMPOUND DOSE INCREASE STUDY

Loadings	Days of operation	Influent Conc. (mg/L)		ICR	ASR
		Dosed	Measured	Effluent Conc mg/L	Effluent Conc. mg/L
1	1 - 13	50	52.8	34.1	34.0
2	15 - 29	100	97.0	69.1	70.5
3	31 - 48	150	152.4	121.0	135.8
4	51 - 75	200	200.7	147.6	177.7
5	85 - 107	250	250.0	224.4	247.9

Flow Rates:

Immobilized Cell Reactor : 2.65 L / day
Activated Sludge Reactor : 10.94 L / day

TABLE VIII

2,4 DAT REMOVAL DURING THE COMPOUND DOSE INCREASE STUDY.

DAT Dose (mg/L)	Days of operation	DAT dose (mg/L) (Measured)	Influent Loading rate (g/L * day)	Immobilized Cell Reactor		
				Effluent Conc (mg/L)	Removal Rate (g/L * day)	% Removal
50	1 - 13	52.8	0.12	34.1	0.04	33.3
100	15 - 29	97.0	0.23	69.1	0.07	30.4
150	31 - 48	152.4	0.36	121.0	0.07	19.4
200	51 - 75	200.7	0.47	147.6	0.12	25.5
250	85 - 107	250.0	0.59	224.4	0.06	10.2

Flow Rates:
Immobilized Cell Reactor : 2.65 L / day
Activated Sludge Reactor : 10.94 L / day

TABLE IX

2,4 DAT REMOVAL DURING THE COMPOUND DOSE INCREASE STUDY

DAT Loadings (mg/L)	Days of operation	DAT dose (mg/L) (Measured)	Influent Loading rate (g/L * day)	Activated Sludge Reactor		
				Effluent Conc (mg/L)	Removal Rate (g/L * day)	% Removal
50	1 - 13	52.8	0.13	34.0	0.05	38.5
100	15 - 29	97.0	0.23	70.5	0.06	26.1
150	31 - 48	152.4	0.37	135.8	0.04	10.8
200	51 - 75	200.7	0.48	177.7	0.05	10.4
250	85 - 107	250.0	0.60	247.9	0.01	1.7

Flow Rates:
Immobilized Cell Reactor : 2.65 L / day
Activated Sludge Reactor : 10.94 L / day

the removal given by the ASR was only 1.7 % compared with a 10.2% value for the ICR (See Figure 11). The highest 2,4-DAT removal rates obtained in this portion of the study were 0.12 gm/L*day for the ICR and occured at influent 2,4-DAT concentrations of 200mg/L (See Table VIII).

From examination of the graph showing the removal of 2,4-DAT during phase I of the study (Figure 12), the effluent 2,4-DAT concentration increased for both reactors as the 2,4-DAT dose was increased. The influent 2,4-DAT concentration was measured just like the effluent samples using HPLC to account for any possibility of its removal due to microbial growth and photodegradation in the feed bottle. Table VII lists both the actual dosed concentration as well as the measured concentration of 2,4-DAT in the influent. There was an initial sharp drop in the 2,4-DAT concentration in the effluent of both reactors at an influent 2,4-DAT concentration of 100 mg/L (See Figure 12), after which it stabilized and reached a steady state. At influent 2,4-DAT levels lower than 150 mg/L, the plots for the effluent 2,4-DAT concentrations for both the ASR and ICR are almost coincidental; but as the influent 2,4-DAT concentration was increased, its removal in both reactors gradually differed with the ICR giving better removals compared to the ASR. However, at an influent 2,4-DAT concentration of 250 mg/L, both the ICR and the ASR exhibited stress in the form of reduced 2,4-DAT removal. Still, at this dose, the removal by the ICR was better than that by the ASR which showed almost no removal toward the end of this run. The diverging plots of the ICR and ASR effluent 2,4-DAT concentrations (Figure 12) indicate that at increased 2,4-DAT concentrations, the ICR exhibited better removals as compared to the ASR. On the 37th day of this phase of the study, the beads in the ICR disintegrated and had to be replaced with freshly manufactured beads. As can be seen in Figure 12, this change in the middle of the run had only a minor impact on the effluent 2,4-DAT removal.

The mechanism of removal of 2,4-DAT is not very clear. 2,4-DAT is known to both biodegrade as well as photodegrade, and for this reason, the feed bottles were kept

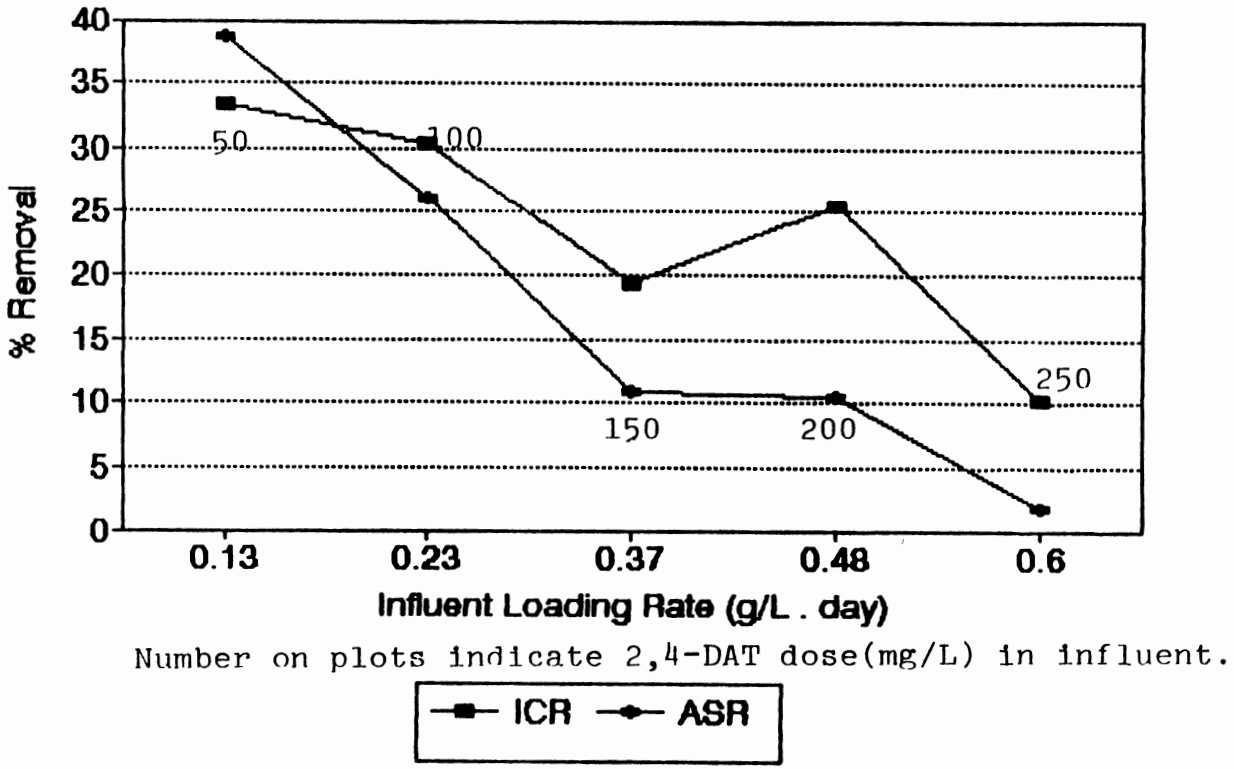


Figure 11. 2,4 DAT Removal Rates vs. Loading Rates

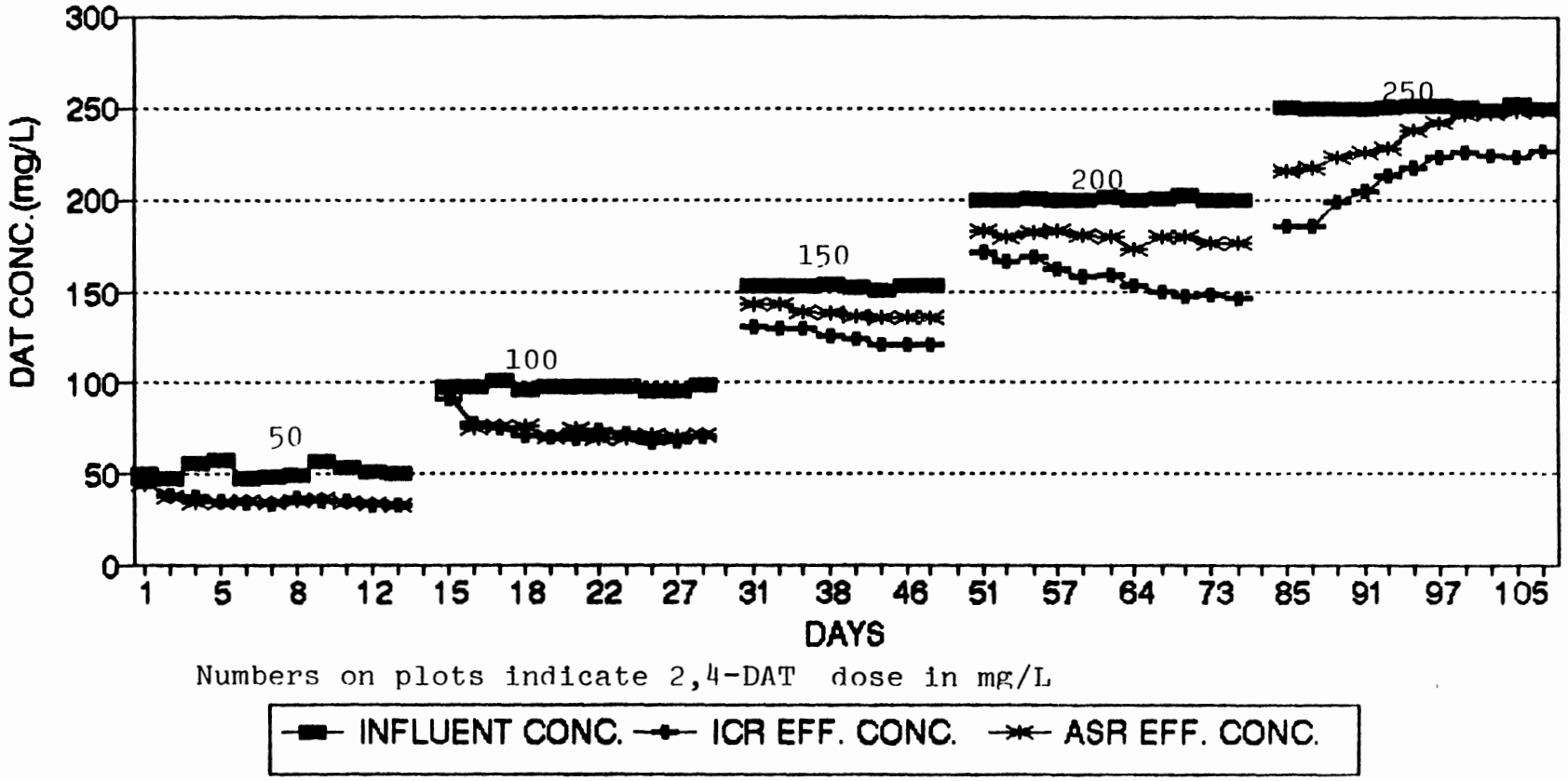


Figure 12. Effluent DAT Concentrations of ICR and ASR

covered during all the experimental runs. In a preliminary experiment where two solutions of 2,4-DAT having concentrations of 50 and 100 mg/L were exposed to ambient light conditions inside the laboratory for 24 hours, no removal of 2,4-DAT was detected. The 2,4-DAT removal plots (See Figure 12) show that both reactors achieved a steady state after an initial period of transition during which the 2,4-DAT in the effluent was larger than the steady state effluent concentration. The only exception to this observation was at an influent concentration of 250 mg/L 2,4-DAT. For both reactors, stabilization took longer at the higher doses.

Effluent Solids and pH.

The feed in this investigation was a simulated synthetic wastewater which was prepared by mixing chemicals (See Chapter III) in tapwater. The solids in the influent were, for this reason, negligible. Varying amounts of solids were produced in the effluent from both the ASR and ICR. The effluent TSS and VSS for both the ICR and ASR increased with the 2,4-DAT concentration up to an influent 2,4-DAT value of 150 mg/L (Table X and Figures 13 and 14). For influent 2,4-DAT concentrations of 50 and 100 mg/L a steady increase in the TSS and VSS values of the ASR can be seen (Figure 13). This increase in more pronounced towards the latter part of the 100 mg/L influent 2,4-DAT concentration run. At the 150 mg/L 2,4-DAT dose, the solids in the effluent remained at a fairly steady level. At an influent dose of 200 mg/L 2,4-DAT, both the ICR and ASR appeared to display a variation in the effluent solids with the TSS and VSS values first decreasing and later increasing. At an influent 2,4-DAT concentration of 250 mg/L, the effluent solids in the ASR dropped steadily. These plots (Figure 13) show that for 150 mg/L 2,4-DAT concentration in the influent, the effluent solid levels were most stable

The presence of solids in the effluent of the ICR at varying 2,4-DAT concentrations is shown in Figure 14. The steady state numerical values are given in Table X. For the

TABLE X

EFFLUENT SOLIDS AND EFFLUENT pH - COMPOUND DOSE INCREASE STUDY

2,4 DAT Loading mg/L	Days of Operation	Immobilized Cell Reactor (ICR)			Activated Sludge Reactor (ASR)		
		TSS (mg/L)	VSS (mg/L)	pH	TSS (mg/L)	VSS (mg/L)	pH
50	1 - 13	10 - 41	3 - 16	8.3 - 7.8	15 - 28	3 - 10	8.0 - 7.7
100	15 - 29	42 - 98	18 - 79	8.5 - 8.2	27 - 65	9 - 46	7.9 - 7.3
150	31 - 48	25 - 92	15 - 76	8.2- 8.0	50 - 62	45 - 49	7.9 - 7 6
200	51 - 75	44 - 63	20 - 57	8.2 - 7.3	40 - 70	26 - 60	7 9 - 7.5
250	85 - 107	37 - 42	27 - 37	8.2 - 7 7	47 - 86	41 - 76	7 9 - 7 4

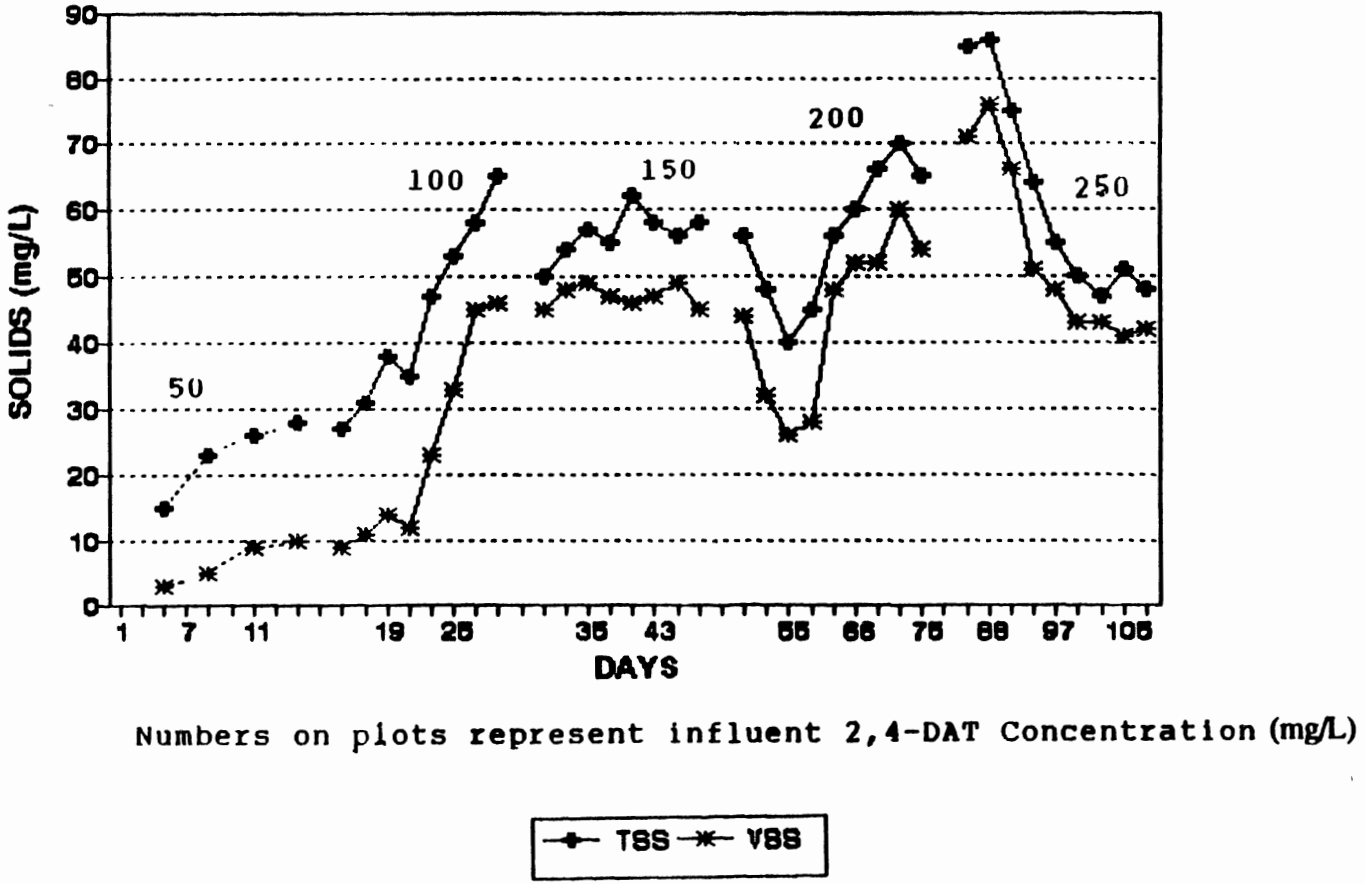


Figure 13. Effluent Solids (ASR)

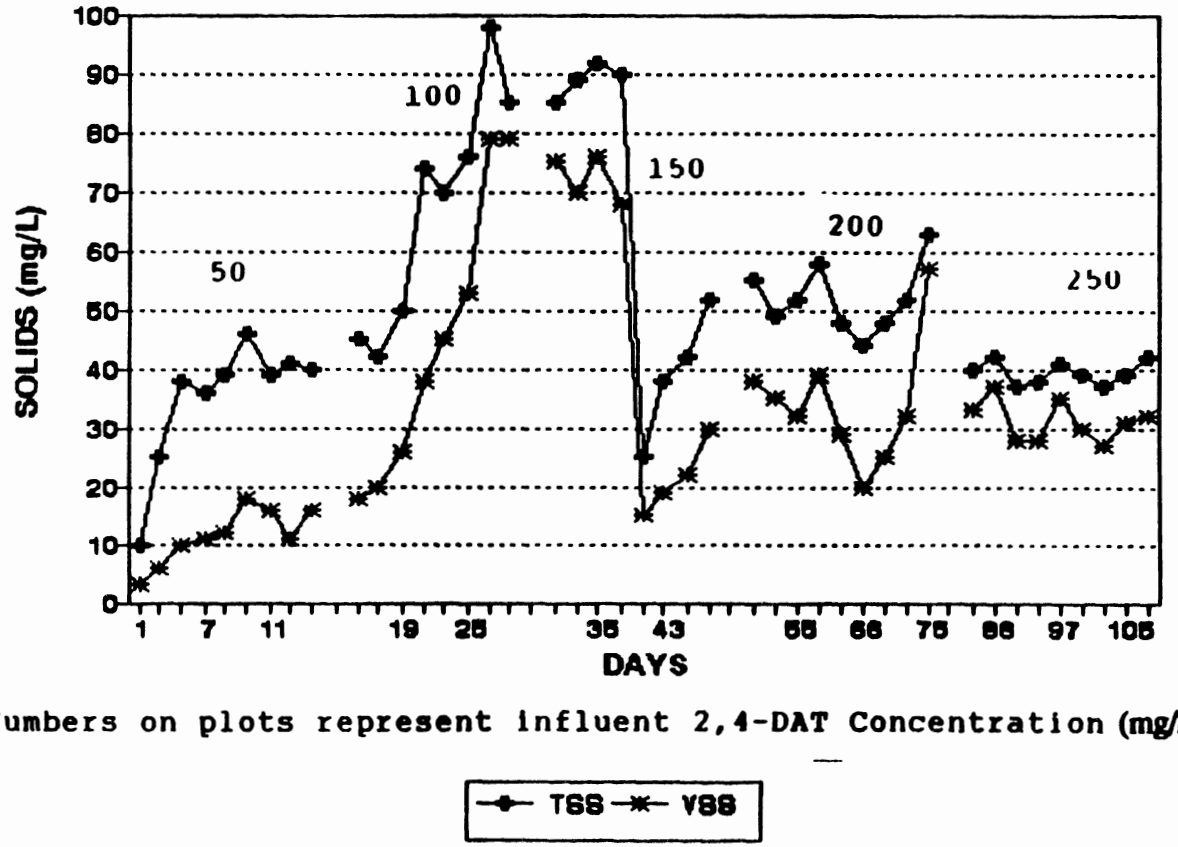


Figure 14. Effluent Solids (ICR)

first two loadings, 50 and 100 mg/L 2,4-DAT, both the TSS and VSS rose steadily. This rising trend continued during the 150 mg/L 2,4-DAT concentration for the first four readings. At this point, on the 37th day of the run, the liquid phase in the reactor became full of solids and there was a large amount of solid deposition on the walls of the reactor and the tubing. A large number of beads had also aged and broken. For this reason, new beads had to be manufactured and the reactor cleaned up. A sharp drop in the TSS and VSS values can be seen at this point (See Figures 9 and 14). Beyond this point the solids started to increase again and at the concentrations of 200 and 250 mg/L 2,4-DAT a virtual levelling off of the effluent solids concentration was seen. Within these ranges, the solids varied with time but a definite overall increase or decrease did not occur.

The pH profiles of the effluent from the ICR and the ASR are shown in Figure 15. The pH of the effluent from the ICR remained, slightly more basic as compared to that of the ASR at influent 2,4-DAT concentrations of 50 and 100 mg/L. The pH values of the ICR effluent remained mostly within the range 7.7 to 8.5 except at 2,4-DAT influent concentration of 200 mg/L when larger than usual deviations were observed. The influent pH varied in the range of 7.8 to 8. The pH of the effluent from the ICR did not show a change before the beads broke (37th day of the run; See Figures 9 and 14). The pH data from either of the reactors did not follow a pattern from which any concrete conclusion could be drawn.

Internal solids in the ASR were measured two to three times a week. The TSS ranged from 600 mg/L at an influent 2,4-DAT dose of 100 mg/L to *ca.* 1980 mg/L at an influent dose of 150 mg/L. These data were used to calculate the solid wastage from the ASR as described in Chapter III. The internal solids data obtained are shown in Figure 16. Under the first three concentrations (50, 100 and 150 mg/L 2,4-DAT), an increase in the internal solids of the reactor was seen. After starting the dose of 200 mg/L 2,4-DAT, the solids in the reactors decreased and stabilized. Maintaining the solids in a narrower range proved difficult because of the anomalous characteristics exhibited by the sludge. These

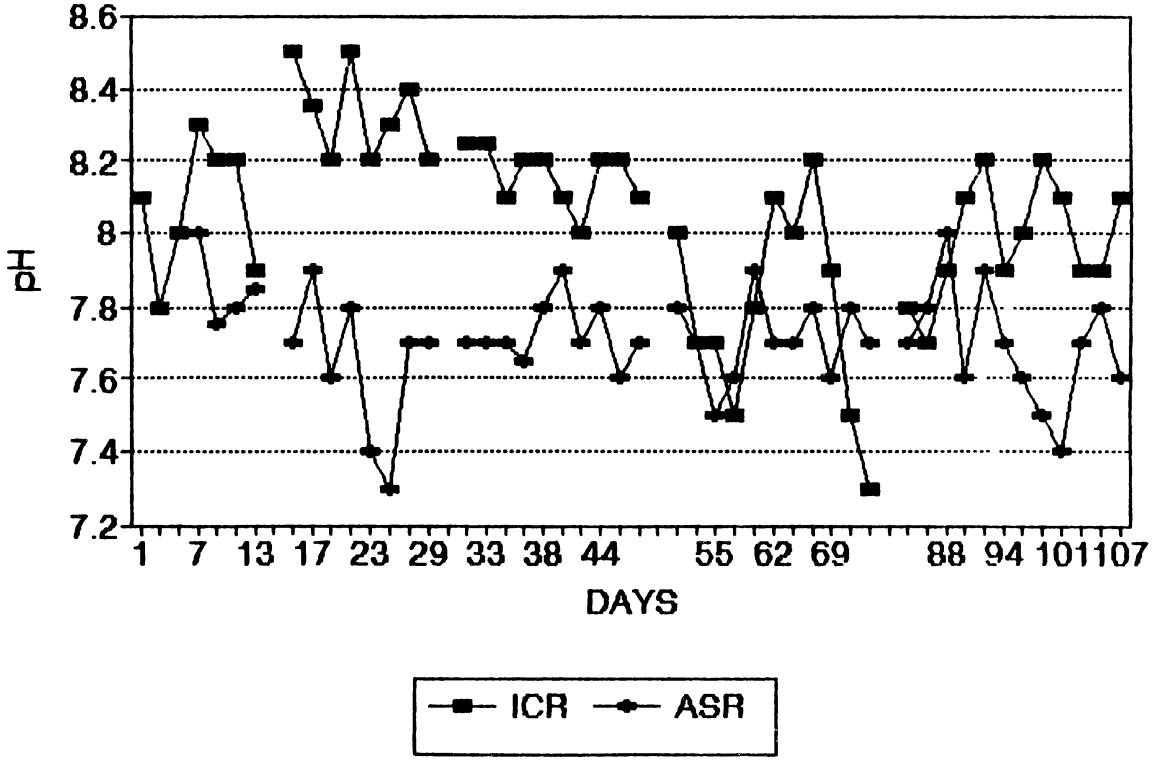


Figure 15. pH Values (Compd. Dose Increase Study)

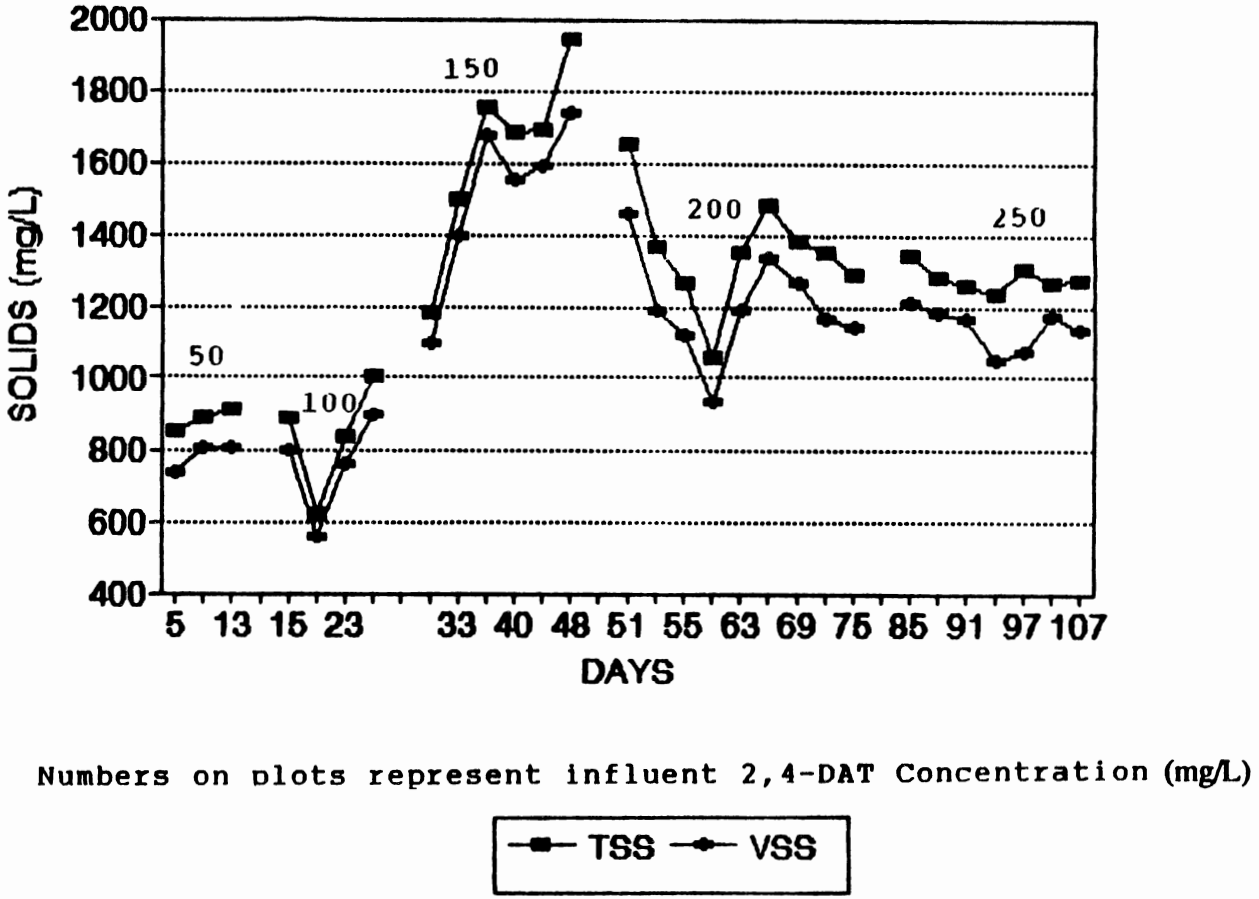


Figure 16. Internal Solids for the ASR

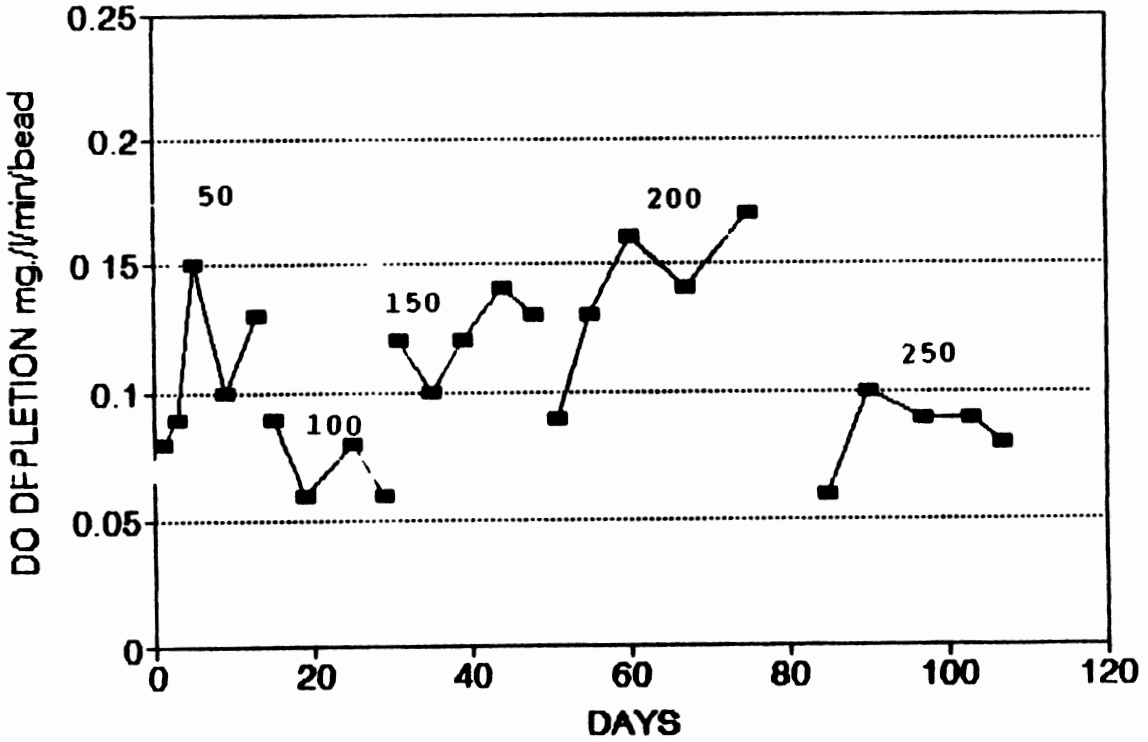
sludge characteristics may have resulted due to the production of extracellular matter by the bacteria which is believed to have caused the anomalous behavior of solids growth in the ASR, resulting in the fluffiness of the biomass. The exact conditions and rate of production of the extracellular solids were not known.

DO uptake by alginate biocatalysts.

The D.O uptake values have been proposed to be an indicator of microbial activity inside the biocatalysts (Sofer et al, 1990; Mattiason, 1983). The DO uptake by the microbes in the beads remained fairly constant throughout the study. The D.O. uptake rates were in the range 0.18 - 0.06 mg/L*min*bead. Only at doses of 100 mg/L and 250 mg/L 2,4- DAT was the DO uptake observed to have lower values than at the other concentrations (See Figure 17). The drop at 100mg/L 2,4-DAT appears to have resulted from the microbes being exposed to a higher concentration of the compound (2,4-DAT) for the first time. The drop at 250 mg/L 2,4-DAT could have resulted from the toxic effect of 2,4-DAT.

The HRT Study

At the end of the phase I of the study, the ASR continued to be operated at 250 mg/L 2,4-DAT concentration until the start of the 8 hour HRT. Since the beads in the ICR had disintegrated partially at the end of the compound dose increase study, fresh beads were prepared and the ICR was started. The ICR was run for two days at an influent 2,4-DAT concentration of 250 mg/L before reducing it to 150 mg/L. Data collection was started two days after this. In this part (phase 2) of the study, the HRT of both the ICR and ASR was sequentially lowered from 8 hr to 3 hr. At all the HRT's, the influent 2,4-DAT concentration was maintained at 150 mg/L and the base mix concentration was the



Numbers on plots represent influent 2,4-DAT Concentration (mg/L)

Figure 17. DO Uptake by the Alginate Biocatalysts

same as used for phase I of the study. The HRT data for 10 hr were taken from the compound dose increase study and have been incorporated in the presentation of the results to enable a better understanding of systems behavior. These data have not been used in drawing conclusions from this phase of the study as the initial conditions of the reactors for both studies were different. The operating parameters for both the ICR and ASR are shown in Table XI. The flow rate for the ICR varied from 2.68 L/day to 8.96 L/day. The flow rate of the ASR varied from 10.68 to 35.64 L/day. After each change in the HRT, the units were run until a steady state was achieved. At least 3 readings were taken during steady state operation of the units. The tabular data included here contain the averages of the three to four steady state values.

TCOD and SCOD removal in HRT study.

The TCOD and SCOD removals in terms of concentration are shown in Table XII. The TCOD loading rate in the activated sludge reactor varied from 2.07 - 6.83 g/L*d and the SCOD varied from 2.02 to 6.61 g/L*d (See Table XIII). The influent TCOD loading to the ICR varied from 2.11 to 6.98 g/L*d, while the SCOD varied from 2.06 to 6.76 g/L*d (see Table XIV). The TCOD and SCOD removals for both ICR as well as the ASR at 8, 5, and 3 hr HRT's are graphically presented in Figures 18 and 19, respectively. Most of the time, the alginate reactor gave the lower effluent COD values. The reason for this appears to be the large amount of biomass immobilized in the ICR. The percentage TCOD removal of the ASR (See Table XIII) fell from 64.2% at 10 hr HRT to 55.5% at an 8 hr HRT, and then rose to 57% at a 5 hr HRT. The corresponding SCOD showed a continuous decrease from 71.8% at 10 hr HRT to 64.1% at 5 hr HRT. The percentage TCOD removals (Table XIV) for the ICR show a steady drop from 67.3% at 10 hr HRT to 25.4% at 3 hr HRT. The corresponding SCOD removal percentages are 83 % and 41.7 %. The ASR showed increased solids washout with decreasing hydraulic retention times, ultimately failing at an

TABLE XI

OPERATING PARAMETERS DURING THE H R T STUDY

Reactor type and related parameters	HRT Hrs	Days	Flow Rate L / d	Amt. of biomass immob.**(g) / MLVSS***(g/L)	COD Loading g/L*d TCOD	COD Loading g/L*d SCOD	SRT days
Immobilized Cell Reactor (ICR)	10*	31 - 48	2.65	20	2.09	2.04	INDETERMINATE
Volume = 1120 ml	8	1 - 20	3.36	20	2.62	2.56	
2,4-DAT concentration =150mg/L	5	22 - 35	5.38	20	4.15	4.02	
	3	38 - 58	8.96	20	6.98	6.76	
	10*	31 - 48	10.94	7.5	2.12	2.07	60
Activated Sludge Reactor (ASR)	8	1 - 20	13.36	5 8	2.56	2.50	5 8
Volume = 4556 ml	5	22 - 35	21.38	7.5	4.06	3.93	60
2,4-DAT concentration =150mg/L	3	38 - 58	35.64	4.4	6 83	6.61	5 6

* 10 hr HRT data has been taken from the the Compound Dose Increase Study
** Mass of immobilized biomass
*** for ASR only

TABLE XII

COD REMOVAL IN HRT STUDY

HRT	Days of operation	Infl TCOD (mg/L)	Infl. SCOD (mg/L)	ICR		ASR	
				Eff. TCOD (mg/L)	Eff. SCOD (mg/L)	Eff. TCOD (mg/L)	Eff SCOD (mg/L)
10 *	31 - 48	883	863	290	148	315	243
8	1 - 20	872	853	370	160	388	290
5	22 - 35	865	837	375	235	375	300
3	38 - 58	873	845	651	492	650	525 ‡
						660	545
						675	530

* Data taken from the 'Compound Dose Increase Study' part of the experiment

‡ The ASR failed (see text) on the 42nd day These three readings were taken during this period (see TCOD and SCOD plots)

TABLE XIII

COD REMOVAL IN HRT STUDY (ASR)

HRT	Days of operation	Influent Loading Rates (g/L * day)		Activated Sludge Reactor (ASR)			
				Removal Rate (g/L* day)		% Removal	
		TCOD	SCOD	TCOD	SCOD	TCOD	SCOD
10 *	31 - 48	2.12	2.07	1.40	1.49	64.6	72.0
8	1 - 20	2.56	2.50	1.42	1.63	55.5	65.2
5	22 - 35	4.06	3.93	2.92	2.51	57.0	64.1
3	38 - 42	6.83	6.61	THE A.S.R FAILED AT AN HRT OF 3 HOURS			

* Data taken from the 'Compound Dose Increase Study' part of the experiment

TABLE XIV

COD REMOVAL IN HRT STUDY (ICR)

HRT	Days of operation	Influent Loading Rates (g/L * day)		Immobilized Cell Reactor (ICR)			
				Removal Rate (g/L* day)		% Removal	
		TCOD	SCOD	TCOD	SCOD	TCOD	SCOD
10*	31 - 48	2.09	2.04	1.40	1.69	67.0	82.9
8	1 - 20	2.62	2.56	1.51	2.08	57.6	81.2
5	22 - 35	4.15	4.02	2.35	2.89	56.6	71.9
3	38 - 58	6.98	6.76	1.77	2.82	25.4	41.7

* Data taken from the 'Compound Dose Increase Study' part of the experiment

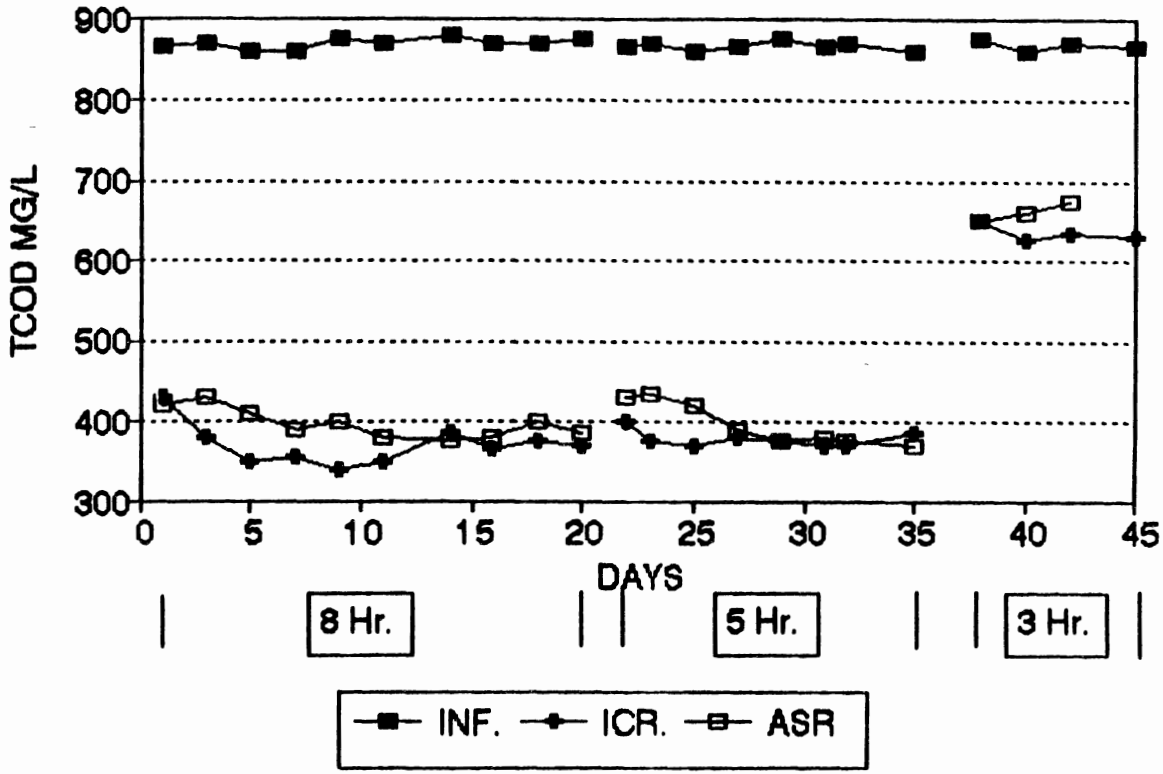


Figure 18. Effluent TCOD Concentrations of ICR and ASR

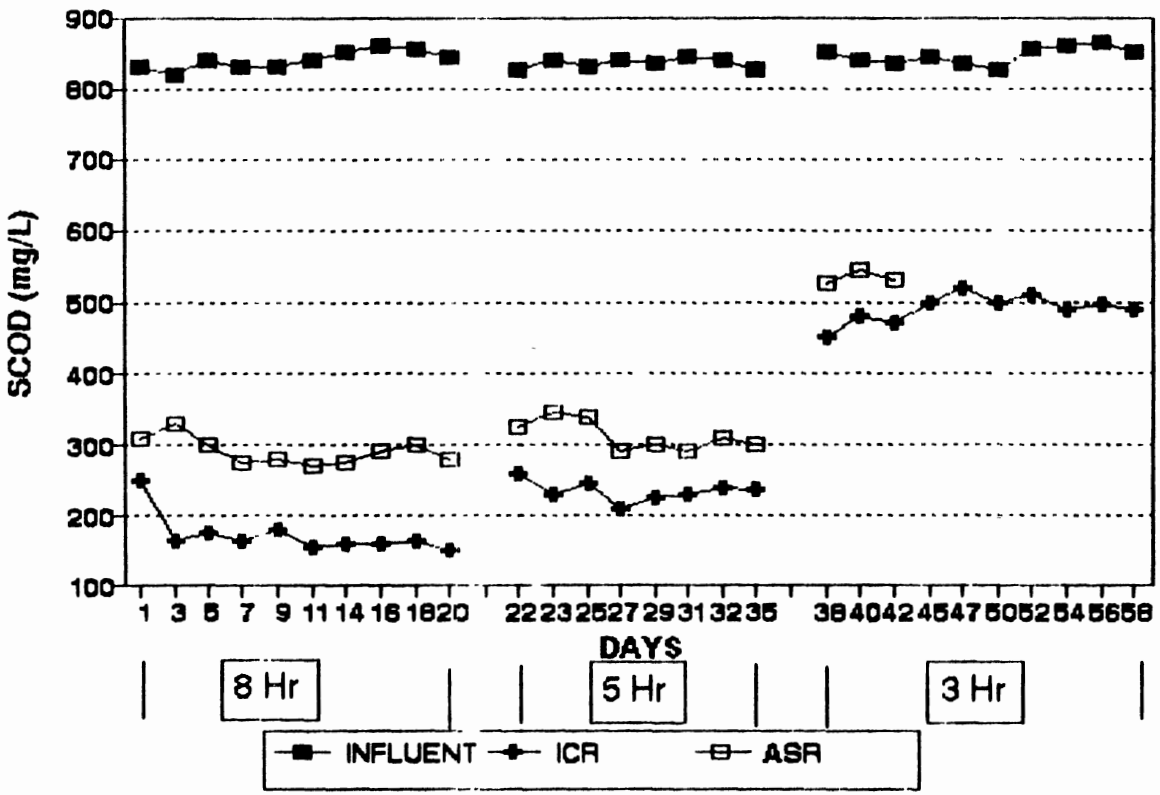


Figure 19. Effluent SCOD Concentrations of ICR and ASR

HRT of 3 hr due to the reactor effluent port becoming obstructed with white fluffy sludge. Based on the identification of the microbial strains for this investigation (Appendix C), it was suspected that *Pseudomonas sp.* synthesized alginate (Brierly et. al., 1985; Moses, 1991) and at least two of the three species stored p-ß hydroxy butyrate. It was observed that just before failure took place, the microbes in the ASR had agglomerated into fairly large loosely knit floc particles, possibly in part due to extracellular matter that could crosslink between smaller particles, and were being physically displaced. The comparisons of percentage TCOD and SCOD removals of both the ICR and ASR are shown in Figures 20 and 21, respectively. While both the ICR and ASR exhibit similar percentage TCOD removals at 10, 8, and 5 hr HRT's (ASR failed at the 3 hr HRT), the ICR shows much better SCOD removal compared to the ASR at all the HRT's where comparison was possible. Although the removal for SCOD was seen to decrease for both the ICR as well as the ASR with shorter HRT's, the treatability achieved by the ASR was slightly less affected as compared to that of the ICR (Figure 21).

Discounting the 10 hr HRT data which were taken from the compound dose increase study, the maximum and minimum TCOD removals for the ICR (see Table XIV) were 57.6 % and 25.4 % at 8 hr and 3 hr HRT's, respectively. These corresponded to influent TCOD loadings of 2.62 and 6.98 g/L*day, respectively. The maximum and minimum SCOD removals were 81.2 % and 41.7 % at 8 and 3 hr HRT's, respectively. These corresponded to influent SCOD loadings of 2.56 and 6.76 g/L*day, respectively. Considering the data for the HRT of 8 hr and below (see Table XIII), the ASR exhibited similar TCOD removals (57.0 %) at an HRT of 5 hr as it did at 8 hr HRT (55.5 %). The SCOD removal was marginally better (65.2 %) at an HRT of 8 hr. This was close to the removal of 64.1% at 5 hr HRT. These results proved that the ICR system performed better in terms of SCOD removal than the ASR system at lower hydraulic retention times because, unlike the ASR, the ICR was not as susceptible to cell wash out.

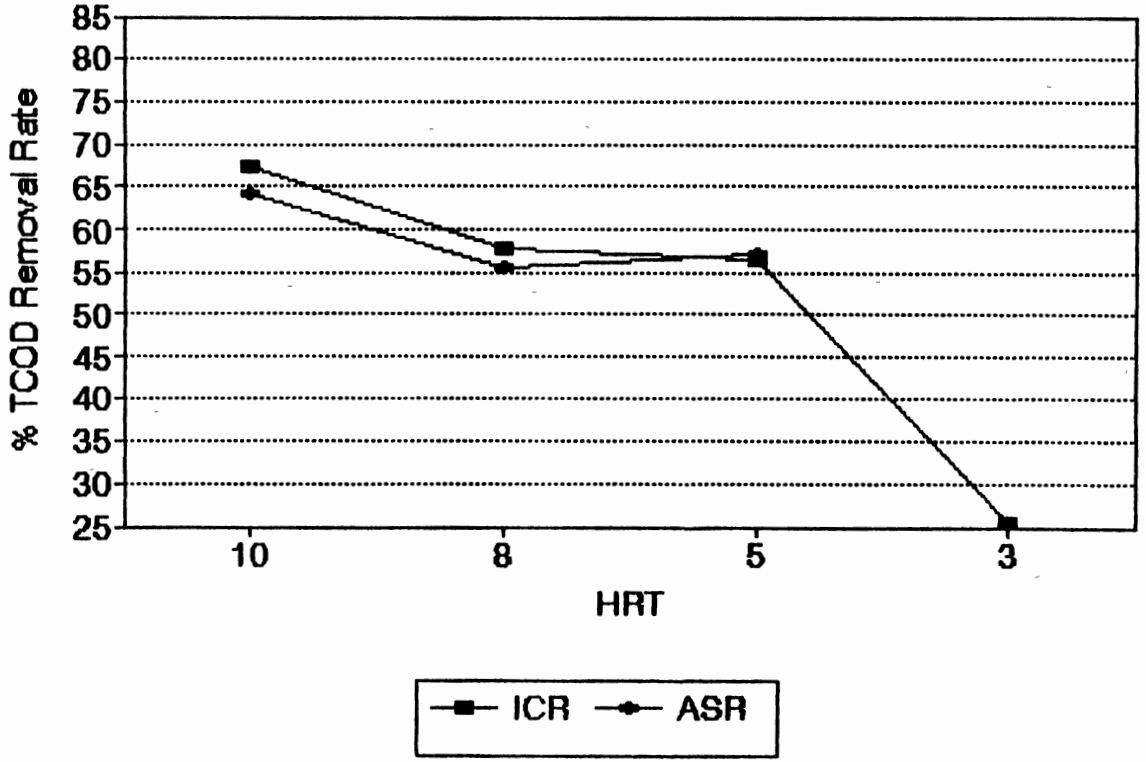


Figure 20. TCOD Removal vs. HRT

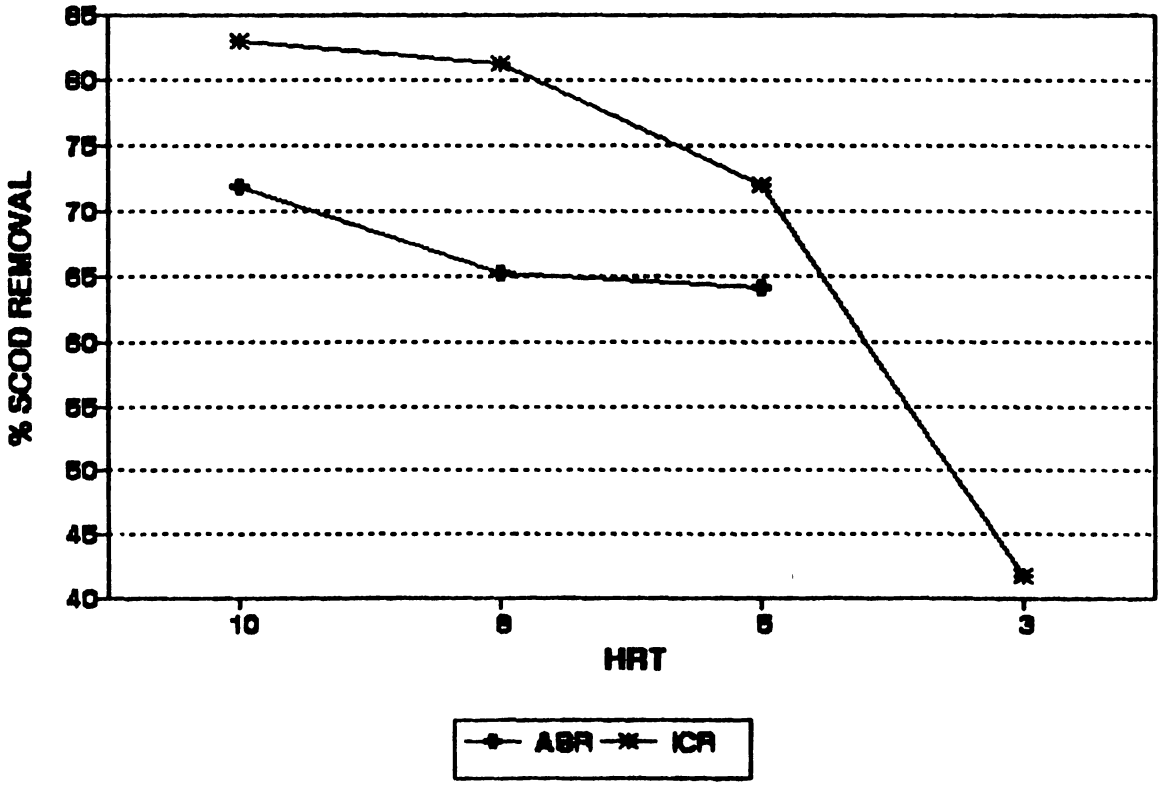


Figure 21. COD Removal vs. HRT

2,4-DAT Removal.

The influent and effluent 2,4-DAT concentrations are shown in Table XV. Discounting the 10 hr HRT values, the removal of the target compound 2,4-DAT was seen to reach a maximum of 16.7 % in the ICR at an HRT of 5 hr during this phase of the study (See Table XVI). The best percentage removals were seen at an HRT of 5 hours for both the ICR as well as the ASR (See Table XVII). Figure 22 shows the removal of 2,4-DAT as it changed with the lowering of the HRT from 8 to 3 hr. The ICR showed a greater amount of 2,4-DAT removal than the ASR at all the HRT's. At an HRT of 5 hr, the effluent 2,4-DAT concentration of the ICR fell sharply, and the system took a week to stabilize and reach a steady state. Product inhibition may have occurred; and with the lowering of the HRT from 8 to 5 hours, the product was washed out at a faster rate, causing the 2,4-DAT removal to improve. The 2,4-DAT removal for the ASR remained fairly constant throughout the 5 hr HRT run. The third data point on the 8 hr HRT plot showed an anomalous behavior in both reactors. This appears to be an experimental error as it shows up in samples from both reactors. In the ASR, the 2,4-DAT removal improved slightly at the 5 hr HRT as compared to the 8 hr HRT. At an HRT of 3 hr, the plot shows an increase in the effluent 2,4-DAT concentration for the ASR. Only three points for this HRT are available because at the end of the 42nd day of the HRT study, the ASR failed due to washout of the cells. At the HRT of 3 hr, the percentage 2,4-DAT removal from the ICR decreased from the 5 hr HRT level (Figure 23).

The maximum and minimum 2,4-DAT removals at HRT's below 8 hr for the ICR (see Table XVI) occurred at 5 hr (16.7 %) and 8 hr (8.9 %), respectively. These removals corresponded to influent 2,4-DAT loading rates of 0.72 and 0.45 g/L*day, respectively. For the ASR (see Table XVII), better 2,4-DAT removal was seen at an HRT of 5 hr (8 %) as compared to an HRT of 8 hr (2.4 %). These removals corresponded to influent 2,4-DAT loading rates of 0.71 and 0.44 g/L*day, respectively.

TABLE XV

2,4 DAT REMOVAL IN HRT STUDY

HRT	Days of operation	Influent Conc mg/L (as measured)	ICR Effluent Conc mg/L	ASR Effluent Conc mg/L
10	31 - 48*	152 4	121 0	135 8
8	1 - 20	150 0	137 1	146 4
5	22 - 35	150 6	124 5	138 7
3	38 - 58	150 3	130 3	135 1 ** 138 7 143 2

* Values taken from the 'Compound Dose Increase Study' part of the experiment

** The ASR failed after these three data points were taken

TABLE XVI

2,4 DAT REMOVAL IN HRT STUDY (ICR)

HRT	Days of operation	Influent Conc. mg/L (as measured)	Influent Loading rate (g/L* day)	Immobilized Cell Reactor (ICR) Removal rate (g/L * day)	% Removal
10	31 - 48*	152.4	0.36	0.07	19 4
8	1 - 20	150.0	0.45	0.04	8 9
5	22 - 35	150.6	0.72	0.12	16 7
3	38 - 58	150.3	1 20	0 13	13 3

* Values taken from 'Compound dose increase study' part of the experiment.

TABLE XVII

2,4 DAT REMOVAL IN HRT STUDY (ASR)

HRT	Days of operation	Influent Conc. mg/L (as measured)	Influent Loading rate (g/L day)	Activated Sludge Reactor (ASR) Removal rate (g/L day)	% Removal
10	31 - 48*	152 4	0 36	0 04	11 1
8	1 - 20	150 0	0 44	0 01	2 4
5	22 - 35	150 6	0 71	0 06	8 0
3	38 - 58	150 3	1 17	A S R FAILED AT THIS HRT	

* Values taken from 'Compound Dose Increase Study' part of the experiment

The data shown in Tables XVI and XVII and graphically represented in Figure 23 show that, for the ICR, the removal of 2,4-DAT was greatest at an HRT of 10 hours. It was unusually low at 8 hr HRT and then improved at the 5 hr HRT before decreasing again at the 3 hr HRT. Product inhibition may be the reason for the poorer 2,4-DAT removal at the 8 hr HRT as compared to 5 hr HRT.

Effluent solids, effluent pH and biocatalyst DO uptake for the HRT study.

The effluent solids from the ICR and ASR are represented in Table XVIII. At an HRT of 5 hr the effluent solids of the ASR increased considerably and then decreased (Figure 24). When the HRT was changed from 8 to 5 hours, a sudden thickening of the contents of the ASR sludge was observed. A possible reason for this was the apparent rapid production of extracellular solids secreted by the microbes. At this point, the sludge looked like a mass of white fluffy flocs which had poor settling characteristics. Efforts were made to control this sudden 'growth' by increasing the wastage of the solids. Only when the amount of solids being wasted was increased did the runaway increase in solids come under control.

The pH value of the effluent during the HRT study stayed between 7.4 and 8.3 (See Figure 25). At all HRT's (in both reactors), the pH gradually rose at the beginning of the run and then reduced near the end of the run. When the systems were undergoing transition from one steady state to another following a change of HRT, the effluent became more basic compared to its values at both the start and the end of the run.

The internal solids of the ASR (See Figure 26) varied greatly with changes in the HRT. Initially, at an HRT of 8 hours, a thickening of the sludge was noticed. Solid wastage was, therefore, increased and the TSS dropped to a steady state level of about 1400 mg/L. At an HRT of 5 hours, another steep increase in the internal solids of the ASR was observed. Solid wastage had to be readjusted to bring the unit back to a steady value.

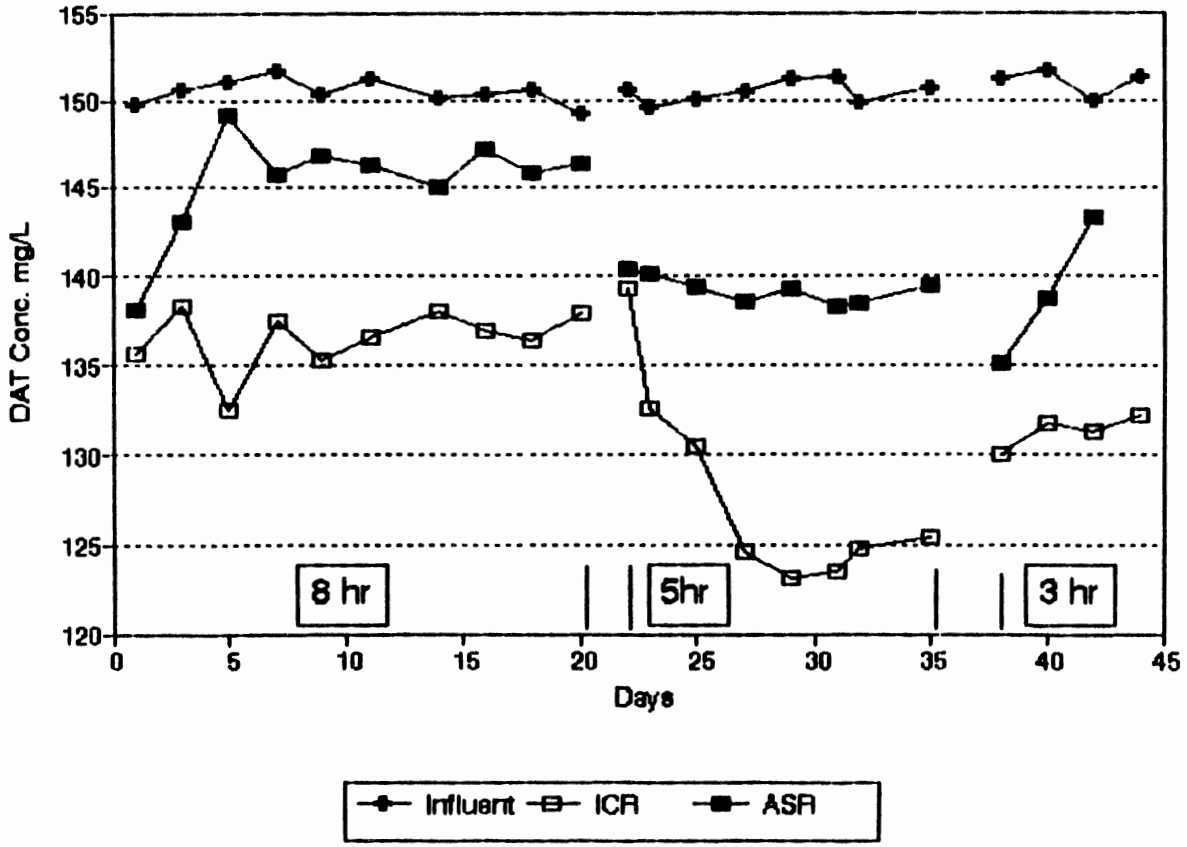


Figure 22. Effluent DAT Concentrations of ICR and ASR

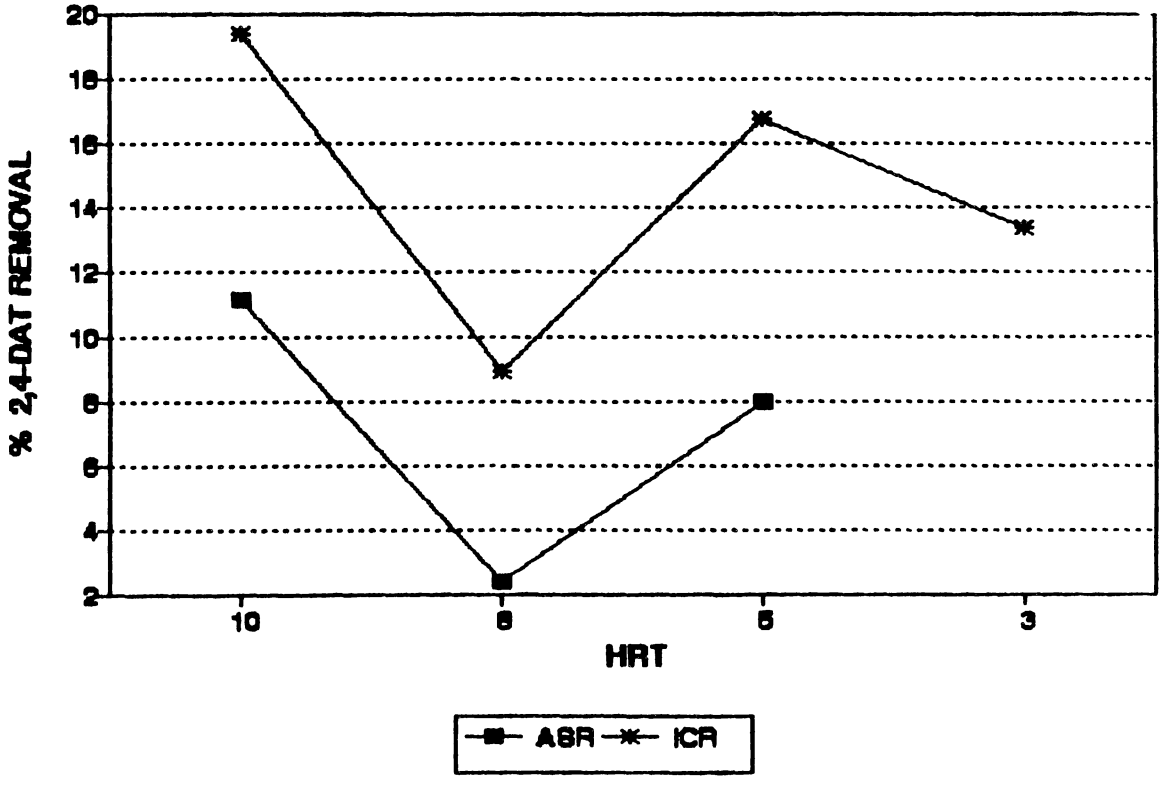


Figure 23. Removal Rate vs. HRT

TABLE XVIII

EFFLUENT SOLIDS AND pH VALUES FOR HRT STUDY

HRT	Days	Immobilized Cell Reactor (ICR)			Activated Sludge Reactor (ASR)			
		TSS mg/L	VSS mg/L	pH	TSS mg/L	VSS mg/L	pH	
10*	31 - 48	25 - 92	15 - 76	8.2 - 8.0	50 - 62	45 - 49	7.9 - 7.6	
8	1 - 20	32 - 80	23 - 73	8.3 - 8.0	33 - 39	20 - 33	7.9 - 7.4	
5	22 - 35	42 - 78	31 - 65	8.1 - 7.7	40 - 378	27 - 365	7.8 - 7.4	
3	38 - 58	40 - 70	31 - 51	7.8 - 7.4	97 - 212	90 - 163	7.6 - 7.5	‡

* Data taken from the 'Compound Dose Increase Study' part of the experiment

‡ The ASR failed at the 3 hr HRT on the 42nd day of the HRT study

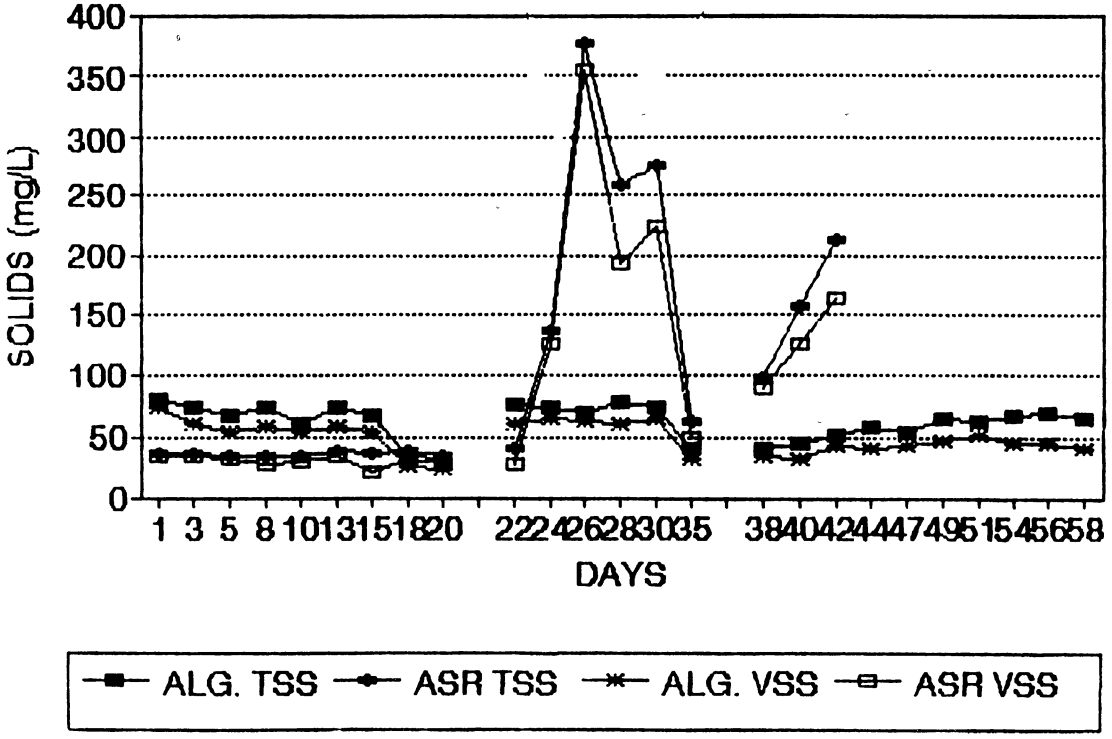


Figure 24. Effluent Solids from the ICR and the ASR

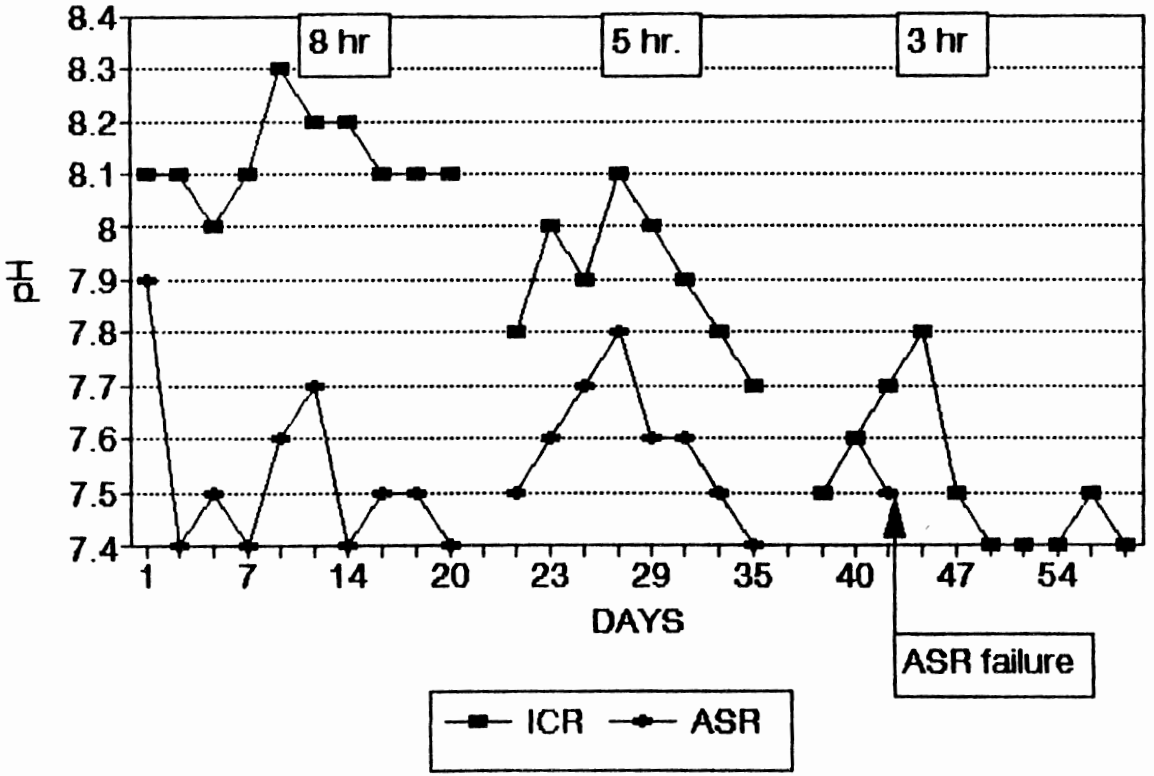


Figure 25. pH Values (HRT Study)

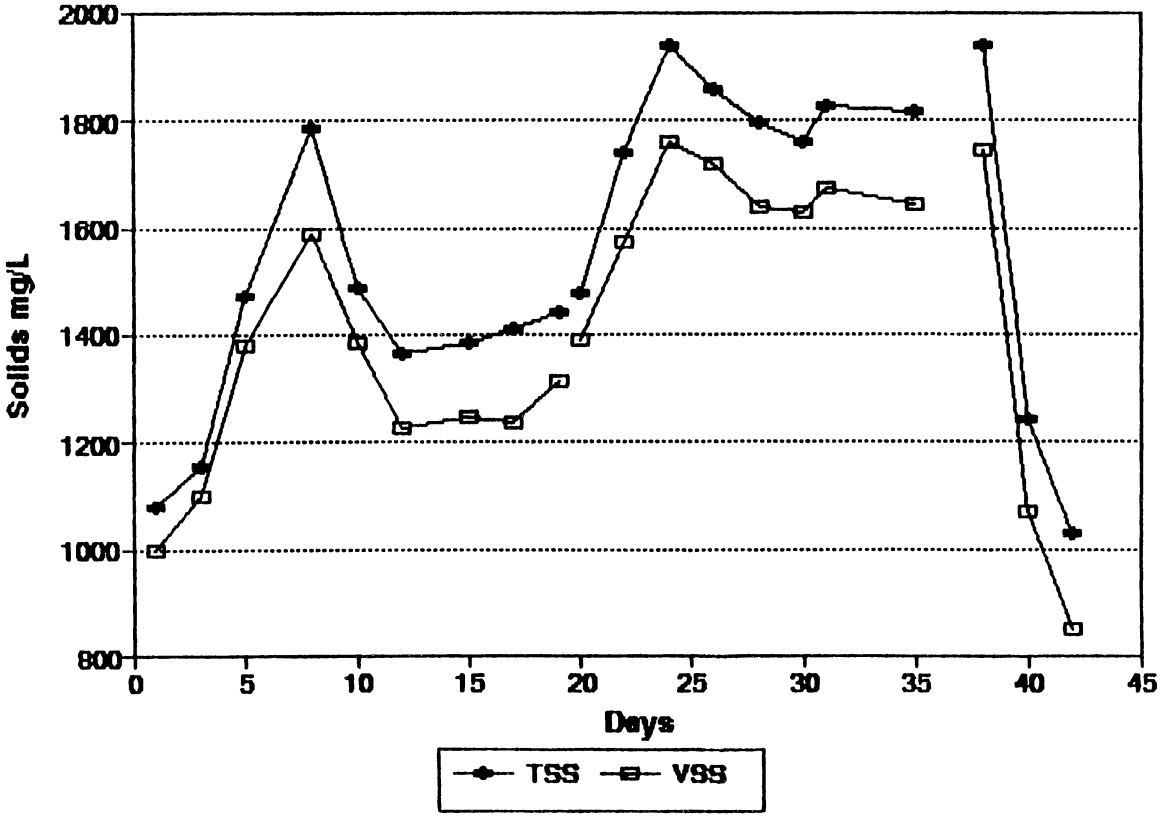


Figure 26. Internal Solids of the ASR

At an HRT of 3 hours, rapid washout of solids from the reactor was observed with the reactor failing after three days of operation at this HRT.

The D.O uptake of the biocatalyst (per bead basis) was stable within the data set obtained at each HRT. As the HRT decreased, the D.O. uptake dropped from 0.13 mg/L*min*bead to 0.07 mg/L*min*bead at an HRT of 3 hours. Figure 27 represents the D.O. uptake activity profile for each range.

Effect of Loading Type

Although the investigation was not designed so that the two systems (ICR and ASR) would have similar loading conditions during the compound dose increase study and the HRT study, some COD loading values were close enough to allow a comparison of the two systems in terms of treatment efficiencies. The SCOD values of the compound dose increase study and the HRT study (Tables VIA, VIB, XIII and XIV) indicate that at an influent concentration of 200 mg/L 2,4-DAT (10 hr HRT) and at an HRT of 8 hours (150 mg/L influent 2,4-DAT dose), the SCOD loading rates to the two reactors were close. These loading values were 2.13 and 2.16 g/L*day for the ICR and the ASR, respectively in the compound dose increase study and 2.56 and 2.50 g/L*day for the ICR and ASR, respectively in the HRT study. From Table XIV and Table V it can be seen that the SCOD removal for the ICR under both phases of the study remained close to 80% (81.2% for the HRT study and 80.8 % for the compound dose increase study). For the ASR, however, the values (65.2% at 8 hour HRT and 50.5% at 200 mg/L 2,4-DAT) were more divergent. It appears, therefore, that (a) the ICR is not as affected as the ASR by the change in the type of loadings and (b) the effect of compound concentration is more predominant for the activated sludge reactor as compared to the HRT variation - at least in the ranges considered.

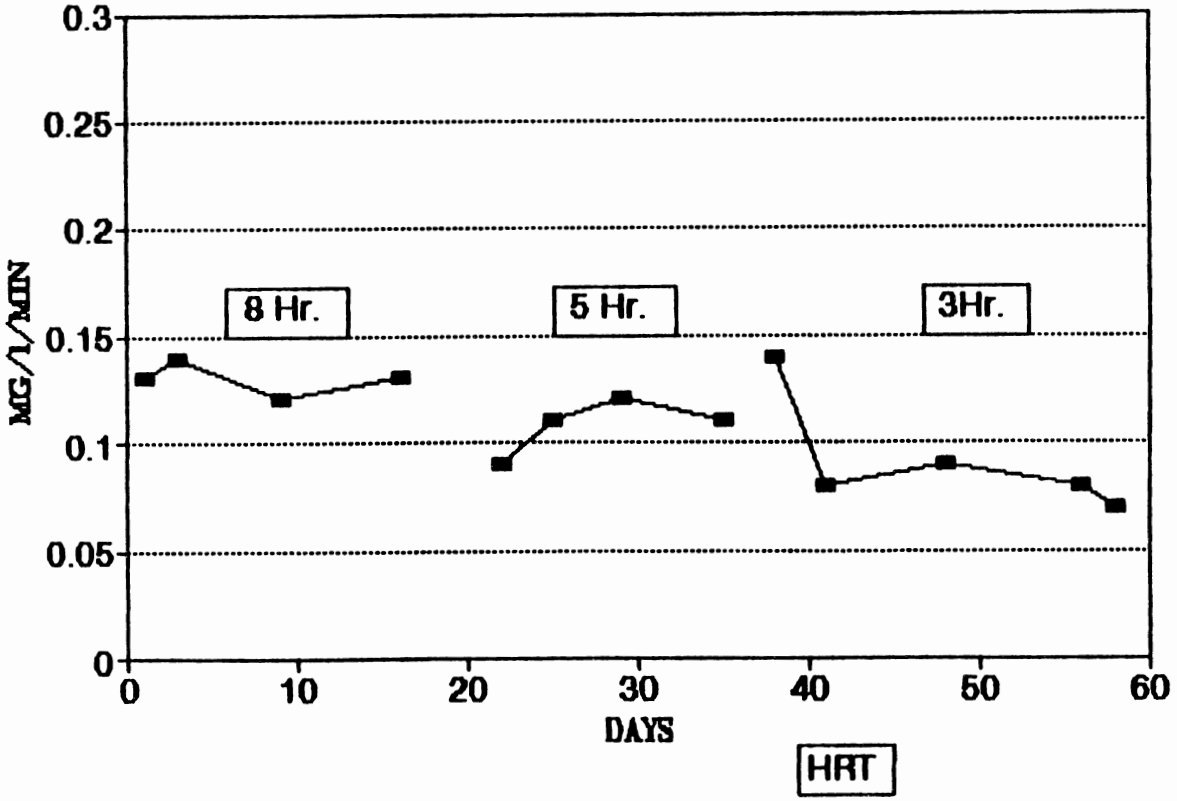


Figure 27. DO Uptake by Alginate Biocatalysts

Product Inhibition

From earlier trial batch studies conducted at the beginning of the investigation where 2,4-DAT had been added in varying doses (50, 100, 150 mg/L) to a nutrient rich culture of the acclimated cells, a possible breakdown product of 2,4-DAT (that could not be detected on the HPLC) gave the solution a tea-like reddish brown color, after which the culture became less conducive to microbial growth. In cultures to which 2,4-DAT had not been added, no coloration was produced. This suggests that some kind of a breakdown product (which caused coloration) was being produced. This breakdown product could have inhibited microbial growth.

From phase II of the study it was seen that at lower HRT's the ICR's performance was better in terms of SCOD and 2,4-DAT removal compared to the ASR. This could have been due to the ICR's inherent resistance to cell washout, to which the ASR was more prone. Also, at lower HRT's product inhibition would have a smaller effect than at high HRT's because the toxic products would be washed out of the reactor at a faster rate. Better 2,4-DAT removal was observed at the 5 hr HRT, in both the ICR as well as the ASR, than at an HRT of 8 hours (Figure 22). With the ability to effectively operate at lower HRT's, the ICR becomes a system of choice wherever the treatment of a compound producing toxic breakdown products (thereby causing product inhibition) is involved.

Physical Characteristics of the Biocatalysts

Biomass growth inside the biocatalyst carriers.

A batch of 40 freshly made alginate beads (with entrapped microbes) was dissolved using a phosphate solution (See Chapter III) and the resulting liquid was filtered using a 0.5 μm glass microfiber filter. The solids were then determined and the VSS was measured

to be 0.0283 g. A batch of 40 beads from this original lot was again taken out of the ICR after 35 days of operation. During this period, the beads had been subjected to varying HRT's of 8 and 5 hours. These beads were then dissolved and the solids in the resulting liquid determined. The VSS was measured to be 0.0308 g. This showed an increase of 0.0025 g / 40 beads over a period of 35 days. The total amount of biomass initially present in an immobilized state inside the ICR was 20 grams. The amount of VSS and TSS produced by the ICR at the end of the 5 hour HRT were 40mg /L and 50mg /L (0.05g / L) , respectively in a grab sample. Since the volume of the ICR was 1 liter, the ratio of biomass inside the bead to the amount of biomass in the liquid phase was 20 / 0.05 = 400. It is, therefore, clear that under conditions of moderate cell growth in the liquid phase of the reactor, the biomass is much greater in the beads as compared to that in the liquid phase. The bulk of the COD and 2,4-DAT removal, therefore, can be assumed to have taken place due to the biomass entrapped in the beads.

The above check for growth in the population of entrapped microbes did not focus on exact quantification of growth, as this was not in the scope of the investigation. The results of this study show that growth of the microbial cells did take place after the beads were made. The results of the Scanning Electron Microscopy of the beads could lend some support to this finding. The micrographs (see Appendix C) show cells in clusters in old beads. At the time of immobilization, centrifuged biomass had been mixed with alginate solution in a blender . The process of blending is believed to have had physically separated the cells before entrapment. The formation of clusters could have most likely taken place only due to the multiplication of the cells after entrapment. A batch of beads (made using 400 ml of Alginate solution) typically contained 13,300 beads.

The specific gravity of the alginate beads.

The specific gravity of the alginate beads play and important role in the determination of the agitative force required to keep a CSTR stirred or the up flow velocity in an expanded / fluidized bed reactor. Since the beads became more buoyant as they aged (while in use), the specific gravity of the alginate beads was thought to vary with time. Freshly prepared beads were heavier in water than the older beads which were seen to swell to about one and a half times their original volume. For this reason, mechanical agitation using a magnetic stirrer was required during the first 10 days of the ICR operation. Afterwards, the agitation caused by the aeration at the preselected air flow rate was sufficient to keep the beads well mixed. The drop in the specific gravity of the beads could have been caused due to the leaching of Ca^{++} ions from the alginate matrix, thus gradually dissolving the bead, and / or due to the growth of microbes (which have a lower specific gravity compared to the freshly prepared matrix) inside the matrix.

The volume of 200 freshly prepared blank (without microbes) beads was determined by water displacement to be 6 mls. These were then dried at 105 ^{o} C and were found to weigh 6.24 g. During the process of drying, the color of the beads changed from a translucent brown to a deep reddish brown. The specific gravity was calculated to be 1.04. This value may not be totally reliable as the process of drying could have altered the chemical characteristics of the matrix.

Life of the alginate biocatalysts.

In this study the beads usually lasted from about four to six weeks. While the maximum life span in this study was seen to be 45 days (from the 37th day to the 82nd day of the phase I study), the minimum was only 26 days (from the 1st day to the 26th day of phase II study). Attempts were made to make the beads using a higher concentration of

alginate, but the high viscosity that resulted made fabrication of the beads physically impossible. Near the end of their life, the beads became friable and cracked to pieces over a period of 5 to 7 days. No measured parameter gave any prior indication of bead breakage. Visual examination of the beads did, however, show surface cracks four to five days before the first bead debris was observed.

Adsorption Studies on the Matrix

At the start of the investigation, 100 ml of a sodium alginate solution was used to make plain beads with no microbes entrapped in them. These beads were used to test the matrix for possible adsorption of 2,4-DAT. The beads were put in 500 ml of base mix (prepared in distilled water) containing 50 mg/L of 2,4-DAT and agitated by stirring for a period of 24 hours at room temperature. Samples were drawn out at intervals of 6 hours and tested using HPLC. No removal of 2,4-DAT could be detected. Similar trials were made using a 2,4-DAT concentration of 100 mg/L and still no removal could be seen. It was concluded from these tests that adsorption was not a major removal mechanism of 2,4-DAT.

CHAPTER V

CONCLUSIONS

The important findings of this study can be summarized as follows:

1. The use of alginate immobilized heterogeneous bacterial culture for treating a complex synthetic wastewater containing a hazardous waste compound 2,4-DAT was successfully demonstrated.
2. The treatment efficiency of the ICR in terms of SCOD and 2,4-DAT removal was superior to that of the ASR under a very wide range of loading conditions.
3. The highest 2,4-DAT removal achieved in this study was 38.5 % at an influent 2,4-DAT concentration of 50 mg/L and at an HRT of 10 hours by the ASR. The highest 2,4-DAT removal by the ICR was 33.3 % - a significantly close value.
4. The highest SCOD removal of 86.7 % was achieved at an influent 2,4-DAT concentration of 100 mg/L at an HRT of 10 hours by the ICR.
5. The maximum 2,4-DAT and SCOD removal efficiency obtained by the ICR were 33.3 % and 86.7 %, respectively. The corresponding values for the ASR were 38.5 % and 85.9 %, respectively.
6. At the influent 2,4-DAT concentration of 250 mg/L, the removals exhibited by both the ICR and the ASR were poor. This points towards either substrate or product toxicity. Although at doses over 200 mg/L of 2,4-DAT, the performance of the ICR deteriorated, still it remained better compared to that of the ASR.
7. The best 2,4-DAT removal efficiency of both ASR and ICR in phase II of the study was at an HRT of 5 hours. A higher 2,4-DAT removal at 5 hour HRT (16.7 %) as

opposed to that at 8 hour HRT (8.9 %) in the ICR suggests product toxicity. The 3 hour HRT value of 13.3 % could be anomalous due to high organic loading on the reactor.

8. The ICR could operate at lower HRT's as compared to the ASR. At low HRT's, the operation of the ASR became difficult due to the transformation of the sludge into white fluffy flocs. Compared to the ASR the ICR was easier to operate as it did not exhibit such problems.

9. The amount of biomass in the liquid phase of the ICR was insignificant in comparison to the biomass entrapped inside the beads. The microbes inside the beads were, therefore, predominantly responsible for the removal of COD and 2,4-DAT.

10. The maximum and minimum life spans of the alginate beads were 45 days (in compound dose increase study at 150 and 200 mg/L influent 2,4-DAT concentration of phase I study) and 26 days (at 8 and 5 hour HRT's of phase II study), respectively.

CHAPTER VI

ENGINEERING SIGNIFICANCE

Wastewaters containing biodegradable organic compounds can be effectively treated by immobilized cell systems. The immobilized cell system tolerated higher concentrations of toxics better than a conventional activated sludge system. Since the microbes in the immobilized cell systems were shielded by the polymer matrix in which they were entrapped, sudden changes in the target compound concentration which could cause a toxic shock to the microbes in free cell biological reactors were better handled by immobilized cell systems. Unlike the activated sludge systems, whose performance deteriorates rapidly with decreasing HRT's due to cell washout, immobilized systems are able to operate without experiencing such a hydraulic instability even at low HRT's. Even though the treatment efficiency of the immobilized system deteriorates at increased toxic concentrations and reduced HRT's, it is still better compared to the treatment efficiency shown by an activated sludge system operated under similar conditions.

Sometimes, the products in a biodegradation process are more toxic than the parent compound itself to the microbes present in the treatment system. In such cases, a low HRT helps in maintaining a low product concentration inside the reactor. The immobilized cell systems are able to handle wastes which exhibit this phenomenon of product inhibition much better as compared to activated sludge systems as they are able to operate at lower HRT's.

Since immobilized cell technology can result in more compact reactors as compared to activated sludge systems, they could find future applications in Superfund type site

remediations where portable / temporary treatment processes might be set up for a specific type of pollutant, provided a more durable matrix can be developed. With the present level of technology, manufacture of long lasting beads is not possible. This is a drawback.

The solids produced in the effluent of immobilized cell systems appear mainly to be microbial cells and are believed to result from cell leakage from the biocatalysts and their consequent proliferation in the nutrient rich liquid medium. Since such sludge has poor settling characteristics, flocculation and sedimentation units might be required to treat the effluents from immobilized cell treatment units.

Since the alginate matrix stability is adversely affected by the presence of phosphates, the need to maintain strict control on the phosphate concentration of the influent shall be required. Alginate is a fairly inexpensive material. Its matrix is mechanically strong, it is easy to work with and yields biocatalysts with high cell viability. For this reason, it could find fairly widespread use in portable or short term dedicated treatment systems in future.

CHAPTER VII

FUTURE RESEARCH NEEDS

The use of immobilized cells in the treatment of wastewaters is a relatively new field. Only a limited amount of work has been done so far. Many questions that were faced during the research need to be studied further :

1. Methods of producing alginate biocatalysts more resistant to phosphate and with better durability characteristics
2. The determination of the nature of the extracellular solids produced by the microbes, and their effect on the stability of the biocatalyst bead
3. Accurate identification of the biochemical pathways of the treatment mechanism including mass spectrophotometry to identify the breakdown products of treatment.
5. The effect of the shape and surface area of the biocatalysts for maximum effectiveness.
6. The effect of other reactor configurations on biocatalyst stability.
7. Possibility of using strengthening agents (supports) inside the polymer beads.
8. Use of other matrices in producing durable biocatalysts

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APPENDICES

APPENDIX A

THE EXPANDED BED REACTOR

Material	:	Glass
Shape	:	Tubular
Mounting	:	Upright
Openings	:	Three (Top open 1/4" inner dia projecting openings, bottom and side)
Size	:	1" dia, 18" height, 3" freeboard above topmost side opening
Bottom	:	Tapered to the bottom opening
Disc	:	Glass disc above tapered portion of the reactor having 9# 2mm dia holes. Functions : To distribute flow and support the medium
Mode of Operation	:	Upflow, continuous, with recirculation
Medium	:	Alginate beads containing entrapped microbes capable of degrading 2,4 Diaminotoluene

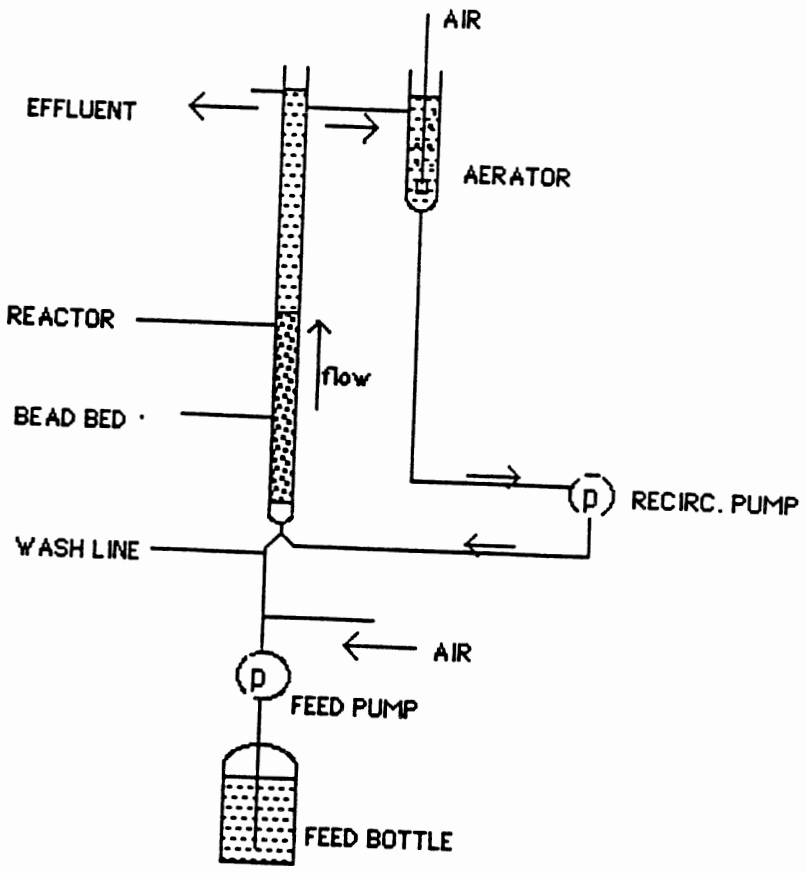


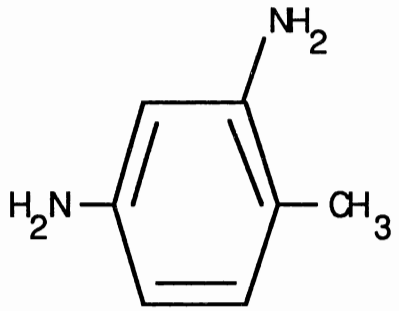
Figure 28. Trial Expanded bed Immobilized Cell Reactor Configuration

APPENDIX B

PHYSICAL AND CHEMICAL CHARACTERISTICS OF

2,4 - DIAMINOTOLUENE

Name	:	2,4 Diamionotoluene (or 2,4 Toluenediamine)
Physical Appearance	:	Dark brown crystals
Health Effects	:	Suspected Carciongen, toxic.
Formula	:	C7 H10 N2
Molecular Weight	:	122.17
Structure		



:

Melting Point	:	99 ^{o} C
Boiling Point	:	292 ^{o} C
Solubility	:	7,470 (mg./L) Aq.
Absorption Max	:	294 nM
Artificial Sources	:	Releases from manufacture of dyes, resins, antioxidants, urethane foams, hydraulic fluids etc.

Volatalization from water	:	Insignificant
Reference	:	Howard P.H. 'Handbook of Environmental fate and exposure data for organic chemicals Vol II', Lewis Publishers (1989)
Major fate mechanisms in soils and water	:	Bio degradation and photooxidation

Description

Major release of this compound takes place during its production and during its major use, the production of diisocynates. Minor releases occur from other sources. high water solubility and low soil sorption partition coefficient cause the compound to be leached rapidly and extensively in soil.

This compound is listed by the EPA as a CERCLA waste and is also listed in the Maryland- New Jersy List. Volatalization from water is not known to be significant. Bioconcentration is not important. In atmosphere it photolyzes and reacts with hydroxyl radicals with an estimated half life of 7.99 hrs.

Sources

No natural sources of this compound are known. This compound is produced in significant quantities. Releases occuring during its production constitute the bulk of the emissions

Fate in the Environment, Biodegradation and exposure

Terrestial fate.

This compound is known to be fairly biodegradagle from whatever little work that has been done on it. It is not expected to volatalize from the soil due to its low vapor pressure and high solubility. With an absorption maximum of 294 nM, it is likely to directly photolyze under environmental conditions, but this would occur only on the top surface and therfore is not expected to be significant inside the soil mass. Biodegradation in soil can occur.

Aquatic fate.

This compound is not likely to hydrolyze significantly. It has been know to degrade in the presence of activated sludge. The compound is likely to get photooxidized in water.

Biodegradation.

A decline of 45% of TOC has been known to occur after a 4 hour exposure to activated sludge.

Occupational Exposure.

Major human exposure of this compoun is likely to occur in occupational settings where this compound is either produced, used in large quantitites or where polyurethane body implants are used

APPENDIX C

IDENTIFICATION OF BACTERIAL STRAINS

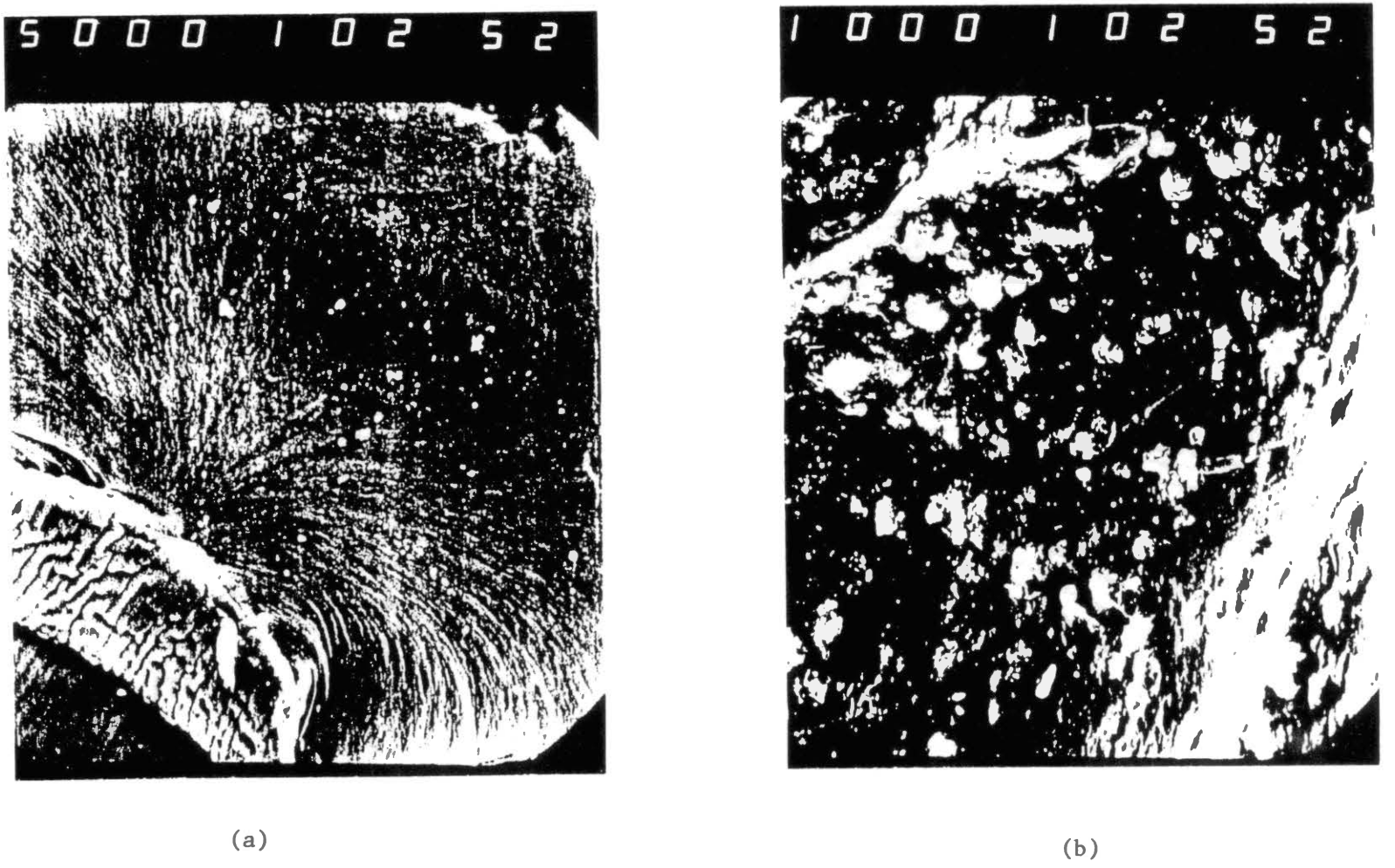
The microbes were taken from the acclimated stock and were grown on agar plates. Three morphologically different colony types were observed. Based on their appearance they were called, White Smooth (WS), Yellow Smooth (YS) and Yellow Ridged (YR). Inocula were taken from each of the three types of colonies and transferred to agar slants where each of the three types of species grew separately. These microbes were then subjected to the following tests. Based on the results of the tests, attempts were made to identify the species present.

TESTS DONE FOR BACTERIAL IDENTIFICATION

TEST	TYPE		
	WS	YS	YR
Gram Stain	Negative	Negative	Negative
Shape	Coccus	Rod	Rod
Motility	Non-motile	Motile	Motile
Aerobic / Anaerobic	Aerobic	Aerobic	Aerobic
Catalase	Positive	Positive	Positive
Oxidase	Negative	Positive	Positive

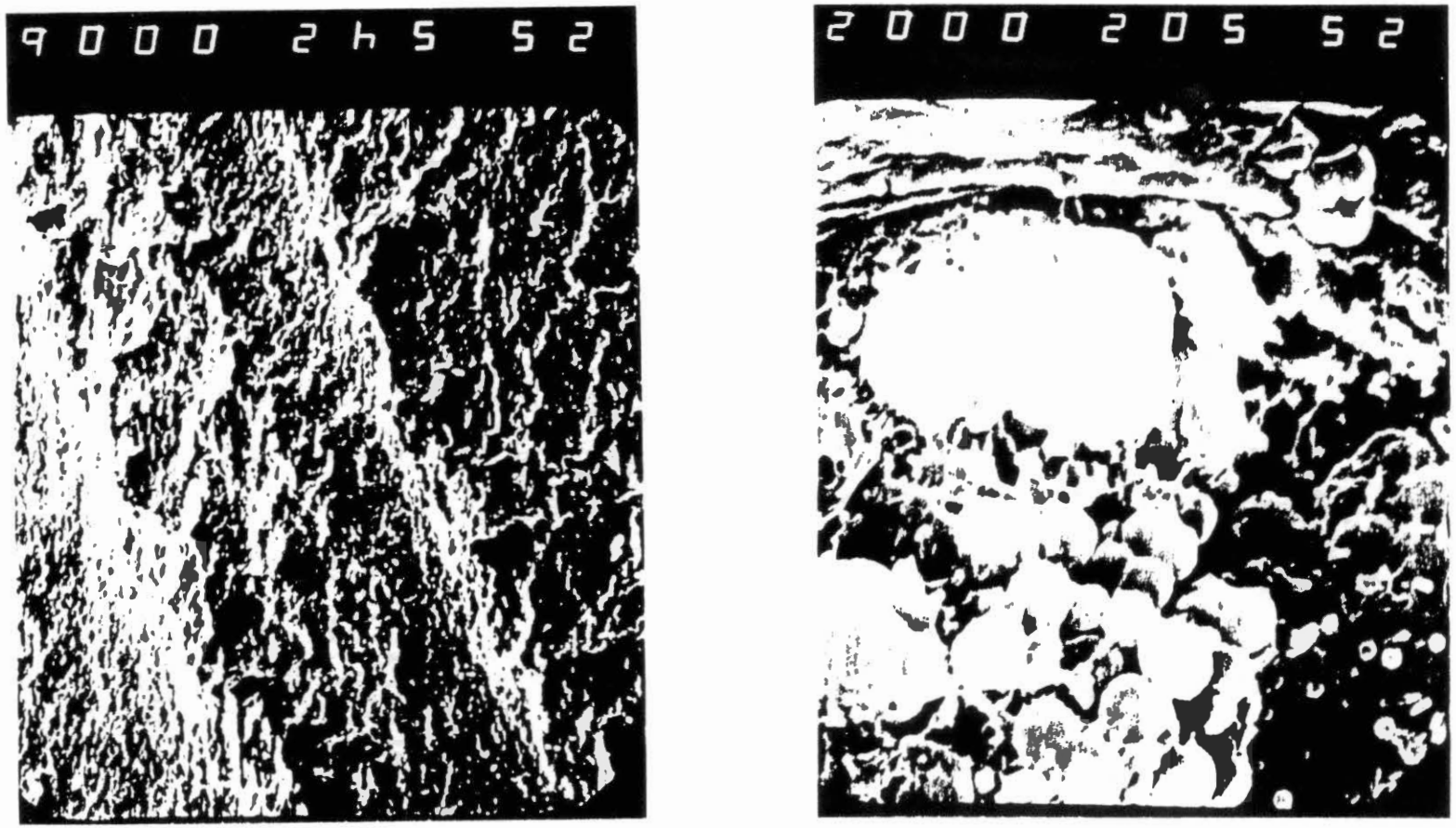
Gel Liquifaction	Negative	Positive	Negative
Denitrification Denitrifies NO_3	Negative	Reduces NO_3 to	NO_2 only to N_2 gas
Indole	Negative	Negative	Negative
Glucose Utilization	Negative	Positive	Positive
King's pH 7.2	Negligible growth	Excellent growth	Excellent growth
p-ß hydroxy butyrate storage	Negative	Positive	Positive
Growth @ 30 deg.C	Positive	Positive	Positive
Growth @ 37 deg C	Negaive	Negligible	Positive
Penicillin High Resistance	High	Low	Low

Based on the tests, it was concluded that there were two species of the *Pseudomonas* genus. Both YS and YR strains accumulated poly-beta-hydroxy butyrate granules and did not form a fluorescent pigment. YS might have belonged to RNA group II whereas, YR might have belonged to RNA group III. YS was also more acid-tolerant and therefore, could be *P acidovorcens*.. Since YR exhibited denitrifying activity and tested negative for proteolysis, it could be *P solanacierum* .WS, although a gram negative, strictly aerobic rod did not appear to be *Pseudomonas* considering other test results. It closely resembled the genus *Acenitobacter* because it was a coccobacilli, was oxidase negative, did not liquify gelatin (proteolysis), did not act on nitrate and exhibited more resistance to penicillin (diameter of inhibition zone was significantly smaller in this case as compared to the other two strains).



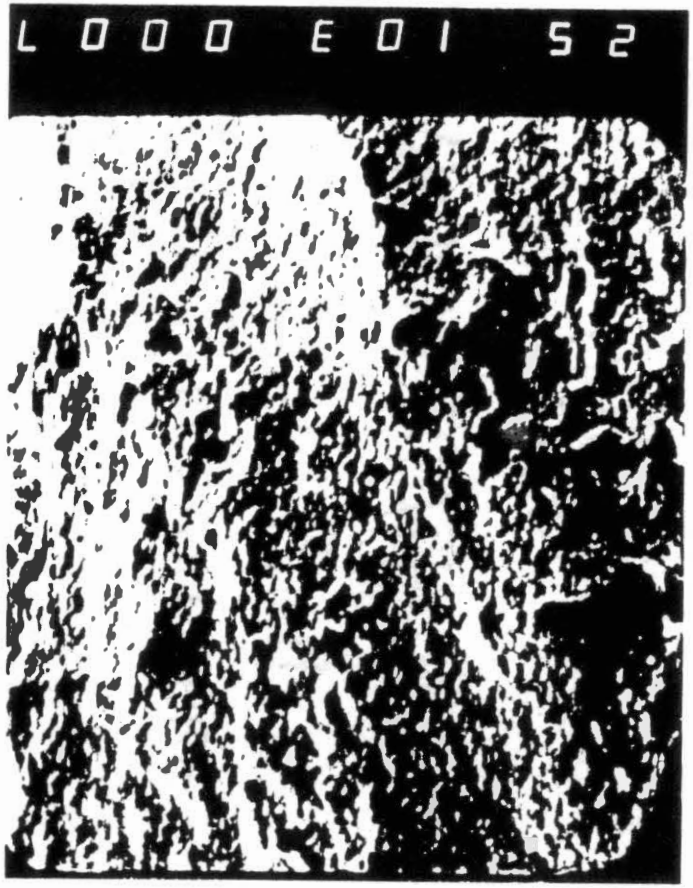
(a) (b)

Figure 29. Electron Micrographs of Alginate Biocatalysts (at 200 X)
(a) Blank bead at 200 X
(b) Bead with immobilized cells at 200X



(a) (b)

Figure 30. Electron Micrographs of Alginate Biocatalysts (at 5,000 X)
(a) Blank bead at 5,400X
(b) Microbes inside bead at 5,000X



(a)

(b)

Figure 31. Electron Micrographs of Alginate Biocatalysts at (10,000 X)

(a) Blank bead at 10,000 X
(b) Microbes inside beads at 10,000X

VITA

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