BIOLOGICAL TREATMENT OF A SIMULATED INDUSTRIAL WASTEWATER USING CHITOSAN - IMMOBILIZED ACTIVATED SLUDGE

By

SURESH SUBRAMANIAN

Bachelor of Science (Engineering)

Birla Institute of Technology and Science

Pilani, India

1988

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
July, 1992

BIOLOGICAL TREATMENT OF A SIMULATED

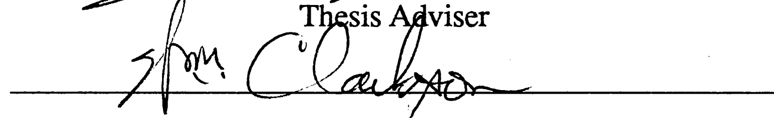
INDUSTRIAL WASTEWATER USING

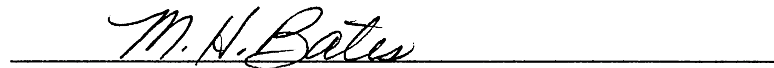
CHITOSAN - IMMOBILIZED

ACTIVATED SLUDGE

Thesis Approved :

Thesis Adviser





Dean of the Graduate College

ACKNOWLEDGEMENTS

I dedicate this thesis to my parents, dear Amma and Appa, who made me understand at a very early age that knowledge is an end in itself and if applied purposefully, results in a virtuous life. I wish to express my gratitude and appreciation to Dr. John N. Veenstra, my principal advisor, for his guidance, support and constant encouragement throughout the project. His dedication to his work and his incredible patience have never ceased to amaze and enlighten me. I also thank Dr. William W. Clarkson and Dr. Marcia Bates for serving on my committee. A special note of appreciation and respect is due to Dr. Clarkson for his fantastic sense of humor and technical brilliance that made all his classes so thoroughly enjoyable. The dog days in the laboratories were made bearable by my dear friend and project mate, Mr. Rakesh Chaubey, whose wit, artistic skills, and knowledge of esoteric trivia have always entertained and educated me.

I am grateful to the Oklahoma State University Water Research Center for financially supporting this project through a research grant (Project No. EN 91 - R - 012).

Let me also thank all my room mates who have coped with and contributed to my highs and lows; especially, Paddy, Anil and Vasu , whose sometimes conflicting personalities have served as a source of great bliss to me. Its impossible for me to list here all the colorful people who have enriched my life - so, thanks everybody. Finally, I am grateful to 'Zen and the Art of Motorcycle Maintenance' for these words: " We want to make good time, but for us now this is measured with emphasis on ' good ' rather than ' time ' and when you make that shift in emphasis, the whole approach changes "

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION 1

Development and Scope of the Experimental Study 1
Objectives.. 3

II. LITERATURE REVIEW 5

Scope of the Review 5
A General Discussion on Immobilized Cell Systems 6
Analysis of a Completely Mixed Reactor used as an
Immobilized Cell Reactor 14
Basic Characteristics of Activated Sludge Reactor with Cell Recycle 27
Background on Waste Characteristics 29

III. MATERIALS AND METHODS 33

Experimental Design.. 33
Experimental Apparatus 37
Base Mix, Target Compound and Dilution Regime for Feed 39
Startup Procedure 41
Analytical Techniques 45

IV. RESULTS 52

Scope 52
Results of Preliminary Studies 53
Results.. 55
Organic Loading Study. 57
Hydraulic Loading Study 74
Supplementary Studies 94

V. DISCUSSION.. 100

Scope.. 100
Performance of the Immobilized Cell Reactor 101
Performance of the Activated Sludge Reactor. 104
Comparison of ICR and ASR Performances. 105
Discussion on Supplementary Experiments... 111

VI. CONCLUSIONS 113

Chapter Page

VII. SIGNIFICANCE OF WORK AND FUTURE RESEARCH NEEDS 115

BIBLIOGRAPHY 118

APPENDIX - RESULTS OF BACTERIAL IDENTIFICATION TESTS. 122

LIST OF TABLES

Table Page

I. Characteristics of Different Reactor Configurations Applied to Immobilized Cells 13

II. Concentration of Organic Compounds in Stock Solution 42

III. Concentration of Nutrients and Salts in Stock Solution 42

IV. Concentration of Organic Compounds in Wastewater 43

V. Concentration of Nutrients and Salts in Wastewater.. 43

VI. Operating Characteristics during Organic Loading Study 58

VII. TDA Removal in Organic Loading Study 59

VIII. TCOD and SCOD Removal in Organic Loading Study 62

IX. Effluent Solids and pH in Organic Loading Study 68

X. DO Uptake by Chitosan Beads and MLSS of ASR in Organic Loading Study 72

XI. Operating Characteristics during Hydraulic Loading Study 77

XII. TDA Removal in Hydraulic Loading Study 78

XIII. TCOD and SCOD Removal in Hydraulic Loading Study 81

XIV. Effluent Solids and pH in Hydraulic Loading Study .87

XV. DO Uptake by Chitosan Beads and MLSS of ASR in Hydraulic Loading Study 91

XVI. Comparison of Substrate Removal Rates in ICR and ASR 107

LIST OF FIGURES

Figure Page

1. Chemical Structure of Chitosan.. ..17
2. Sampling Flowchart used in Data Collection ...36
3. Schematic Diagram of Immobilized Cell Reactor Set-up. .38
4. Schematic Diagram of the Bench-Scale Activated Sludge Reactor Set-up 40
5. Schematic Diagram of Recirculation Reactor used in Preliminary Study .54
6. Influent and Effluent TDA Profile for Organic Loading Study ..60
7. TDA Removal Efficiency Profile for Organic Loading Study 61
8. Influent and Effluent TCOD Profile for Organic Loading Study. 63
9. TCOD Removal Efficiency Profile for Organic Loading Study... 64
10. Influent and Effluent SCOD Profile for Organic Loading Study.. . 65
11. SCOD Removal Efficiency Profile for Organic Loading Study.. .66
12. Effluent TSS Profile for Organic Loading Study 69
13. Effluent VSS Profile for Organic Loading Study.. .70
14. Effluent pH Profile for Organic Loading Study .71
15. DO Uptake Profile of Beads during Organic Loading Study 73
16. MLSS in ASR during Organic Loading Study. 75
17. Influent and Effluent TDA Profile for Hydraulic Loading Study .79
18. TDA Removal Efficiency Profile for Hydraulic Loading Study 80
19. Influent and Effluent TCOD Profile for Hydraulic Loading Study .. 82
20. TCOD Removal Efficiency Profile for Hydraulic Loading Study.. 83
21. Influent and Effluent SCOD Profile for Hydraulic Loading Study .84

Figure | Page

22. SCOD Removal Efficiency Profile for Hydraulic Loading Study 85

23. Effluent TSS Profile for Hydraulic Loading Study 88

24. Effluent VSS Profile for Hydraulic Loading Study 89

25. Effluent pH Profile for Hydraulic Loading Study 90

26. DO Uptake Profile of Beads during Hydraulic Loading Study 92

27. MLSS in ASR during Hydraulic Loading Study 93

28. SEM of Unseeded Chitosan Matrix (5400 X) 96

29. SEM of Seeded Chitosan Matrix (5400 X) 97

30. SEM of Unseeded Chitosan Matrix (10,000 X) 98

31. SEM of Seeded Chitosan Matrix (10,000 X) 99

32. Comparative TDA Loading and Removal Rates for ICR and ASR 108

33. Comparative TCOD Loading and Removal Rates for ICR and ASR 109

34. Comparative SCOD Loading and Removal Rates for ICR and ASR. 110

CHAPTER - I

INTRODUCTION

Development and Scope of the Experimental Study

Immobilized cells may be defined as cells that are physically confined or localized in a certain region with retention of their catalytic activities, and which can be used repeatedly and continuously (Klein and Wagner,1983). Conventional wastewater treatment systems such as trickling filters and rotating biological contactors are just two examples of immobilized cell systems. Immobilization is a general term that includes the following:

(a) attachment to solid surfaces;

(b) entrapment of cells within three-dimensional polymer matrices;

(c) confinement of free cells in a reactor with semi-permeable membranes;

(d) site-specific bonding resulting from chemical interaction between the cell and the support; and,

(e) pelletization or flocculation of cells.

Immobilization by entrapment has been the most popular technique used in the pharmaceutical industry chiefly because of mild immobilization conditions as well as immobilization without chemical reaction between cells and support; thus ensuring preservation of viability and activity (Klein and Wagner, 1983 ; Kennedy and Cabral,1983; Mattiasson,1983).

In the present work, a mixed microbial biomass was entrapped in a three dimensional polymer network of chitosan, a polymer derived from a naturally occurring substance

called chitin. The resulting immobilized cells, which were in the form of beads of approximately 3 mm diameter, were then used in treating a simulated complex wastewater. Recent research has revealed the great potential of using entrapped microbial cells within a polymer / gel matrix for wastewater treatment (Sofer et al,1989 ; Kennedy and Cabral, 1983).This study was undertaken to explore the feasibility of applying chitosan-immobilized cells in the treatment of complex, synthetic industrial wastewater by comparing the treatment efficiency of the system with that of a bench-scale internal recycle activated sludge reactor operated under similar conditions.

In a continuous-flow, completely-mixed, suspended-growth treatment system without recycle, the overall efficiency may be improved by one or both of the following methods: (a) by increasing the loading rate (b) by increasing the viable biomass concentration in the reactor. However, the above methods are limited by the occurrence of cell washout (Kennedy and Cabral, 1983 ; Mattiasson.,1983). One way to circumvent this problem is by confining a large amount of active biomass within porous, solid, freely-moving, macro-particles. Adopting this entrapment method will reduce the washout problem in this type of system while retaining some of its intrinsic advantages. In the past, several naturally occurring polymers such as alginate and k-carrageenan have been used for immobilizing whole cells and the resulting biocatalysts applied to wastewater treatment (Sofer et al, 1990; Kennedy and Cabral, 1983).

Chitosan is a deacetylated form of chitin, a material that forms the exoskeleton of sea-animals such as shrimp and crab. Chitin is potentially a waste product obtained from the seafood industry (de la Noue & Proulx,1988 ; Muzzarelli et al, 1986). Chitosan has been used in immobilizing homogeneous bacterial cultures and used in conversion or formation of specific organic compounds (Vorlop and Klein, 1981,1987; Wagner et al, 1982). In the present study, a mixed microbial culture immobilized in chitosan beads was used for the first time to treat a complex industrial wastewater containing several organic compounds.

One of the advantages entrapped cell systems have over the other conventional systems is that the cell density per volume of supporting matrix may be greatly increased. This increases the viable cell concentration and consequently, the efficiency of the system. It also prevents failure from occurring due to washout conditions resulting from high hydraulic loadings, which is a common problem in suspended growth systems. Entrapped cell technology is therefore highly suitable for not only designing smaller treatment units but also in upgrading an existing system (Klein and Wagner, 1983).

This research also involved treating a simulated complex industrial wastewater containing chemicals normally present in chemical, plastics, petrochemical and petroleum industry wastewater. The synthetic mix was used as a base for evaluating the biodegradability of the target compound 2,4 Toluene diamine (2,4 TDA), a toxic organic compound that can occur in such complex wastewaters. The above strategy was intended to provide information on overall treatment versus removal of a specific toxic compound in a situation where both easily biodegradable as well as toxic or recalcitrant compounds occur together (Stover and Kincannon, 1981).

In this thesis, the terms immobilization and immobilized cells mean only entrapment of cells and entrapped cells, respectively - unless stated otherwise. The terms beads or biocatalysts define the solid-phase treatment medium containing immobilized cells.

Objectives

The goals of this research were

1. To compare the overall performance of the biocatalyst system and the control activated sludge reactor with respect to wastewater treatment efficiency.
2. To study the responses of both the systems to increasing compound loading rates at a constant hydraulic retention time (HRT) and to establish the HRT at a

fixed organic loading at which the systems first exhibit severely stressed conditions.

3. To achieve a better understanding of the special problems and limitations associated with start-up, operation and maintainance of immobilized cell systems, specifically, chitosan biocatalysts.

CHAPTER II

LITERATURE REVIEW

Scope of the Review

This review surveys the available literature in order to give the reader a general background of the present research with emphasis on the immobilization technology. The use of immobilized microbial cells for the purpose of wastewater treatment is a new area. Research done in the past two decades has provided a better understanding of this technique as applied to wastewater treatment. Although chitosan-immobilized bacterial cells have not been used to date in treating complex wastewaters as well as specific toxic organics, researchers in the past have successfully used other immobilizing matrices such as calcium alginate for the above purpose (Sofer et al,1989; Yang and Wang,1990). In this review, an overview of immobilized cell applications in wastewater treatment is delineated. Attention is focussed on the benefits of immobilized cells, typical reactors that may be used in such systems, their general characteristics and associated limitations. Since a bench-scale activated sludge reactor was used in the present study for the purpose of comparing its performance with that of the immobilized-cell reactors, the basic theoretical and operational characteristics of such a reactor are briefly described.

The origin, characteristics and significance of the complex, synthetic, industrial wastewater that was used in the present work is related to previous research conducted with it. The physical and chemical properties and environmental significance of 2,4,TDA is included along with notes on its biotic and abiotic degradation.

A General Discussion on Immobilized Cell Systems

Definition of Immobilized Cells

Immobilized cells may be defined as cells that are physically confined or localized in a certain defined region of space with retention of their catalytic activity and their viability and which can be used repeatedly and continuously (Klein and Wagner, 1983). In this definition, three important aspects need to be explained i.e.,

(a) confinement ;

(b) retention of catalytic activity; and,

(c) repeated and continuous use.

The cells are confined to a macroscopic solid support that is in contact with the reactant medium or in this case,the complex wastewater. This type of localization of cells makes it possible to have a large amount of biomass in a much smaller volume compared to a free cells reactor.

During and after the immobilization process, the catalytic activity and viability of cells can be maintained by carefully controlling the environmental conditions (Klein and Wagner, 1983). It should be understood, however, that viability may not be required in cases where only enzymatic activity need be retained.

The immobilized cells may be used continuously and repeatedly because the biocatalysts are clearly separated from the reactant medium and therefore may be retained within the reactor, especially in a continuous flow mode (Kennedy and Cabral, 1983; Klein and Wagner,1983).

Immobilized Cells versus Free Cells

Advantages. Immobilized cells have intrinsic advantages compared to the use of suspended or free cells. These are as follows:

(a) reuse and continuous use of the cells in the form of biocatalysts without the problem of cell washout;

(b) immobilized cell systems enable exploitation of the advantages of various reactor configurations;

(c) immobilized cells are easier to handle, are potentially less prone to contamination, and are clearly separated from the reactants and products in the liquid medium;

(d) immobilized cells make it possible to have high cell numbers per unit reactor volume and thus enable significant reduction in reactor volumes for a required treatment efficiency;

(e) the application of immobilized cells results in lower fluid viscosity in the reactor than if a comparable free-cell biomass is used; and,

(f) free cell batch reactors may be replaced by equivalent immobilized cell continuous flow configurations which offer better process control, reduced O&M costs, minimal downtime and product uniformity (Kennedy and Cabral, 1983; Klein and Wagner, 1983; Brodelius and Vandamme, 1988).

Disadvantages.There are several drawbacks associated with immobilized cells that have resulted in the rather slow introduction of this technology into commercial industry even though laboratory research on immobilized cells has unequivocally proved their several advantages (Sofer et al,1990). With specific reference to entrapped cells, these disadvantages may be listed as follows:

(a) carrier / reagent costs are relatively high compared to free-cell technology;

(b) low efficiency of immobilization;

(c) operational and mechanical stability problems;

(d) toxicity to cells due to immobilizing matrix or reagents;

(e) efficient conventional technology that obviates the need for immobilized cell systems;

(f) lack of adequate research at pilot-plant scale;

(g) undesirable side reactions such as matrix effects;

(h) toxicity to entrapped cells due to substrate or product accumulation; and,

(i) cell leakage from the supports (Kennedy and Cabral,1983; Cochet et al, 1990; Venkatasubramanian et al, 1983).

Methods of Immobilization

Cell immobilization may be achieved by two types of methods, (a) bonding, and (b) physical entrapment. Bonding involves either physical or chemical or both types of interaction between the cell and the support or among cells. A fixed-film system is a good example of this type of immobilization (Cochet et al, 1990).This method is not relevant to the present work and therefore will not be elaborated.

In physical entrapment, whole cells are physically restrained in a porous support that serves two principal purposes, i.e.,(a) confining the whole cells in a polymer network, and (b) allowing the passage of reactants and products to and from the cells.

Physical entrapment differs from bonding methods of immobilization in that the cells are not affected by cell-cell or cell-support interaction. This implies that the structure of the cell membrane is preserved intact during immobilization. However, the cell activity and viability may be affected by environmental changes during the process of immobilization. It has been shown in the past that immobilized cells must be kept in a living state in order to effect complex biochemical reaction pathways. Most entrapment methods offer mild

immobilization conditions that do not adversely affect viability and growth (Kennedy and Cabral, 1983).

The most popular method used to entrap whole cells is immobilization in the course of carrier preparation applying a mild chemical process called ionotropic gelation. Ionotropic gelation may be defined as network formation by ion exchange due to ionic crosslinking of poly-ionic chains with multi-valent counterions. Generally, in this process, a precultured cell suspension is mixed homogeneously with a linear polyelectrolyte, usually a long-chain polymer. This mixture is then dropped into a solution of multi-valent counterions. Ionotropic gelation takes place resulting in a microporous network that hold the cells within. For example, sodium alginate prepolymer may be crosslinked with calcium chloride (Ca^{2+} ions) to form the porous polymer calcium alginate. Chitosan beads are also a product of ionotropic gelation (Kennedy and Cabral, 1983; Mattiasson, 1983).

Previous research on ionotropic gelation reports:

(a) matrix formation by ionotropic gelation may be a reversible process in that certain adverse conditions such as a change in pH, presence of certain ions or interaction between the low molecular weight counterions and the components of the reaction medium may lead to the dissolution of the matrix;

(b) the mechanical stability of the biocatalysts are good under normal, non-mechanical agitation and in packed column conditions;

(c) it is possible to form regular beads of varying diameters;

(d) macroporous networks are formed at the completion of gel formation;

(e) cell loading capacity depends on mechanical stability and catalyst efficiency; and,

(f) entrapment method is usually under mild conditions which helps in preserving the cell viability (Klein and Wagner,1983; Kennedy and Cabral, 1983).

Possible Effects of Immobilization on the Kinetics and Properties of Living Cells

Even though immobilized cells offer a number of unique advantages over the application of free cells for wastewater treatment, there are some inherent problems associated with the kinetics of these biocatalysts which put limitations on their applicability. These may be broadly classified as follows: (a) partitioning effects, and (b) mass transfer and diffusional effects.These two effects in turn influence the overall kinetic behavior of the system.

Partitioning Effects. Partitioning effects arise from electrostatic or hydrophobic interactions between the matrix and low-molecular weight compounds present in the liquid medium. This may result in a change in the micro-environment of the entrapped cells. However, this is significant only in the case of bonding between cell and support (Kennedy and Cabral, 1983).

Mass Transfer Effects. Since the cells are entrapped inside the carrier in the ionotropic gelation method, the porous matrix acts as the interface between the liquid wastewater medium and the active cells. Substrates and products pass through the matrix during the treatment process. Diffusional mass transfer effects of two types may occur in this case, i.e. external mass transfer and internal mass transfer. External mass transfer occurs due to the catalytically active outer surface, in contact with the liquid medium, being surrounded by a stagnant film called the Nernst layer, across which actual substrate - product diffusion takes place. The corresponding driving force creates a concentration difference between the liquid and the solid media. The effect of internal mass transfer within beads is unique to cells immobilized within a porous support. This effect is caused by internal diffusion of the substrates and the products. The substrate concentration decreases from the surface of the matrix towards its center; while a corresponding product concentration gradient occurs in the opposite direction (Kennedy and Cabral, 1983).

Other effects, such as changes in the physiology and metabolism and increase in the respiration rates of immobilized cells have been reported. Considerable growth of cells, both internal or solid phase growth as well as bulk-phase growth, has been observed especially in the case of growth-associated product formation. This phenomenon has led to periodic fluctuations in the product and biomass output. It has been proposed that these changes are a function of the dilution rate or HRT (Mattiasson,1983).

Engineering Aspects of Immobilized Cell (IC) Systems

Three factors that govern the overall design of an IC system are given below:

(a) shape of the biocatalysts;

(b) mechanical properties of the biocatalysts; and,

(c) reactor design (Kennedy and Cabral, 1983; Venkatasubramanian et al,1983).

Shape of Biocatalysts. In the past, most researchers who worked with ionotropic gels for immobilizing cells used spherical beads (Sofer et al,1990; Bettmann and Rehm,1984; Vorlop and Klein,1981). Spherical beads offer some advantages over other possible shapes. They are as follows:

(a) easy to manufacture because of simple equipment needs;

(b) easy to handle; may be handled just like ion-exchange material in most reactor designs;

(c) surface area may be increased easily by decreasing the size, depending on the application; and,

(d) least damage-prone due to rounded surface.

Mechanical Properties. Compression behavior in packed-bed systems is an important property to be considered. The extent of abrasion in a stirred tank reactor is also very important for bead stability. This depends on the following factors,

(a) matrix type;

(b) cell loading; and

(c) reactor shape.

Reactor Design. The selection and design of a reactor system employing immobilized cells (ICs) need be done after careful consideration of the following factors:

(a) cell viability requirements;

(b) type of matrix and method of immobilization;

(c) nature of wastewater;

(d) kinetics of various reactions involved;

(e) operational requirements of the process;

(f) ease of biocatalyst replacement;

(g) hydraulic characteristics required;

(h) simplicity of reactor design and process control; and,

(i) cost of reactor set-up and O & M costs. Table I gives a summary of various reactor configurations that have been used and their advantages and disadvantages with regard to the above factors (Venkatasubramanian et al, 1983; Kennedy and Cabral, 1983; Klein and Wagner, 1983; Mattiasson, 1983).

Factors in the Selection of the Matrix

The factors to be carefully considered in selecting a suitable matrix are given below:

(a) the material should be easily available on a large scale at a low price;

(b) the process of immobilization must be simple and effective;

(c) the activity of the immobilized cells should be preserved or minimally affected;

(d) mechanical stability of the supporting matrix must be high; and,

(e) reactor design with respect to mechanical handling of the matrix should be simple (Klein and Wagner, 1983; Mattiasson,1983).

TABLE I

CHARACTERISTICS OF DIFFERENT REACTOR CONFIGURATIONS APPLIED TO IMMOBILIZED CELLS

	Packed bed reactors	CSTR	Fluidized bed reactors	Hollow fiber reactors
Ease of operation	Easy	Easy	Difficult	Easy
Ease of catalyst replacement	Difficult	Easy	Easy	Easy
Pressure drop	High	Low	Low	Low
Ease of cell removal	Difficult	Easy	Easy	Easy
Mixing characteristics	Poor	Good	Good	Fair
Oxygen transfer	Poor	Good	Good	Fair
Distribution of viable cells	Uneven	Uniform	Uniform	Uneven
Catalyst attrition	Low	High	Low	Low
Ease of scale-up	Easy	Easy	Difficult	Difficult
Cost	Low	Low	High	Low
Suitability for:				
1. Product inhibited kinetics	Good	Poor	Fair	Good
2. Substrate inhibited kinetics	Poor	Good	Fair	Poor
Ease of reactor control	Difficult	Easy	Easy	Difficult

(adapted from Mattiasson (1983))

Organic polymers - both naturally occurring and synthetic - were used in previous research. At present, the focus has shifted to naturally occurring polymers such as alginate, k-carrageenan and chitosan. Synthetic polymers such as polyurethane have unique advantages in that they are more chemically resistant as well as superior in strength. However, they are more expensive and add to the pollution problem (Kennedy and Cabral, 1983).

Analysis of a Completely Mixed Reactor used as an Immobilized Cell Reactor

In the present study, a continuous flow complete mix reactor (a simple chemostat) was used as the reactor containing the biocatalysts. This system is identical to a Complete-mix Stirred Tank Reactor (CSTR) without recycle.

In a chemostat, substrate as well as product concentration is the same at any point in the vessel and in the effluent. The substrate concentration is minimized with respect to the final conversion in a chemostat, unlike, for instance, in a plug-flow reactor. Therefore, the average reaction rate is lower in this reactor type. It is obvious that the chemostat system is more suitable for application in situations where substrate inhibition occurs (Venkatasubramanian et al, 1983).

Strengths and Weaknesses of a CSTR applied to ICs

The CSTR configuration offers some advantages that are particularly amenable to IC applications. They are as follows:

(a) since the CSTR has a very simple construction, it is easy to replace biocatalysts;

(b) the complete mix provides uniform environmental conditions throughout the reactor;

(c) preferable where substrate costs are not important;

(d) relatively greater gas and mass transfer efficiency compared to other types of reactors;

(e) plugging due to biomass or suspended solids will not occur; and,

(f) pressure drop will not occur.

The disadvantages of this reactor are as given below:

(a) since the average reaction rates are lower, compared to other conventional configurations, more biocatalysts are required;

(b) if product inhibition occurs, it will be more pronounced in this case since the product will be in contact with all of the biocatalyst; and,

(c) the biocatalyst should be strong enough to withstand the high shear forces existing in the reactor (Kennedy and Cabral,1983; K. Venkatasubramanian et al,1983; Mattiasson, 1983).

Previous Applications of Immobilized Cells - Some Examples

Scope of Review

Results from various studies involving different types of IC systems treating wastewater are presented in this section. Some general facts about application of immobilized cells to wastewater treatment are highlighted with background on the specific research that was conducted. Chitosan-immobilized microbial cells have not been used to date in treating complex wastewaters. However, chitosan-immobilized algal biomass was successfully employed in the tertiary treatment of urban wastewaters. In this study, fine chitosan flakes were added to the secondary effluent to form chitosan-algae aggregates. This material was then used in batch and semi-continuous reactors to remove inorganic nitrogen and phosphorus (de la Noue and Proulx, 1988). This application was

fundamentally different from the present study in that (a) algae was used, and (b) chitosan was used in the raw form as a cohesive agent.

Chitosan-entrapped pure bacterial cultures have been successfully used by several research groups to synthesize or convert pure organic compounds (Vorlop and Klein,1981; Wagner et al, 1982; Stocklein et al, 1983). Even though this type of application is well outside the realm of wastewater treatment involving mixed bacterial populations and heterogeneous wastes, these studies have provided very useful information on the manufacture as well as the performance characteristics of chitosan beads.

Origin, Chemical and Physical Properties of Chitosan

Chitosan is a partially deacetylated form of chitin, which occurs naturally in the exoskeletons of aquatic animals such as crab and shrimp (Muzzarelli, 1986). Chitin is a waste product from the sea-food industry (de la Noue and Proulx, 1988). It is a high molecular weight, polycationic, linear polymer, consisting of glucosamine linkages as shown in Figure 1 . Chitosan is available commercially in the form of flakes or powder. It is soluble in weak organic acids such as acetic acid, though not so soluble in mineral acids. A chitosan-acetate solution, formed by dissolving chitosan in dilute acetic acid, can form ionotropic gels upon contact with multi-valent anionic counterions or crosslinking agents such as tripolyphosphates, hexametaphosphates and ferrocyanides (Vorlop and Klein, 1981; Vorlop and Klein, 1987). This property is exploited in the manufacture of chitosan biocatalysts used in wastewater treatment.

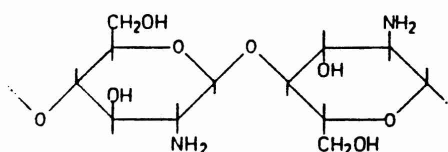


Figure 1. Chemical Structure of Chitosan
(adapted from Vorlop and Klein, 1987)

Advantages and Disadvantages of Chitosan Biocatalysts

The method to make chitosan biocatalysts was first developed by Vorlop and Klein (1981). In a study by the above workers, E-coli cells having tryptophan synthetase activity were immobilized in chitosan-acetate. These biocatalysts were then used for synthesizing L-tryptophan from L-serine and indole. This method was developed in order to overcome several disadvantages of other matrices. Three different procedures were put forth by these workers. For living cells, the suggested technique was to follow the method of ionotropic gel formation. In this method, the chitosan-acetate -cell mixture was dropped into a cross-linking solution having a pH less than 6. The beads were collected for use after 30 minutes of curing in the solution and subsequently washed using 0.1 M phosphate buffer at a pH 7.5. At this stage, a great number of the free amino groups in chitosan (Figure 1) are totally protonated and ionotropic gelation occurs. In the second method,the biocatalysts were formed in a cross-linking solution which was maintained at a pH of 8.2

using 5 M NaOH. After 4 hours,the beads were removed. At a pH > 7.5, beads can also form; however, this is due to the precipitation of chitosan and deprotonation of the amino groups. An important aspect of chitosan immobilization is the shrinking of the beads that occurs during the curing process (Vorlop and Klein, 1987). The third method seems to be a combination of the above two methods. Beads were first formed in a tripolyphosphate solution at a pH of 5.5 and after 30 minutes, these particles were transferred to another tripolyphosphate solution at a pH of 8.5 for curing. The beads were cured in pH 8.5 solution for three hours after which biocatalysts of 2mm diameter were obtained. They were further dried for 3 hours at 20 ^{o} C for hardening. At the end of the drying phase, 1.3 mm diameter beads were obtained (Vorlop and Klein, 1981).

Chitosan gels have several advantages over other ionotropic gels such as calcium alginate and k-carrageenan. The calcium alginate matrix is structurally unstable in phosphate-buffered solutions but, since phosphate is essential for sustenance of microbial viability; lifespan of alginate carriers is limited. Carrageenan is unstable in solutions containing Na+ ions (Sofer et al, 1990; Vorlop and Klein,1981). Polyacrylamide, another polymer gel, has a crosslinking agent (N,N'-methylene-bis-acrylamide or BIS) that is toxic to microbial cells. Also, the gelation has to be carried out at low temperature in order to preserve the viability of cells. This makes the procedure more difficult . Cellulose triacetate, yet another popular immobilizing matrix, needs toluene for cross-linking. Toluene is a known toxic chemical which could seriously affect the activity of the entrapped cells. The cellulose triacetate bead-making procedure is also relatively complex compared to the chitosan or alginate procedures (Yang and Wang,1990). Chitosan beads are very stable in phosphate-buffered solutions and are not affected by other ions. Further, the immobilization conditions are mild and do not adversely affect the activity of the entrapped cells. These biocatalysts have good mechanical properties and are excellent for high cell loading and high activity (Vorlop and Klein, 1987). However, it has been reported that the neutral chitosan matrix is stable at pH values greater than ~ 7.5. At lower pH values,

crosslinking ions are necessary for its structural stability. In order to circumvent the above problem, further hardening of the chitosan matrix may be carried out using glutaraldehyde or other bifunctional agents (Vorlop and Klein, 1981).

Chitosan beads were observed to be more mechanically stable than alginate beads when crosslinked with tetra-sodium pyrophosphate. However, the same beads were not as strong when crosslinked with potassium ferrocyanide. The mechanical stability of chitosan beads was shown to be directly proportional to the magnitude of shrinking that takes place during the curing process in the crosslinking solution. The shrinking, in turn, depends inversely on the cell loading. The chitosan beads dissolved in the absence of phosphate ions and swelled in acid conditions (Stocklein et al, 1983). In a study conducted by Wagner et al (1982), it was mentioned that the final cell density (cell mass / unit matrix volume) after curing was four times greater than the initial cell density of the chitosan-cell mixture. On completion of shrinking, the original diameter had decreased by about fifty percent.

The activities and operational stability of chitosan beads with resting or living cells have been studied by some researchers. It was stated by Vorlop and Klein (1987) that the two most important aspects of a good industrial biocatalyst are its activity and its operational stability. In converting Penicillin-G solutions to 6-APA (6-aminopenicillanic acid) using immobilized resting cells (non-growing enzymatically active cells) in batch studies, it was demonstrated that chitosan biocatalysts retained nearly 90% of their conversion efficiency even after 70 batches. In the case of living E-coli cells (actively growing cells) immobilized for L-tryptophan production, it was determined that the activity of the cells increased over a 40 hour period. The growth of cells inside the beads based on the L-tryptophan productivity and by dry weight comparisons of beads at different times was estimated by Wagner et al (1982). By this method, it was determined that the cell mass increased 400 times after 40 hours of incubation of the beads in the growth medium used. A linear relationship was also established between the cell concentration within beads and

the L-tryptophan productivity. Growth experiments conducted with two Bacillus sp. by Vorlop and Klein (1987) proved that the cells within the chitosan tripolyphosphate grow at a rate one-third that of free cells.

The conversion of phenylalanine to tyrosine using Pseudomonas sp. cells immobilized in chitosan beads was studied by Stocklein et al (1983). The stability and activity of chitosan and alginate beads were investigated and their performance (with reference to their phenylalanine hydroxylation activity) was compared with that of free cells cultivated under similar environmental conditions . This study revealed that the activity of chitosan beads was only 50% that of free cells and approximately 25% less than the activity of alginate beads. It was concluded that the loss of activity in chitosan beads could be due to pyrophosphate toxicity during cross-linking. In most studies with chitosan biocatalysts, sodium tripolyphosphate has been used as the cross-linking agent since it is non-toxic to microbial cells (Vorlop and Klein,1981; Vorlop and Klein,1987).

Polymer beads of this type have a common limitation which affects their efficiency to a large extent - the porosity of the matrices. This factor directly influences the diffusion of substrate and product into and out of the matrix as well as cell leakage from the beads. Several studies have been conducted in this direction although extensive research on chitosan carriers have not been accomplished to date (Mattiasson, 1983; Kennedy and Cabral, 1983; Klein and Wagner, 1983).

Based on scanning electron micrographs of chitosan beads, it was proved that the matrix has a microporous surface with larger cavities inside. This has been stated to be an ideal matrix because it retains large microbial cells but allows smaller substrate and product molecules to pass into and out of the bead matrix quite freely (Vorlop and Klein,1981).

A study conducted by Stocklein et al (1983) revealed that cell leakage from chitosan biocatalysts was less than that of calcium alginate carriers by a factor of 100. However, chitosan immobilized cells showed reduced activity (phenylalanine hydroxylation activity) compared to alginate-entrapped cells. Chitosan beads had only 50 % of the activity of free-

cells while the alginate beads had 80% activity. Diffusional limitations for the substrate and / or the product, pyrophosphate toxicity and oxygen deficiency were identified as the possible reasons for the reduced activity. Based on these results, it was concluded that chitosan was the most suitable matrix for the immobilization of Pseudomonas sp. cells.

Applications of Immobilized Cells in Wastewater Treatment

An extensive study of wastewater treatment by immobilized cells was conducted by Yang and Wang (1990). A number of experiments utilizing various matrices was performed. Two significant aspects of this work were (a) application of mixed microbial cells to degrade easily biodegradable and toxic/inhibitory synthetic wastewaters; and (b) using cellulose triacetate monocarriers and alginate-cellulose triacetate bicarriers.

For the readily biodegradable wastewater, glucose was used as the sole carbon source. COD loadings in the range of 1.2 - 12 g COD / L.day were applied to the bicarrier to study the effect of COD loading on COD removal efficiency. The monocarrier beads were enclosed in a 250 mL column while the bicarrier beads were used in a 500 mL column, with substrate administered in a downflow mode and air diffuser at the bottom of the columns. In this set-up, it was shown that alginate (monocarrier) beads did not have sufficient mechanical strength to withstand the compression in the packed column. The system exhibited a maximum SCOD removal efficiency of 98 % in the loading range 4 - 6 g COD/L.day. Over the entire loading range of 1.2 - 12 g COD / L.day, an average SCOD removal efficiency of 90 % was achieved. It was observed that the monocarrier was mechanically more stable than the bicarrier. Hence, the monocarrier was concluded to be more suitable for long-term operations.

In their long term study (Yang and Wang, 1990) conducted with glucose wastewater and the monocarrier, it was observed that the effluent SS contributed to an apparent increase in the effluent TCOD values of 40 - 65 mg/L. However, after one month of

operation, the effluent SCOD and SS concentrations were in the range of 5 - 20 mg/L and 2 - 25 mg/L respectively. In this study, TCOD removal efficiency was greater than 93 % and SCOD removal efficiency was above 95 %. To prevent clogging due to biomass, the beads were washed. The frequency of washing was based upon the organic loading rate.

In the experiments conducted by Yang and Wang (1990) on a synthetic wastewater using phenol as the sole carbon source, COD loadings in the range 3 - 9.5 g.COD/L.day were applied to the bicarrier in order to evaluate the effluent quality. The loading was increased from 3 to 9 g COD / L.day over 32 days. After 10 days, clogging was observed and the beads were washed. During the first 20 days, effluent SCOD and SS concentrations were below 50 mg/L and 35 mg/L, respectively. Removal efficiencies for TCOD and SCOD were above 85 % and 90 %, lower than those for glucose synthetic wastewater. The difference was attributed to phenol toxicity. At a COD loading of 9 g/L.day on day 22, the SCOD and SS concentrations in the effluent increased sharply to about 100 mg/L and 50 mg/L, respectively. The system, however, returned to the previous state after one week. This loading was considered to be the critical point for both substrate and cell washout. A further increase in COD loading to 18 g/L.day at an HRT of 0.77 hour led to washout and the system did not recover during the 9 days of observation. In the long term study using phenol synthetic wastewater and the monocarrier beads, the loading rate was maintained in the range of 2.5 - 3.5 g COD/L.day. The SCOD and TCOD removal efficiencies after 10 days were consistently greater than 90% with effluent SS less than 25 mg/L. Based on the consistently high effluent quality, it was concluded that an external settler was unnecessary for this system in this organic loading range.

Since there is no definite method to estimate biomass within the matrix, the SRT was calculated by performing a mass balance on the NH_4-N entering and leaving the system. The amount of nitrogen utilized in cell synthesis was determined by protein assay and this value was then used in calculating the SRT. It was observed that the SRT gradually increased with the days of operation ; internal cell growth was reported as the causal factor

for this increase. It was determined that an SRT of 30 days could be achieved with a start-up period of 8 days, which is comparable with a typical extended aeration process. On an average, however, SRTs in the range of 8 - 15 days could easily be maintained.

Several experiments were conducted by the above workers (Yang and Wang,1990) to determine the operational stability of cellulose triacetate beads under concentration shock loads. In this study, in order to facilitate direct comparison, the same composition of phenolic wastewater and dilution rates that were applied by a previous research team (Rozich and Gaudy,1985) on an activated sludge process with cell recycle were used. This comparative study showed that the entrapped cell system performed comparably or even slightly better than the activated sludge system, with respect to effluent SCOD and SS concentrations.

Hydraulic shock load studies on cellulose triacetate beads were also conducted by Yang and Wang (1990), keeping influent COD concentrations constant at 570 and 250 mg/L. It was reported that when the HRT was decreased stepwise from 6 to 1.5 hours, effluent TCOD increased due to increased effluent SS (cell washout) while SCOD increased initially but decreased to the normal range after some time. It was clearly demonstrated that the immobilized cell system performed well even at very low HRTs. It was reported that hydraulic shock loads had a smaller effect on overall performance than did concentration shock loads. This property has been stated to be an advantage when designing treatment facilities for factories discharging phenolic wastewaters with changing or fluctuating hydraulic flow rates.

The results of a series of experiments on the biodegradation of 2- Chlorophenol (2-CP) using calcium alginate -immobilized activated sludge was reported by Sofer et al (1990). In this study, two types of reactors, air-sparged and recirculation reactors, were employed to determine various characteristics of the bead system. 2-Chlorophenol, a toxic organic compound, was used as the sole carbon source. The mixed microbial population taken from a water pollution control plant was acclimated to phenol and 2-CP by spiking it

with 5 doses of each compound alternately over 10 consecutive days. Acclimation was considered to be achieved when the dissolved oxygen (DO) consumption of the culture stabilized. Biomass drawn from this culture was centrifuged at 3000 rpm to obtain concentrated pellets whenever needed. These pellets were used to make the beads. The ratio of dry biomass to wet pellets was determined to be 45 mg dry biomass / g pellets. When making the beads, a constant ratio of 5 : 2 by weight of distilled water to pellets was maintained (Sofer et al, 1990).

The air-sparged reactor was used to quantify the biodegradation as well as physical removal of 2-CP. A vessel 5.08 cm in diameter and 20.32 cm in length with a working volume of 300 mL was used as the reactor. Thirty grams of beads were placed in the reactor and sparging was achieved by diffusing water -saturated air at 1 L / min.; the air kept the beads in suspension as well as aerated the liquid medium. One significant fact emerged from this study ; air sparging severely affected the mechanical stability of the biocatalysts. For instance, in this case, the alginate beads did not last more than 36 hours (Sofer et al, 1990).

In order to overcome this problem, a recirculation reactor in which aeration was performed outside the main reactor in a separate reservoir, was used. The results of the study may be summarized as follows:

(a) the immobilized cells were able to tolerate significant fluctuations in pH brought about by the stripping of free HCl due to aeration and biodegradation of 2-CP;

(b) bead stability is inversely dependent on recirculation velocity; for example, in the above experiment, alginate beads disintegrated within 48 hours at a cross sectional upward velocity of about 30 cm/min and even at the operational velocity of approximately 7.5 cm/min, the beads lasted only one month; and,

(c) an increase of biomass loading does not give a proportional increase in biodegradation rates.

These workers also used a Clark-type DO electrode enclosed in a 1.8 mL jacketed vessel for measuring beads activity and biodegradation in terms of DO uptake by the beads (Sofer et al, 1990).

The toxicity of phenol on free and calcium alginate -immobilized cultures of E.coli, Pseudomonas putida and Staphylococcus aureus.was investigated by Keweloh et al (1989). Some general aspects of the effects of toxic compounds on the free and immobilized states of essentially homogeneous cells were stated. It was reported that when free cells and immobilized cells of E.coli were cultivated in a glucose-containing growth medium and 1 g/L of phenol was added after 4 hours of exponential growth, the free cell growth was inhibited 3 to 4 times more strongly than that of immobilized cells. Another finding was that, different species had different levels of tolerance. It was reported that P.putida, which was originally derived from a phenolic wastewater, was more sensitive to phenol than the other two species. When all the species were grown in free and immobilized states in the presence of glucose as the primary carbon source, they exhibited a higher tolerance to phenol when it was added. Growth studies conducted with E.coli revealed that cells that grew inside the beads showed a higher tolerance to phenol. SEM studies showed the micro-organisms were confined to the outer areas of the inside of the bead. This was attributed to a possible oxygen transfer limitation. It was postulated that the immobilized microbes built an extracellular layer around their colonies and this structure aided in protecting against toxicity. In order to prove this, the immobilized cells were liberated using sodium hexametaphosphate after a 4 hour growth period in the medium. Then the freed cells were exposed to phenol. It was shown that the cells lost most of their previous phenol tolerance within one hour of exposure to phenol. Interestingly, it was also reported that compared to the above cells, cells that were never immobilized had a lower phenol tolerance. Based on the above result, it was concluded that the immobilization process itself endows resistance to toxicity to cells. In another experiment, it was demonstrated that biocatalysts that were highly loaded originally with biomass, could

develop only small colonies and therefore exhibited lesser tolerance to phenol compared to beads that had a lesser biomass loading in which more new cells were able to grow (Keweloh et al, 1989). This implies that biomass loading has to be optimized with respect to treatment performance. A similar conclusion was reported by Sofer et al (1990) after bead design studies with calcium alginate-immobilized activated sludge.

A comparative study of two bead systems - alginate and polyacrylamide hydrazide (PAAH) - treating a phenolic wastewater was conducted by Bettmann and Rehm (1984). A Pseudomonas sp. was entrapped in these two matrices and employed in a special airlift fermenter to treat phenol. It was reported that the immobilized microbes were able to degrade up to 2 g/L phenol in two days, while at this concentration, free cells did not grow. Immobilized cells were found to be more efficient in degrading phenol and more tolerant to high concentrations compared to free cells. The reasons behind the protective capacity of the matrices were not known. PAAH was found to offer more protection against phenol compared to alginate - immobilized cells. For instance, free Pseudomonas cells degraded 0.1 % phenol in 48 hours, while alginate and PAAH biocatalysts took only 35 and 25 hours respectively. The immobilized cells were also able to degrade 0.3 % phenol while 50 % of the free cells were killed in two days at only 0.2 % phenol concentration. The difference in degradation rates of the alginate and PAAH was attributed to the total surface area of the two beads. The average diameter of the alginate bead was 1.4 times that of the PAAH and interestingly enough, the activity of the PAAH biocatalyst was observed to be approximately 1.4 times more than that of the alginate biocatalyst. Furthermore, there was no significant difference among the growth parameters such as pH and DO consumption between the two beads. It has been proposed by these workers that formation of micro-colonies by the microorganisms in the outer regions of the beads offer protection to the cells inside by creating a diffusion barrier to phenol.

Basic Characteristics of Activated Sludge Reactor with Cell Recycle

The activated sludge process utilizes a fluidized mixed microbial biomass under aerobic conditions to use organic materials in wastewater as substrates, thus removing them by microbial respiration and synthesis (Reynolds, 1982). A general model for describing the parameters for a complete-mix system with cell recycle was developed Lawrence and McCarty (1970). This model, based on Monod kinetics, rests upon two key assumptions i.e.,(a) all waste utilization occurs in the aerated portion of the reactor, and (b) the total biomass in the system is same as the biomass in the aerated section i.e. the non-aerated settler volume is small and recycle is continuous. It was proposed that the biological solids retention time (Sludge Retention Time or SRT) is fundamentally related to process performance of a continuous flow system. Even though the following equations were developed essentially for pure-culture, homogeneous substrate systems, they have been shown to apply to heterogeneous culture, mixed substrate systems also. SRT (θ_c)has been successfully employed in full-scale biological wastewater treatment processes for exercising control. Conceptually, θ_c, was defined as follows:

$$\theta_c = Xt / (dX/dt)t \qquad (1)$$

where,

θ_c = sludge retention time

Xt = mass of active organisms

dX/dt = mass of active organisms lost in a specific time period including biomass wasted purposely and biomass lost in the effluent

Unlike in a CSTR without recycle, this method allows operational control of the system independent of θ, the space time or HRT. This is achieved practically by controlled wastage of reactor biomass on a daily basis.

To determine the amount of solids to be wasted daily, an equation that is based on the Lawrence and McCarty's model was applied by El-Gamal (1985). For the bench-scale

activated sludge reactor with internal cell recycle, the quantity of biomass to be wasted daily in order to maintain a required θc (days) is given by,

$$Fw = \frac{VX/\theta_c - FXe}{X - Xe} \qquad (2)$$

where,

Fw	=	volume to be wasted (L/day)
V	=	volume of reactor (L)
X	=	mixed liquor VSS concentration (mg/L)
Xe	=	effluent VSS concentration (mg/L)
F	=	feed flow rate (L/day)
θ_c	=	required sludge age (days)

The use of a laboratory type bench-scale ASR with a built-in settler intrinsically precludes accurate control of recycle rate. However, Reynolds (1982) has suggested several empirical guidelines for the start-up and operational control of this type of activated sludge reactor. These may be summarized as follows:

(a) the sludge may be acclimated to a particular wastewater by feeding the reactor in a step-wise manner or in a batch mode. Sludge should be wasted to maintain the desired SRT;

(b) proper recycle may be achieved by keeping the space below the baffle between 1/4 - 3/8 inch;

(c) the baffle should be pulled and the contents allowed to mix thoroughly before drawing samples for MLSS analyses and wasting sludge; and,

(d) the detention time (HRT) should be calculated based on the total volume of the reactor i.e.including the volume of the settler.

Background on the Waste Characteristics

Base Mix

Toxic or inhibitory components could be found in combination with non-toxic or " conventional " wastes. In the presence of easily biodegradable compounds such as glucose, glutamic acid etc., several types of interactions could take place. Two very important types of such interaction are, (a) diauxic phenomenon, and (b) cometabolism.

In diauxic phenomenon, the substrates are utilized sequentially depending on the preference of the degrading bacterial community. The toxic substrate is usually degraded only after the preferable substrate has been exhausted.During the lag period that exists between the two events, the bacterial population switch their enzyme systems to metabolize the next most preferred substrate. In cometabolism, substrates are metabolized simultaneously, though with different degradation rates (Yang and Wang, 1990).

Effluent limitations for wastewaters containing organic compounds have typically been developed in terms of " indirect " indicators such as BOD 5, COD, TOC etc. However, its quite possible that effluent limitations will, in future, also include specific organic compounds found in wastewaters (Stover and Kincannon, 1981). To date, research has only focussed on treatability of specific toxic organic compounds when present alone or at best with one or two other compounds (Valo et al, 1990; Bettmann and Rehm,1984; Rozich and Colvin,1986).

It was proposed by Stover and Kincannon (1981) that more logical substrate mass balance models that encompass both physical and biological removal mechanisms of specific organic compounds from complex wastewaters should be developed. In this connection, the treatment of a complex synthetic wastewater containing chemicals that are typically present in chemical, plastics, petrochemical and petroleum industry wastewaters was studied by these workers. This complex mixture, referred to as the " base-mix "

contained ethylene glycol, ethyl alcohol, glucose, glutamic acid,acetic acid, phenol, ammonium sulfate, phosphoric acid and other salts. It was reported that these compounds were included in the wastewater in order to provide a "broad range biopopulation" and reasonable kinetic rates, settleability effluent quality etc. Further, this base mix was intended to serve as a base for evaluation of specific toxic organics that can appear in " real-world" industrial wastewaters. These toxic organics were added singly or in combination with other organics to the base mix to make the final complex industrial wastewater. The treatment performance of the activated sludge reactors used in this study were monitored with respect to BOD_5, TOC,COD and specific organic compound removal. Other typical operating parameters such as pH, TSS, VSS etc. were also monitored (Stover and Kincannon, 1981).

The Test Compound - Characteristics, Significance and Determination of 2,4 Toluenediamine

2,4 Toluenediamine (2,4 TDA) is an industrial intermediate used in the manufacture of products such as polyurethane industrial dyes, hydraulic fluids, fungicide stabilizers, and toluene isocyanates (Howard, 1984; Unger and Friedman, 1979). It is a large production chemical; approximately 16.5 million pounds were released during production in 1977. Major release of the compound to the environment occurs during production.

2,4 TDA has been reported to be mutagenic and carcinogenic to rodents (Unger and Friedman, 1979). Recently, it was reported in Time magazine (Time, 1992) that TDA was released into the bloodstream from silicone-gel breast implants. It has been shown that leakage of the gel due to rupture of the gel sac may result in cancer. However, it has not been confirmed to be acutely dangerous to human life though it is a toxic substance and is harmful to human health.

The compound has a maximum estimated solubility of 7470 mg/L in water. Due to its high water solubility, it can undergo extensive leaching if released into soil. The Henry's Law Constant for 2,4 TDA is very low and this property coupled with a high water solubility makes loss by volatilization from soil and water negligible. 2,4 TDA may photolyze under direct sunlight due to reaction with singlet oxygen, alkoxy or hydroxyl radicals, which are generated by sunlight. It is amenable to biodegradation which has been stated to be one of the major means of TDA degradation in water and soil (Howard, 1984).

Extensive biodegradability studies were conducted by Matsui et al (1975) with several toxic organics present in the wastewater discharged by petrochemical and petroleum industries. Activated sludge, drawn from the joint wastewater treatment plant of a group of factories was used in these studies. The purpose of this study was to investigate whether or not, activated sludge previously acclimated to the complex wastewater could degrade individual test substances.

One of the compounds investigated by the above team was 2,4 TDA. The MLVSS of the activated sludge at the time of the test was reported to be 2360 mg/L. A fill-and-draw method was used and samples were taken for analysis after 2, 4 and 24 hours.

The COD and TOC due to 2,4 TDA at the start of the experiment were reported to be 105 mg/L and 58 mg/L respectively. After 4 hours, COD and TOC were determined to be 69 mg/L and 32 mg/L; representing removal efficiencies of 34 % and 45 % respectively. Results for longer test durations were not reported (Matsui et al , 1975).

The determination of TDA in aqueous extracts of boil-in bags and retortable pouches using reversed-phase HPLC was reported by Snyder and Breder (1982). Samples were analyzed on a reversed-phase , 5 μm, C-8 column with 10 % acetonitrile aqueous solution as the mobile phase. The mobile phase was buffered at pH 7.4 using mono- and di-basic phosphates and pumped at a flow rate of 1 mL/min. The method was used successfully to analyze down to 1 ng/L of TDA. The compound was detected using a UV detector set at a

wavelength of 254 nm and 0.002 aufs (absorbance unit full scale). The authors have also suggested that the same method may be employed using a reversed-phase C-18 column instead of a C-8 column. In this case, however, it has been proposed that a " slightly longer " analysis time may result with a concomitant " marginally broader " 2,4 TDA peak.

CHAPTER - III

MATERIALS AND METHODS

Experimental Design

Scope of the Design

In order to evaluate any new system used for wastewater treatment, one of the efficient means of gathering basic performance characteristics of the system is to test it for stressed conditions where there is a significant decrease in treatment efficiency. This may be achieved by two different approaches. They are, (a) increasing the organic loading on the system until it exhibits stress conditions keeping the hydraulic residence time (HRT) fixed at an arbitrary value; from the results an optimum loading range may be deduced; (b) reducing the HRT from the above fixed value in steps until the system is stressed hydraulically, keeping the loading fixed in the optimum loading range deduced from the first phase. In the present study, a steady state condition for each unit was operationally defined as the condition at which the TDA and SCOD concentration of the effluent stabilizes within a range having a variability of 10 %, following continuous operation for at least 10 days from the start of the current organic or hydraulic loading range. By using the above strategy, it is possible to define the limits of the system, while at the same time procuring vital information on the different factors that affect the overall performance of the system.

Reactor Selection

Two different types of reactors were chosen for this study. A simple bench-scale activated sludge reactor (ASR) with internal recycle was used as the conventional,free-cell, control reactor while a chemostat (complete mix without recycle)was used as the immobilized cell reactor (ICR)

The activated sludge system was selected for the following reasons:

(a) it is a widely used conventional suspended growth system;

(b) its system characteristics have been extensively studied and therefore well understood;

(c) it is operationally simple; and,

(d) it has a built-in settler with internal recycle to minimize biomass wash-out.

A completely-mixed air-sparged reactor was used because complete mix offers the following advantages over other conventional reactor configurations that may be employed for immobilized cells:

(a) allows uniform distribution and instantaneous dilution of the influent;

(b) permits uninhibited circulation of the biocatalysts within the reactor;

(c) has simple once-through configuration without biomass recirculation;

(d) precludes clogging due to biomass;

(e) does not require a separate external aerator and mechanical stirrer;

(f) is easy to operate and maintain; and,

(g) control ASR unit was also a complete mix system.

Selection of the Immobilization Matrix

Chitosan gel offers several advantages over materials which are potentially beneficial to wastewater treatment. Chitosan beads are:

(a) unaffected by phosphate buffers, sodium and potassium ions;

(b) formed at room temperatures;

(c) non-toxic to micro-organisms;

(d) amenable to high cell-loading capacity; and,

(e) mechanically strong (Vorlop and Klein, 1981; Stocklein et al, 1983; Vorlop and Klein, 1987).

Chitosan-entrapped bacterial cells have not been used to date in treating industrial wastes. Chitin, the raw material used in the manufacture of chitosan, is easily obtained. Based on all these points, it was decided to apply chitosan - immobilized cells for this study (Muzzarelli, 1986).

Experimental Design

In the first phase, both ICR as well as the ASR were operated at an HRT of approximately 10 hours by adjusting the feed flow rates to each unit. At this point, the reactors were fed with the synthetic wastewater containing the base mix and 50 mg/L of 2,4 TDA. When the systems reached steady state, the base mix was maintained at the same level while the TDA concentration was increased in steps of 50 mg/L until one of the systems showed a significant difference in SCOD removal efficiencies. Base mix composition is given in a later section. Since 2,4 TDA also contributed to the total influent SCOD, SCOD removal was considered as the best indicator of overall performance of the system. The different types of data gathered during the course of the experiments and the sampling flowchart used for procuring such data are given in Figure 2.

In the second phase, the TDA concentration was maintained at 150 mg/L with the same base mix concentration, and the HRTs of the systems were gradually lowered to 8, 5, and 3 hours by increasing the feed flow rates to the units. This procedure was continued until significant cell washout occurred in the activated sludge unit. In the case of the

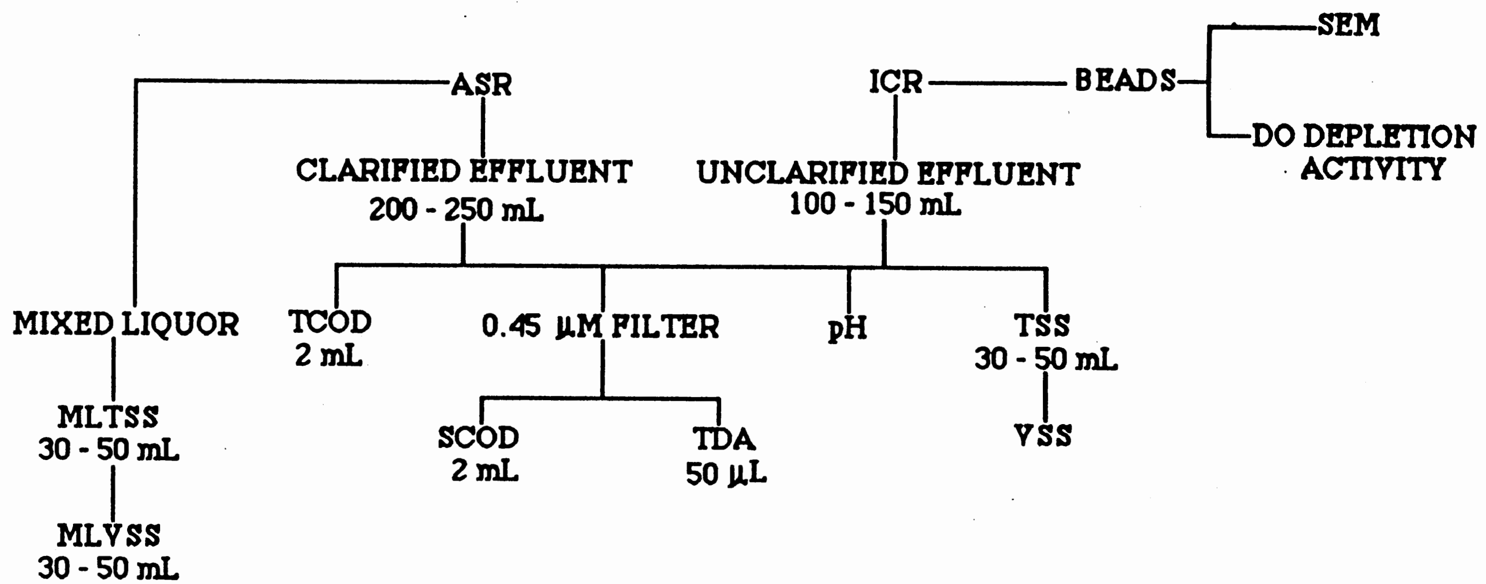


Figure 2. Sampling Flowchart Used in Data Collection

biocatalyst reactor, since it was expected that cell washout will not significantly affect treatment efficiency, the unit was operated until steady-state was achieved; the experiment was terminated at this point. At each HRT, the different parameters were monitored during the transition phase as well as at steady state in order to understand how each system responds to hydraulic shock loads and how it ultimately recovers.

Experimental Apparatus

Immobilized Cell Reactor

The reactor set-up (Figure 3) consisted of a 1000 mL side arm Kimax Erlenmeyer flask which functioned as the reactor vessel containing the biocatalysts. The opening at the top was sealed with a two-holed rubber stopper through which the influent tube and the tube connected to the diffuser stone passed. The side-arm of the flask, which served as the effluent port, was connected directly, without employing a solids trap, to a 25 L glass effluent collection bottle using Tygon tubing. The reactor was placed on a magnetic stirrer, which was employed only during start-up procedure with freshly-manufactured beads. Air supply was maintained at approximately 1 L / min. by a flow rate control device.

The feed was pumped into the reactor using a Cole Palmer peristaltic pump (Model No.7553-30) fitted with a standard pump-head (Model No. 7014-20). Feed rate was controlled using an external solid state Masterflex control device. Masterflex tubing.was employed in the pumping system and corrosion-resistant Tygon tubing was used in the rest of the system.

The feed was injected 5 inches inside the reactor in close proximity to the diffuser stone and the effluent removed by overflow from the unit. The air used for sparging escaped from the effluent port, thereby preventing clogging of the effluent tubing due to biomass wash-out.

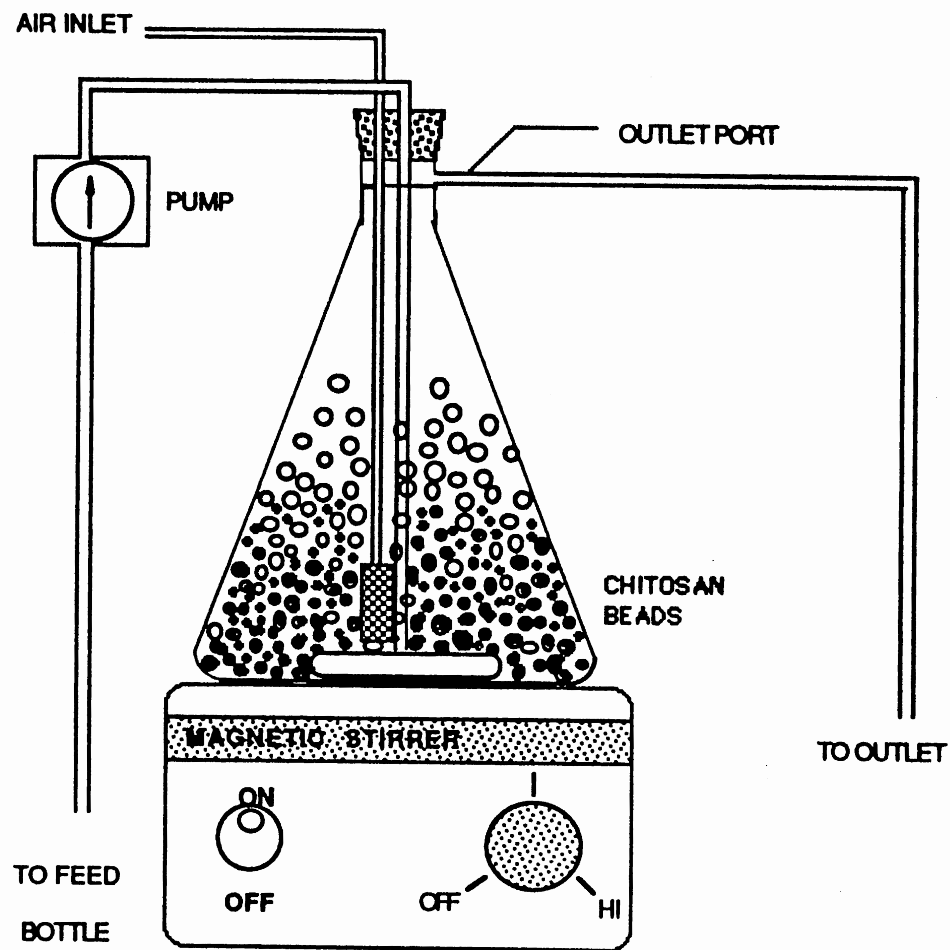


Figure3. Schematic Diagram of Immobilized Cell Reactor Setup

Activated Sludge Reactor

A bench-scale activated sludge reactor with an internal clarifier and a provision for internal solids recycle served as the conventional suspended-growth reactor (Figure 4). The reactor vessel was made out of plexiglass. The total volume of the tank was 4.6 liters of which the aerated section occupied 2.6 liters while the clarifier occupied approximately 2.0 liters. The feed was pumped into the aerated section using a piston-driven pump (Milroyal, Model No. DB-2-117R). The clarifier had an effluent port at the top edge of the quiescent zone which was connected by Tygon tubing to a 25 L effluent collection bottle. The diffuser stone was connected to an air-flow control device which supplied approximately 1.5 L / min. of air to the reactor; both aeration and complete-mixing was performed by this air.

Base-mix, Target Compound and Dilution Regime for Feed

Base mix

The synthetic wastewater fed to the reactors was made up of a readily biodegradable base mix combined with the appropriate concentration of the target compound, 2,4 TDA (99 % purity; Aldrich Chemical Co, Milwaukee, WI.). This recipe was based on the type of complex waste used by Kincannon et al (1982).

Two stock solutions, one for supplying the carbon source and another for supplying the nutrients and salts, were prepared and stored at 4 ^{o} C. The constituents and their concentrations in the stock solutions are given in Tables II and III. For making one liter of the base mix, 4.5 mL carbon.stock and 3.0 mL salt stock were diluted to a volume of 1 L using tap water. The total amount of TDA required to achieve the desired concentration was then dissolved in this base mix to yield the synthetic wastewater. The pH of the synthetic

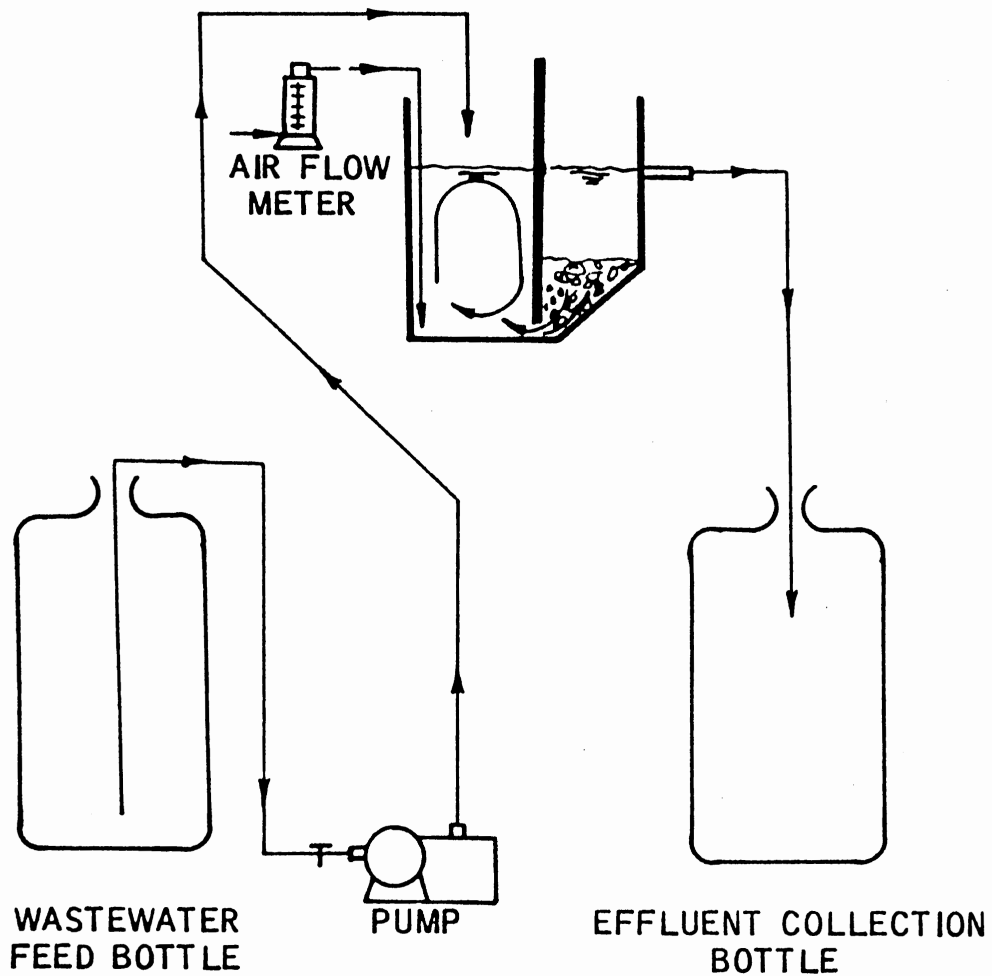


Figure 4. Schematic Diagram of the Bench-scale Activated Sludge Reactor Setup

wastewater was in the range of 7.8 - 8.0 throughout the study. The final concentration of the different constituents in the feed are given in Tables IV and V.

Start up procedure

The biomass used for immobilization was harvested from an 8 liter fill-and-draw stock culture maintained in the laboratory through the research. The inoculum for this culture was originally obtained from the return activated sludge line of the secondary clarifier at the wastewater treatment plant at Ponca City, Oklahoma and from the industrial wastewater treatment plant at the CONOCO refinery in Ponca City, Oklahoma. It was decided to draw the inoculum from a mixture of these two mixed liquors in order to make available a wide range of micro-organisms; it was expected that acclimation would then result in a mixed microbial population that could biodegrade the complex waste.

The 8L sludge 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 every day for a period of 10 days,with continuous aeration at room temperature. During this phase, the culture was also spiked with 50 mg/L 2,4 TDA every alternate day so that the biomass could be acclimated to this compound. After this procedure, and throughout the research, the stock culture was fed with the base mix daily and 50 mg/L 2,4 TDA once every three days. An SRT of approximately six days was maintained by the fill-and-draw method.

The control activated sludge reactor was started with inoculum drawn from the acclimated stock culture. Whenever required, biomass was harvested from the stock culture by centrifuging 50 mL aliquots of the sludge at 2800 rpm and room temperature for 10 minutes in an IEC Centra-7 centrifuge (Damon Inc., MA.). Concentrated wet biomass thus obtained was, if necessary, cooled and stored at 4 ^{o} C for not more than 12 hours to preserve its viability until needed.

TABLE II

CONCENTRATION OF ORGANIC COMPOUNDS IN STOCK SOLUTION

Source	Concentration (g / L)
Ethylene glycol	24.5
Ethyl alcohol	17.5
Dextrose	22.2
Glutamic Acid	8.4
Acetic Acid	30.4
Phenol	3.5

TABLE III

CONCENTRATION OF NUTRIENTS AND SALTS IN STOCK SOLUTION

Source	Concentration (g / L)
Ammonium chloride	17.8
Phosphoric Acid	3.1
Calcium chloride	0.0014
Manganese sulfate	0.0012
Ferric chloride	0.066
Magnesium sulfate	6.6

TABLE IV

CONCENTRATION OF ORGANIC COMPOUNDS IN WASTEWATER

Source	Concentration (mg/L)
Ethylene glycol	110.4
Ethyl alcohol	78.6
Dextrose	99.7
Glutamic Acid	37.9
Acetic Acid	136.6
Phenol	15.8

TABLE V

CONCENTRATION OF NUTRIENTS AND SALTS IN WASTEWATER

Source	Concentration (mg/L)
Ammonium chloride	53.5
Phosphoric Acid	9.3
Calcium chloride	4.2×10^{-3}
Manganese sulfate	3.6×10^{-3}
Ferric chloride	0.2
Magnesium sulfate	19.9

Manufacture of Chitosan Biocatalysts

The chitosan beads were manufactured according to the procedure proposed by Vorlop and Klein (1987). In order to obtain 100 mL of chitosan-acetate solution, 1 gram of high viscosity chitosan (VNS #311;Vanson Chemical Co., Redmond, VA) was dissolved in 99 mL distilled water with 0.7 mL concentrated acetic acid.The mixture was stirred overnight on a magnetic stirrer in order to achieve complete dissolution. Five grams of concentrated biomass were then added to this solution and homogenized using a blender. This solution was pumped dropwise using a simple pneumatic pump into a 2% pentasodium tripolyphosphate (Sigma Chemical Co.) crosslinking solution. During this procedure, the pH of the tripolyphosphate solution was maintained at 8.2 for 4 hours by continuous addition of 5M NaOH. This is very important because proper gelation occurs at a pH greater than 8.0. The beads shrink to about 50% of their original size at the end of curing (4 hours). At this point, the beads were collected using a nylon net, washed with distilled water and subsequently used in the reactor. During the curing process, viability of the entrapped biomass was maintained by adding a small amount of the base feed and aerating the crosslinking solution. Further, in order to confirm their viability, the beads were tested for oxygen depletion using the Clark D.O electrode. A total volume of 400 mL chitosan-acetate solution and exactly 20 grams of concentrated biomass were used to make a single batch of beads which was subsequently employed in the reactor.

Startup and Control of the ASR

The bench-scale continuous flow activated sludge reactor was started by transfering 4.8 liters of the mixed liquor from the acclimated 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 a diffuser stone. Care was taken to ensure that the biomass was

completely mixed; a circular motion of the activated sludge was established and maintained (see Figure 4). The baffle was adjusted to facilitate proper and adequate recycling from the settler into the main reactor at the same time ascertaining that the settler functioned well.

From the start, a typical SRT of 6 days was maintained by wasting a calculated amount of the sludge every day. This volume was calculated using the model equations based on Monod kinetics that were devised by Lawrence and McCarty (El Gamal,1985). The biomass concentration within the reactor was monitored by determining the MLVSS concentrations once every three days. This value, along with the effluent VSS data , was used in determining the wastage rate. However, the recycle rate could not be determined due to the internal recycle system. Before wasting as well as drawing samples for solids analysis, the baffle wall was removed and the sludge was thoroughly mixed (Reynolds, 1982).

Analytical Techniques

Scope

Effluent samples were routinely analyzed for determining the various performance parameters such as 2,4 TDA removal, TCOD and SCOD removal, TSS, VSS and pH. The complete sampling flowchart of procedures was given in Figure 2. In addition to the above analyses, a tentative identification of the bacterial strains present in the biomass used in this research was also accomplished. The chitosan biocatalysts were also examined using Scanning Electron Microscopy (SEM) as part of the study.

Analysis of 2,4 TDA using High Performance Liquid Chromatography

The concentration of 2,4 TDA in the influent and effluents of the reactors was

quantified using High Performance Liquid Chromatography (HPLC) based on the work done by Snyder and Breder (1982). A reversed phase Beckman C-18 octa-decyl silyl column (dimensions: 250 X 4.6 mm I.D) with 5μm pore size was used in isocratic elution mode for this purpose. A 20 / 80 solution of HPLC-grade methanol (Fisher Scientific Co.) in deionized water buffered at pH 7.4 with mono- and di- basic sodium phosphates served as the mobile phase. After making the buffered solution, it was filtered through a 0.2 μm Nylon -66 Rainin filter (Rainin Instrument Co. Inc., Woburn, MA.) using clean vacuum filtration equipment. After filtration, helium gas was bubbled through the mobile phase to remove any dissolved air present, before putting it to use. The mobile phase was stored at $4\,^{o}$C when not in use. Before use, the solution was allowed to return to room temperature and helium was sparged once again. The mobile phase was pumped through the column using an electronically-controlled precision pump (Beckman, Model No. 110 A) at a flow rate of 1 mL / min. Samples were prepared by filtering the influent or effluent through a 0.45 μm Nylon-66 Rainin filter. Each sample was injected manually into an Altex 210 injection port attached to the pump. The sample volume was maintained at a constant 50 μl throughout the experiment. A fixed-wavelength UV detector (Waters Associates, Model No.440) operated at 254 nm and 0.5 aufs and an integrator (Hewlett Packard, Model No.3380 A) served as the output devices.

In order to maintain accuracy and flush out trace contaminants such as salt residues from the column, 25 - 30 column volumes of mobile phase were pumped through it before starting an operational run (Dolan and Snyder, 1989). The column was also flushed regularly between successive injections and after each run with pure HPLC-grade methanol and then with micro-filtered deionized water, in that order.

A standard curve was obtained for 2,4 TDA by injecting known concentrations (50 - 300 mg/L TDA in increments of 50 mg/L) of the compound and plotting the concentration against the peak area. This procedure was repeated once every 20 runs. Linear regression of the values consistently yielded a correlation coefficient greater than 0.95. In order to

account for changes in column, mobile phase characteristics and environmental conditions, a filtered influent sample of known concentration was injected before each run and its peak area compared to the appropriate area on the standard curve; the actual concentration was recalculated based on this comparison. The filtered samples were stored, for not more than a week at a time, at 4 ^{o} C in order to minimize any amine oxidation (Unger and Friedman, 1979).

Determination of Biocatalyst Activity by the D.O Uptake Method

The activity of the biocatalysts were evaluated by a dissolved oxygen (D.O) uptake method as described by Sofer et al (1990). A Clark D.O electrode enclosed in a Gilson's water jacket, a DC-power driven amplifying circuit and a strip chart recorder were employed for this purpose. The Clark electrode is a compact, highly sensitive probe that is designed to fit into the Gilson's water jacket with its Teflon covered tip reaching into the 1.6 mL sealable cavity. A magnetic micro-stirrer bar (Fisher Scientific Co.)was placed in the cavity to agitate the beads during the test.

The Clark electrode (YSI, Model No. 5775) was connected to the strip chart recorder by an electronic amplifying circuit driven by a 3V D.C power source. The probe and the strip chart recorder were calibrated using solutions having known D.O concentrations. The known D.O concentrations were obtained by decreasing gradually the dissolved oxygen concentrations in oxygen-saturated distilled water using sodium sulfite. These DO levels were determined with a DO electrode (Orion Research, Model No.97 - 08 - 00) connected to a calibrated analog pH meter (Orion Research Model No. 301) The strip chart recorder calibration curve was used to convert chart readings into DO values in mg/L.

For the DO uptake test, five chitosan beads were taken out of the ICR along with the well aerated feed substrate and placed inside the micro-vessel of the Gilson's water jacket.

A capillary-holed stopper was used to provide a water seal. The DO uptake by the beads was recorded on the chart for a sufficiently long time, for example, ten minutes and the total DO consumed was later converted into DO consumed per bead per minute. All the DO uptake tests were performed at room temperature.

Total and Soluble Chemical Oxygen Demand

Influent and effluent samples from the immobilized-cell and the activated sludge reactors were routinely tested for Total Chemical Oxygen Demand(TCOD) and Soluble Chemical Oxygen Demand (SCOD). The HACH COD procedure was employed for this purpose (HACH, 1992).

For determining TCOD, 2 mL samples were taken directly from the influent or effluent, and for SCOD analyses, the influent or effluent samples were filtered using 0.45 μm Nylon-66 Rainin filters in order to remove all suspended material including microbial matter. Two mL of the prepared sample was then added to a HACH COD High Range vial and placed in the HACH COD reactor (Model No. 16500-10). The vials were heated for 2 hours in this reactor and subsequently cooled to room temperature before analyzing them using a HACH DR/3 spectrophotometer at 620 nm. COD values were read off directly from a HACH High Range COD template which was used in the spectrophotometer.

pH Measurement

The pH was measured using an analog pH meter (Fisher Scientific, Accumet, Model No. 90) fitted with a Fisher electrode.

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

The TSS and VSS of effluent samples from both the ICR and ASR as well as the MLVSS of the ASR were determined by the HACH methods described in HACH's Handbook for Waste Analysis, 1991. TSS was determined using the Hach Method No. 8158 and VSS by the Hach Method No. 8164 (HACH, 1991). For this analysis, 100 mL samples were collected directly from the effluent ports of the reactors and were filtered using 47 mm diameter glass microfiber filter (Whatman, No. 934-AH) on a vacuum filtration unit. A Fisher Scientific XA Analytical Balance was used for all mass measurements.

Scanning Electron Microscopy of Chitosan Biocatalysts

Scanning Electron Microscopy (SEM) of the chitosan beads was performed with the help of the Department of Veterinary Medicine at Oklahoma State University. The purpose of this examination was to study the growth pattern and distribution of microbes inside the polymer matrix. Plain chitosan beads without entrapped biomass were also examined for comparison. Both the plain and the biomass loaded beads were manufactured from the same chitosan-acetate solution and were cured under identical conditions.

Approximately twenty-day old beads were taken out of the ICR and were put in distilled water after carefully washing them to remove attached biomass. Then, they were put in a fixative consisting of 3 % glutaraldehyde and 3 % sucrose for 3 hours. The beads were then washed thoroughly with a 0.2 M solution of sodium cacodylate buffer (pH 7.4) and were stored overnight in a refrigerator. The beads were subsequently dehydrated in a graded series with 50, 70, 90, 95 and 100 % ethanol and liquid nitrogen and then fractured with a razor blade. The fragments were put back in 100% ethanol and dried to the critical point after which they were subjected to SEM (Pennington, 1991). The SEM was

performed using a JEOL 35-U Scanning Electron Microscope at an accelerating voltage of 25 kV.

Identification of Bacterial Strains in the Stock Culture

Microbiological assays were conducted in order to identify the viable strains of bacteria in the acclimation culture. These tests were carried out with the help of Dr. Mary Grula of the Department of Microbiology at Oklahoma State University. It was initially suspected that the bacterial culture consisted predominantly of Pseudomonas species, based on the constituents of the complex synthetic wastewater used in the present study. Therefore, specific tests for confirmation of these bacteria were done in addition to other more general ones that covered a broad range of genera (Bergey's Manual, 1980).

A sample from the acclimated culture was taken and several dilutions in the range 10^{-3} to 10^{-7} viable cells / mL were made using distilled water. Then, three pour plates of each dilution were made with nutrient agar media. Two of these were incubated at $30\,^{o}$ C and the remaining one at room temperature. Each colony was streaked onto fresh nutrient agar slants and by successive streakings, essentially pure, homogeneous cultures were obtained. Agar slants of each culture were made and stored at $4\,^{o}$ C. For all the following test procedures, seed was taken from these slants. These tests are given as follows:

(a) Gram;

(b) shape;

(c) motility;

(d) aerobic / anaerobic;

(e) catalase;

(f) oxidase;

(g) gel liquefaction;

(h) denitrification;

(i) indole;

(j) glucose utilization;

(k) King's pH 7.2 and pH 4.5;

(l) poly-beta hydroxy storage;

(m) growth at 30 ^{o} C and 37 ^{o} C;

(n) fluorescence; and,

(o) resistance to penicillin.

All the above tests were performed under sterile conditions and the test results were interpreted using Bergey's Manual (1980).

CHAPTER IV

RESULTS

Scope

The primary goal of this research was to study and compare the treatment of a simulated complex synthetic wastewater containing the toxic compound 2,4 TDA by a bench-scale immobilized-cell reactor (ICR) and compare its overall performance with that of a control bench-scale activated sludge reactor (ASR) operated under the same conditions. A simple chemostat served as the ICR containing the chitosan biocatalysts. This investigation addressed the maximum influent TDA concentrations that could be treated, maximum possible TDA, TCOD and SCOD removal rates that could be achieved and establishment of other operating parameters (see Chapter III) at steady state conditions. Other aspects peculiar to the immobilized cell system were also evaluated. These objectives were pursued by conducting the experiments in two phases.

The first set of operations were conducted at a constant HRT of 10 hours in order to determine the highest possible TDA concentration that could be treated before failure occurred. The TDA concentration was varied from 50 to 250 mg/L in steps of 50 mg/L.

The second part of the study was conducted at a TDA concentration of 150 mg/L but with the same concentration of base-mix, to achieve the highest loading and removal rates in terms of TDA, TCOD and SCOD, before treatment efficiency dropped significantly. The hydraulic loading rates were increased by increasing the feed flow rates into the units; this resulted in a systematic decrease in the HRTs. Both units were operated at HRTs of 8, 5,

and 3 hours during this phase.

Results of Preliminary Studies

Reactor Design Study

For the biocatalyst system, initial trials were conducted in an upflow, expanded bed reactor with an external aerator. This unit consisted of a 1 inch internal diameter glass column with three ports, the effluent and recycle port situated laterally at the top of the column and the influent port at the bottom.(see Figure 5) The beads were placed inside this column, and upflow expansion was maintained inside the reactor using the recycle pump (peristaltic type) which was used to circulate the waste through the reactor and the aerator. An influent peristaltic pump was used to supply the complex waste into the reactor. The effluent was drawn by overflow from the topmost port of the reactor.The port just beneath the effluent port was connected to the external aerator which was connected to the recycle pump.The fresh waste was mixed with the recycled waste at the inlet end as illustrated in Figure 5.

After a few days of operation in this manner, it was noticed that excessive biomass growth in the tubing and the reactor vessels caused the system to fail due to clogging. Several trials were conducted with the same results occuring consistently. Although backwashing and cleaning the entire system was temporarily successful in alleviating this problem, a permanent solution could not be found. Other problems that were encountered with repeated backwashing were, (a) the bead structure was weakened, and (b) beads were washed out. Gentle mechanical agitation was essential to keep it from clogging and subsequently failing. However, such a provision could not be incorporated in this type of system. Air sparging, as a means of agitation, was employed through the feed inlet port of the main reactor vessel, but this process resulted in bead damage.

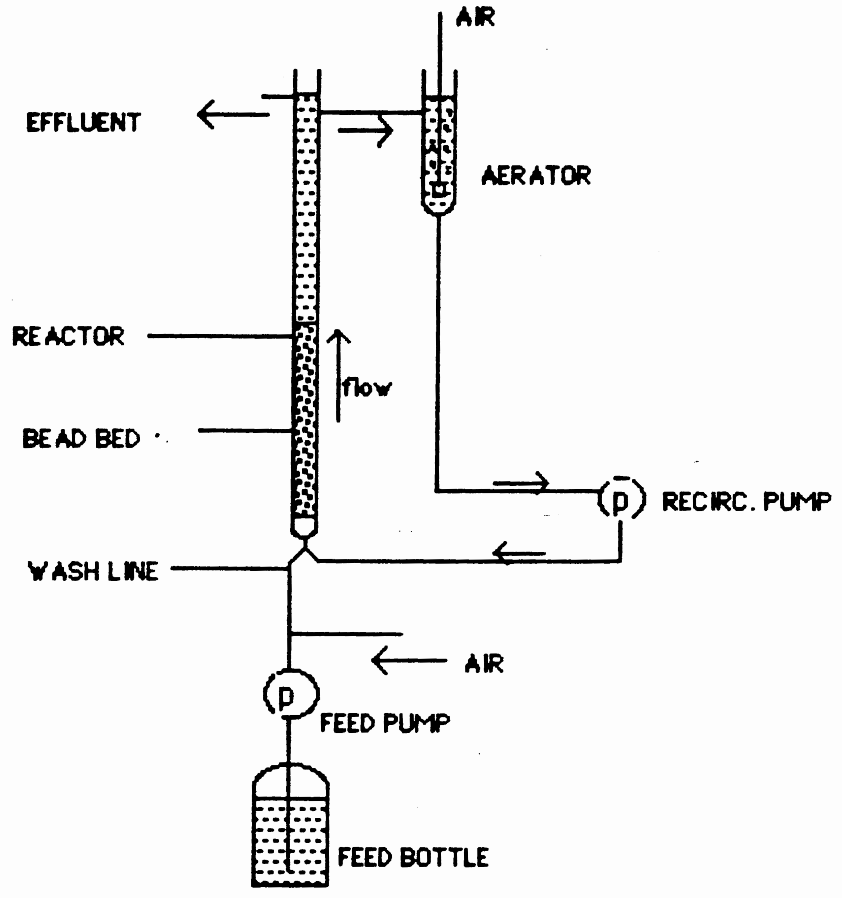


Figure 5. Schematic Diagram of Recirculation Reactor used in Preliminary Study

The above system was abandoned for a chemostat system which was successfully employed as the ICR throughout the research. The new system eliminated the serious problems encountered with the recirculation reactor; as evidenced by the following results. An upflow, recirculation reactor was used in an earlier experiment by Sofer et al (1990); however, problems of excessive biomass growth and clogging were not reported.

Adsorption Study using Unseeded Beads

Chitosan beads without entrapped biomass were made using 100 mL of chitosan-acetate solution. These beads were used to test any possible adsorption of 2,4 TDA by the matrix. The beads were put in 500 mL of synthetic wastewater (made using distilled water) containing 50 mg/L TDA and agitated for 24 hours. Samples were drawn at intervals of 6 hours and analyzed using HPLC. No TDA removal could be detected. A similar test with a new batch of beads at 100 mg/L TDA yielded the same result. The tests could not be continued for longer durations due to excessive microbial contamination in the test samples.

Results

For both reactors, feed flow rates for a given HRT were calculated based on the total volume of the respective reactor. In the case of the ASR, this was done according to guidelines set down by Reynolds (1982) (see Chapter II). However, for the ICR, the effective reactor volume is less than the total volume of the reactor since the beads occupy a certain volume. In spite of this fact, the total volume was finally adopted for all calculations due to the following observations:

(a) chitosan beads shrink considerably during the curing procedure; therefore, final volume could not be estimated from the volume of the chitosan-cell mixture used;

(b) the chitosan beads are highly porous; therefore, it was impossible to estimate the actual volume occupied by the matrix; and,

(c) it was observed that the size of the chitosan beads varied every time the beads were manufactured, although not considerably.

Based on the above, the matrix volume was assumed to be negligible compared to the total volume of the reactor.

Data for both studies were collected according to the flowchart given in Chapter III. Since one of the objectives of this research was to compare the responses of the two units, data were collected during the transition period from one loading to the next as well as at steady state of a particular loading. The performance of each system with respect to the various parameters described in Chapter III is given in this chapter.

Since many readings were recorded for each steady-state run, the average values of all relevant steady-state data are reported here in the form of tables. In contrast, the figures depicting performance profiles versus the total days of operation include the complete set of data points gathered during each run. This has been done in order to focus attention on transitional responses. Unless specifically mentioned, all data mentioned in the text represent steady-state data. Each continuous run (at a given loading) depicted in the figures is represented by roman numerals that correspond to the loading numbers given in the tables.

Several times during the study, the chitosan beads disintegrated.and were replaced by fresh beads. The reactor vessel was cleaned before biocatalyst replacement. After this procedure, data procurement for the run was resumed usually after two or three days. It was presumed that possible toxic effects on the new beads would be minimal because of the protection offered by the matrix. This assumption was eventually corraborated by the DO uptake activity of the beads.

Due to the organically - rich nature of the synthetic wastewater, external biomass growth in the liquid phase was observed in the biocatalyst reactor throughout the entire

study. In a concurrent study conducted in this lab with alginate biocatalysts under the same conditions (Chaubey, 1992), it was conclusively proved that the concentration of the liquid-phase biomass is negligible compared to the immobilized biomass. Under most loadings, there was no apparent biomass growth on the bead surface.

Organic Loading Study

In the first phase of the study, the influent TDA concentration was varied from 50 mg/L to 250 mg/L in steps of 50 mg/L at an HRT of about 10 hours. The corresponding TCOD and SCOD concentrations varied from 625 to 936 mg/L and 600 to 913 mg/L, respectively. At each TDA concentration level, the reactors were operated until steady state conditions were achieved at which at least 3 readings were obtained for all parameters. A summary of operating parameters for the two reactors during this phase is given in Table VI.

TDA Removal

The influent TDA concentration was varied from 50 mg/L to 250 mg/L and the effluent concentrations varied from approximately 35 mg/L to 227 mg/L (for the ICR) and 33 mg/L to 248 mg/L (for the ASR); representing removal efficiencies of 32 to 9% (ICR) and 35 to 1% (ASR) respectively. The data gathered for this part of the study are summarized in Table VII. The corresponding data are represented in Figures 6 and 7.

TCOD and SCOD Removal

Influent TCOD and SCOD increased in the range 625 - 936 mg/L TCOD and 600 - 913 mg/L SCOD; this corresponded to the increase in influent TDA concentration. In the

TABLE VI

OPERATING CHARACTERISTICS DURING ORGANIC LOADING STUDY

Reactor	Reactor Volume (mL)	Total Days of Operation	Loading No.	Influent TDA* (mg/L)	Influent COD TCOD (mg/L)	Influent COD SCOD (mg/L)	HRT (hours)	Flow Rate (L/d)	SRT (days)	Amt.Biom (g)** or MLVSS (g)***
ICR	1120	1 - 13	I	50	625	600	10.14	2.69	-	20
		15 - 29	II	100	788	768	10.14	2.69	-	20
		31 - 48	III	150	876	857	10.14	2.69	-	20
		51 - 75	IV	200	927	907	10.14	2.69	-	20
		85 - 107	V	250	936	913	10.14	2.69	-	20
ASR	4556	1 - 13	I	50	625	600	9.99	10.94	6.0	3.7
		15 - 29	II	100	788	768	9.99	10.94	6.0	3.8
		31 - 48	III	150	876	857	9.99	10.94	5.8	7.5
		51 - 75	IV	200	927	907	9.99	10.94	6.0	5.6
		85 - 107	V	250	936	913	9.99	10.94	6.1	5.3

* by mass determination; ** mass of biomass immobilized; *** for ASR only

TABLE VII

TDA REMOVAL IN ORGANIC LOADING STUDY

Loading.No.	Total Days of Operation	Influent TDA Concentration* (mg/L)	ICR Effluent		ASR Effluent	
			TDA Concentration (mg/L)	Removal Efficiency (%)	TDA Concentration (mg/L)	Removal Efficiency (%)
I	1 - 13	51.3	34.8	32.2	33.3	35.0
II	15 - 29	97.1	70.5	27.4	70.9	26.9
III	31 - 48	152.8	120.8	20.9	136.4	10.7
IV	51 - 75	200.6	148.9	25.8	178.4	11.0
V	85 - 107	250.1	227.4	9.1	247.6	1.0

* by HPLC determination

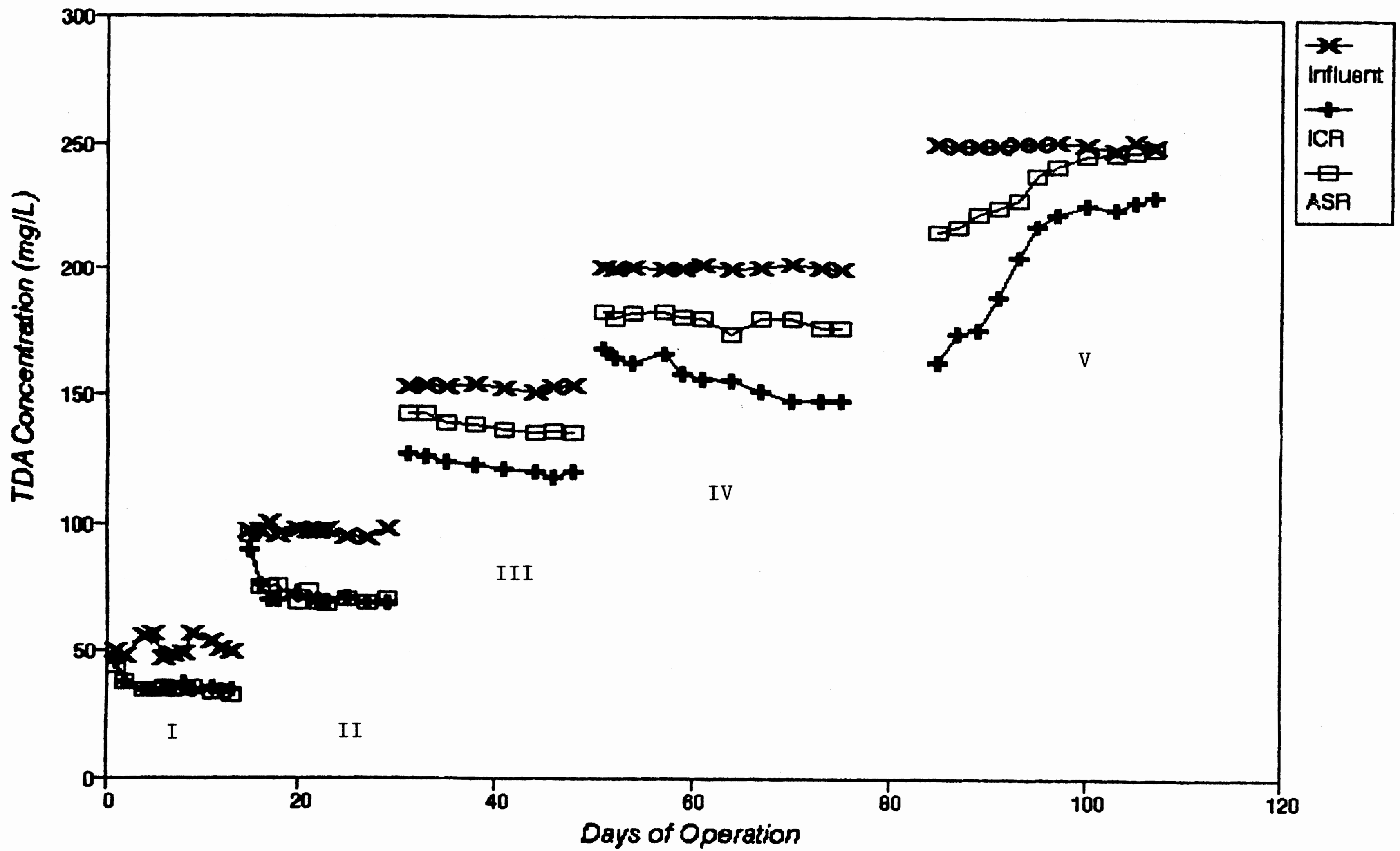


Figure 6. Influent and Effluent TDA Profile for Organic Loading Study

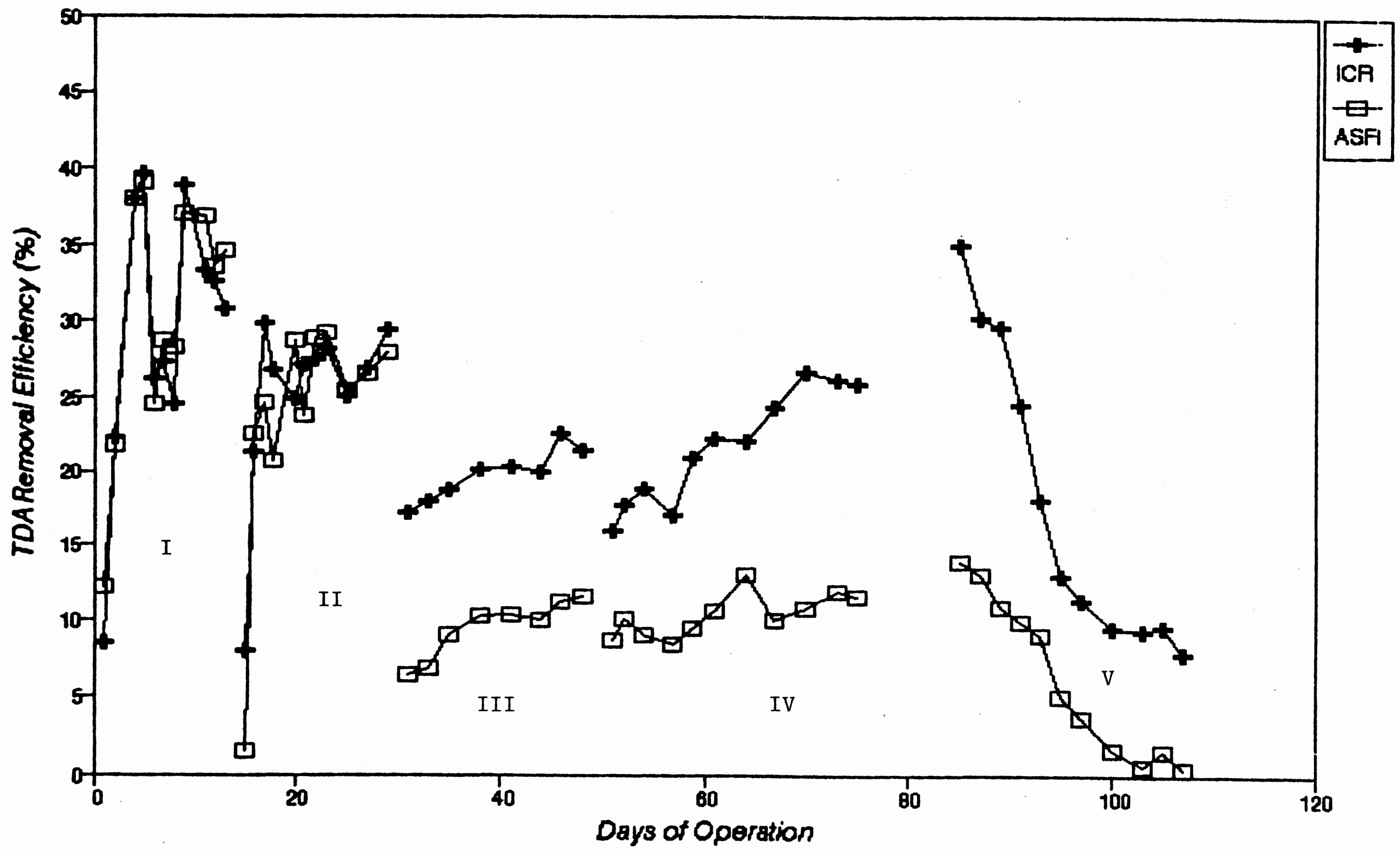


Figure 7. TDA Removal Efficiency Profile for Organic Loading Study

TABLE VIII

TCOD AND SCOD REMOVAL IN ORGANIC LOADING STUDY

Loading No.	Total Days of Operation	Influent		ICR Effluent				ASR Effluent			
		TCOD (mg/L)	SCOD (mg/L)	TCOD (mg/L)	Rem.Eff (%)*	SCOD (mg/L)	Rem.Eff (%)*	TCOD (mg/L)	Rem.Eff (%)*	SCOD (mg/L)	Rem.Eff (%)*
I	1 - 13	625	600	167	73.3	85	85.8	185	70.4	118	80.2
II	15 - 29	788	768	200	75.5	131	82.8	228	72.1	165	77.9
III	31 - 48	876	857	289	66.9	154	81.9	315	64.0	240	71.9
IV	51 - 75	927	907	398	56.9	189	79.0	483	47.8	439	51.5
V	85 - 107	936	913	490	47.6	236	74.1	696	25.6	635	29.9

* removal efficiency of parameter in immediate left column

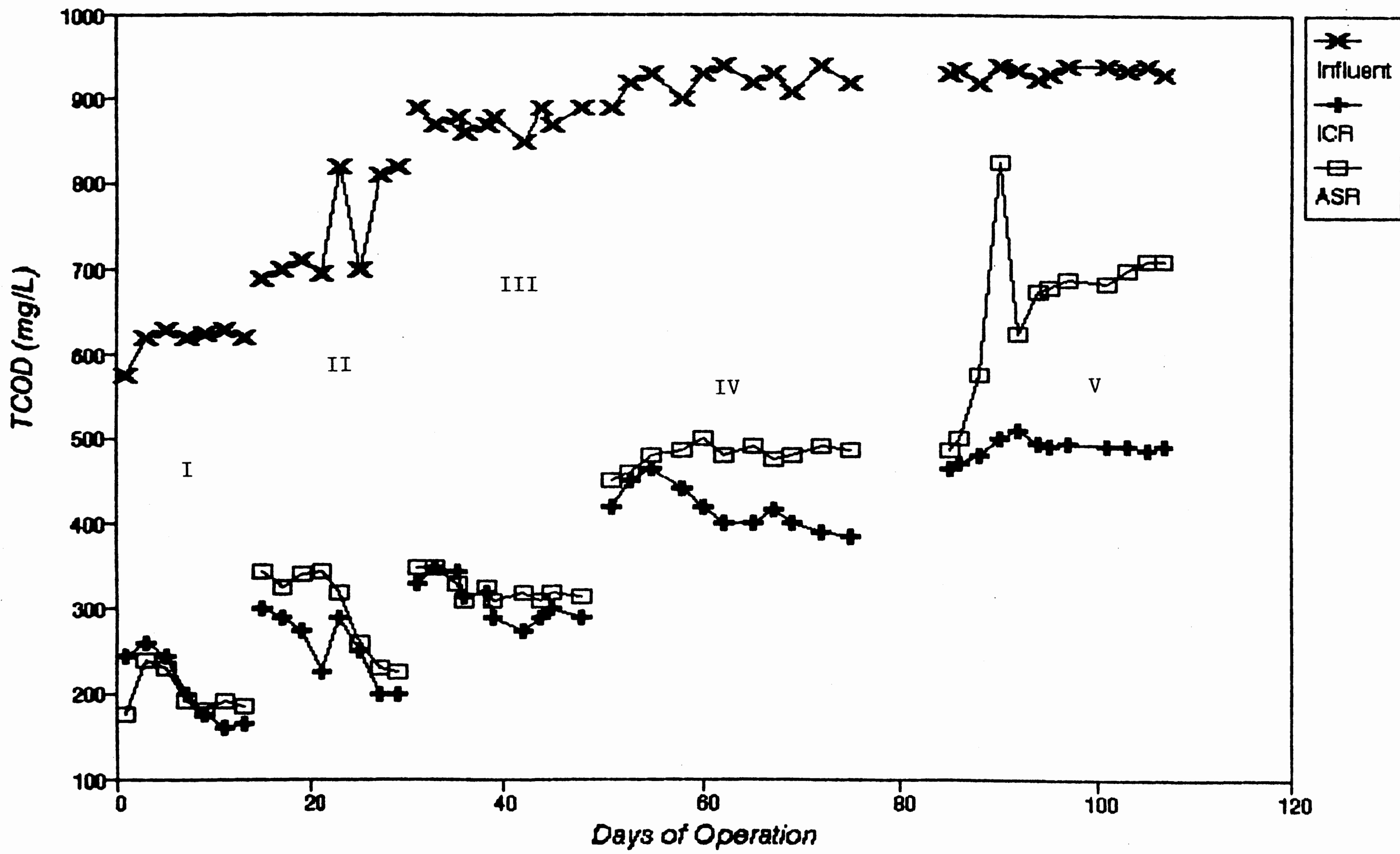


Figure 8. Influent and Effluent TCOD Profile for Organic Loading Study

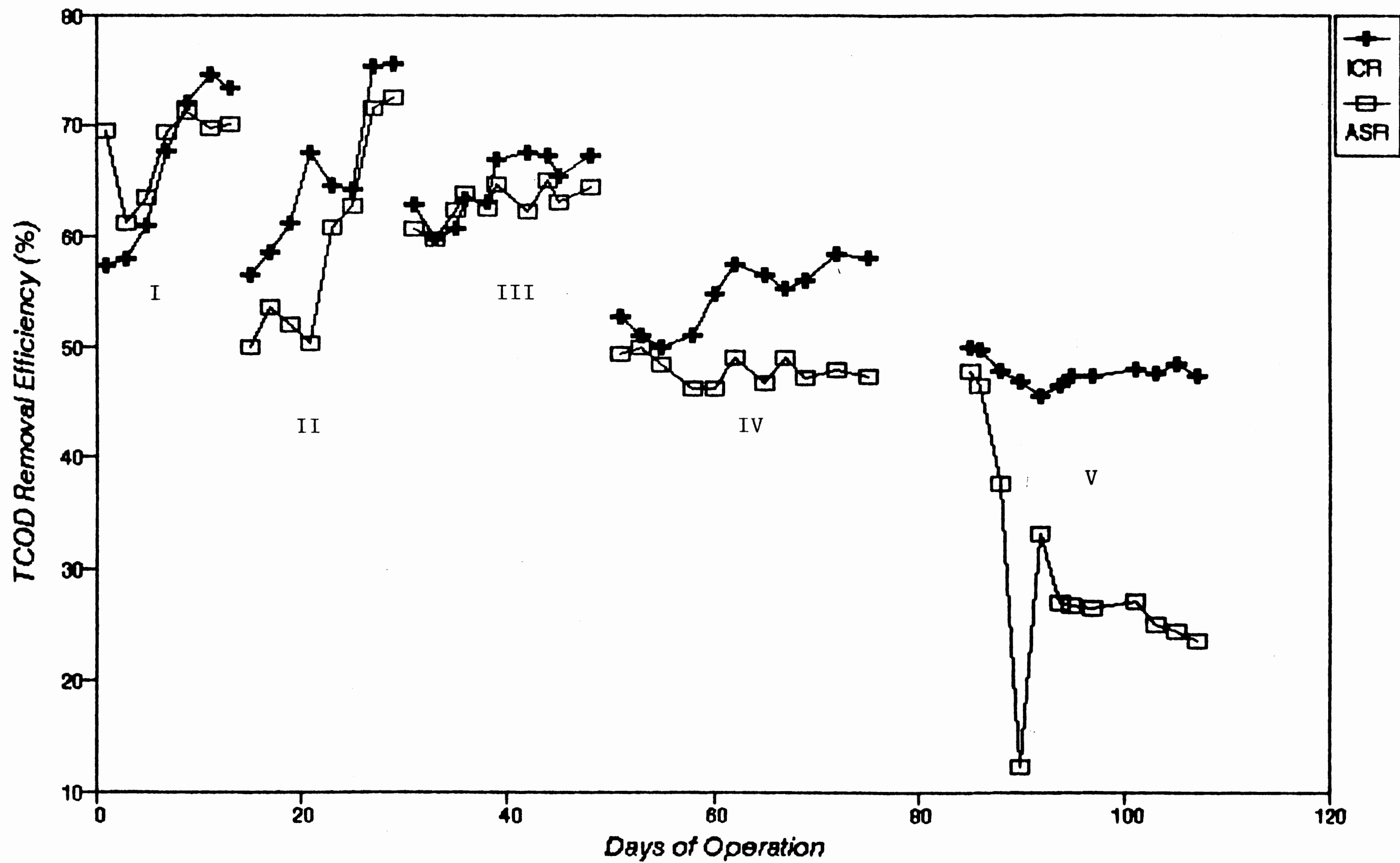


Figure 9. TCOD Removal Efficiency Profile for Organic Loading Study

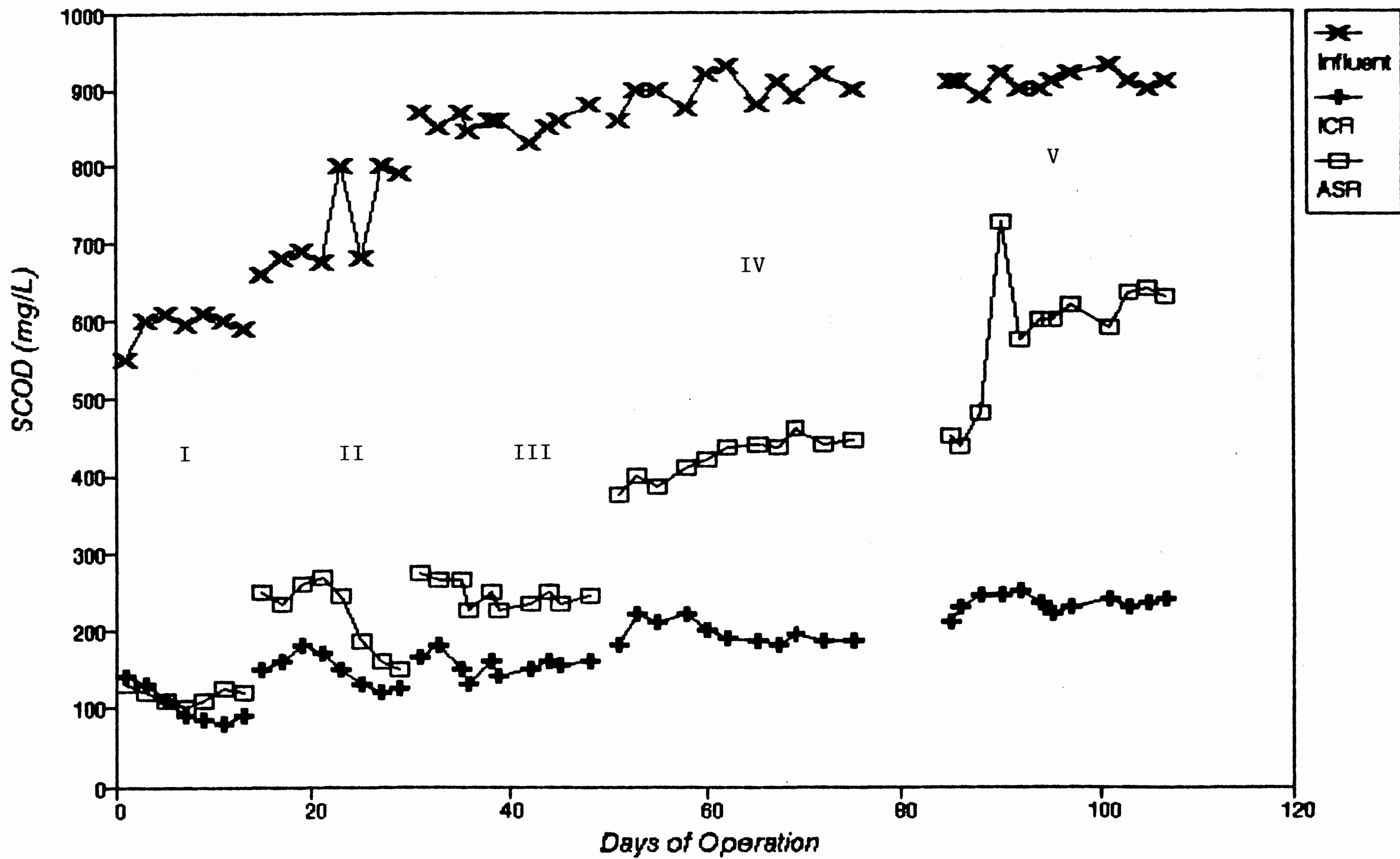


Figure 10. Influent and Effluent SCOD Profile for Organic Loading Study

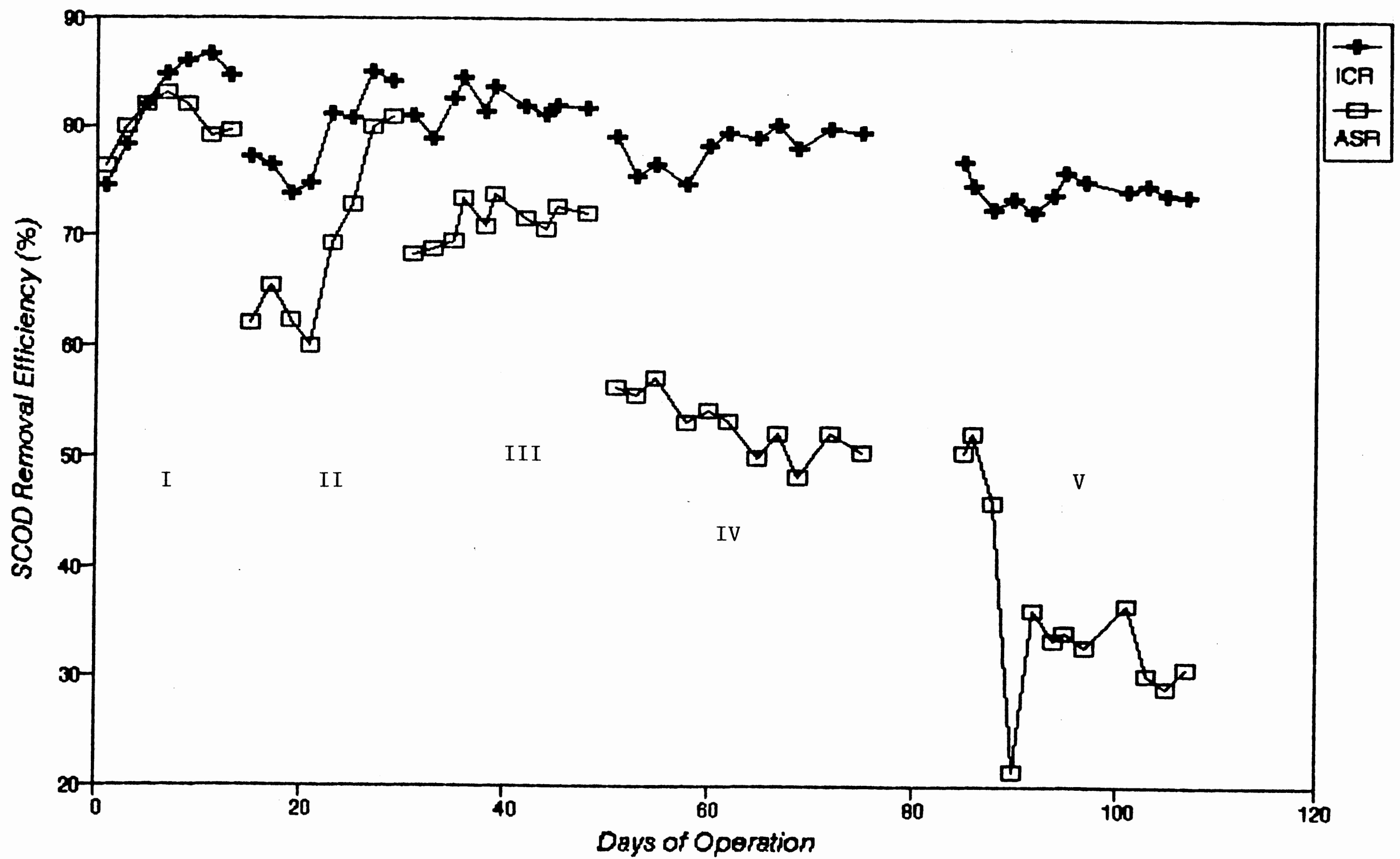


Figure 11. SCOD Removal Efficiency Profile for Organic Loading Study

ICR, the effluent TCOD and SCOD concentrations varied in the range of 167 - 490 mg/L TCOD (73 - 48 % TCOD removal) and 85 - 236 mg/L SCOD (86 - 74 % SCOD removal) As for the ASR, the corresponding effluent concentration levels were 185 - 696 mg/L TCOD (70- 26 % removal) and 118 - 635 mg/L SCOD (80 - 30 % removal).

The liquid phase biomass growth in the ICR apparently influenced the effluent TCOD readings considerably as is evident from the difference between TCOD and SCOD values given in Table VIII; in the ASR, the presence of a clarifier mitigated this effect significantly. Therefore, it is proposed that SCOD removal is a better tool for comparison of the reactor performances. The above results are delineated in Table VIII and Figures 8 - 11.

Effluent Solids and pH

The intrinsic nature of the biocatalyst reactor as well as the ASR resulted in continuous washout of solids in the effluent due to bead damage and liquid-phase biomass loss in the former, and cell-washout in the latter. Table IX shows the steady state values of effluent TSS and VSS in each run. Figures 12 and 13 depict the complete effluent solids profile in each run. It can be seen that the peaks in the ICR plots were caused by bead-breakage and subsequent washout of matrix debris and immobilized cells. The exact times of bead disruption are indicated in Table IX.

Effluent pH in both the reactors did not vary significantly throughout this entire phase. The range of pH values under steady-state conditions and complete pH profiles for each run are presented in Table IX and Figure 14.

DO Uptake by Chitosan Biocatalysts in ICR

The rate of DO consumed by immobilized cells has been proposed by previous

TABLE IX

EFFLUENT SOLIDS AND pH IN ORGANIC LOADING STUDY

Loading No.	Total Days of Operation	ICR Effluent				ASR Effluent		
		TSS (mg/L)	VSS (mg/L)	pH Range	Remarks	TSS (mg/L)	VSS (mg/L)	pH Range
I	1 - 13	28.0	9.0	7.5 - 8.0	Days 12-13-Bead breakage	27.0	9.5	7.8 - 7.0
II	15 - 29	68.5	54.0	8.0 - 8.2		55.8	36.8	7.3 - 7.7
III	31 - 48	67.5	57.0	7.3 - 8.2	Day 45-Bead breakage	58.5	46.8	7.6 - 7.8
IV	51 - 75	79.2	59.2	7.1 - 8.2	Day 55-Beads washed.Day 72-Bead breakage	63.4	53.2	7.6 - 7.8
V	85 - 107	102.3	90.0	7.4 - 8.3	Day 107/108-Bead breakage	49.0	42.3	7.4 - 7.8

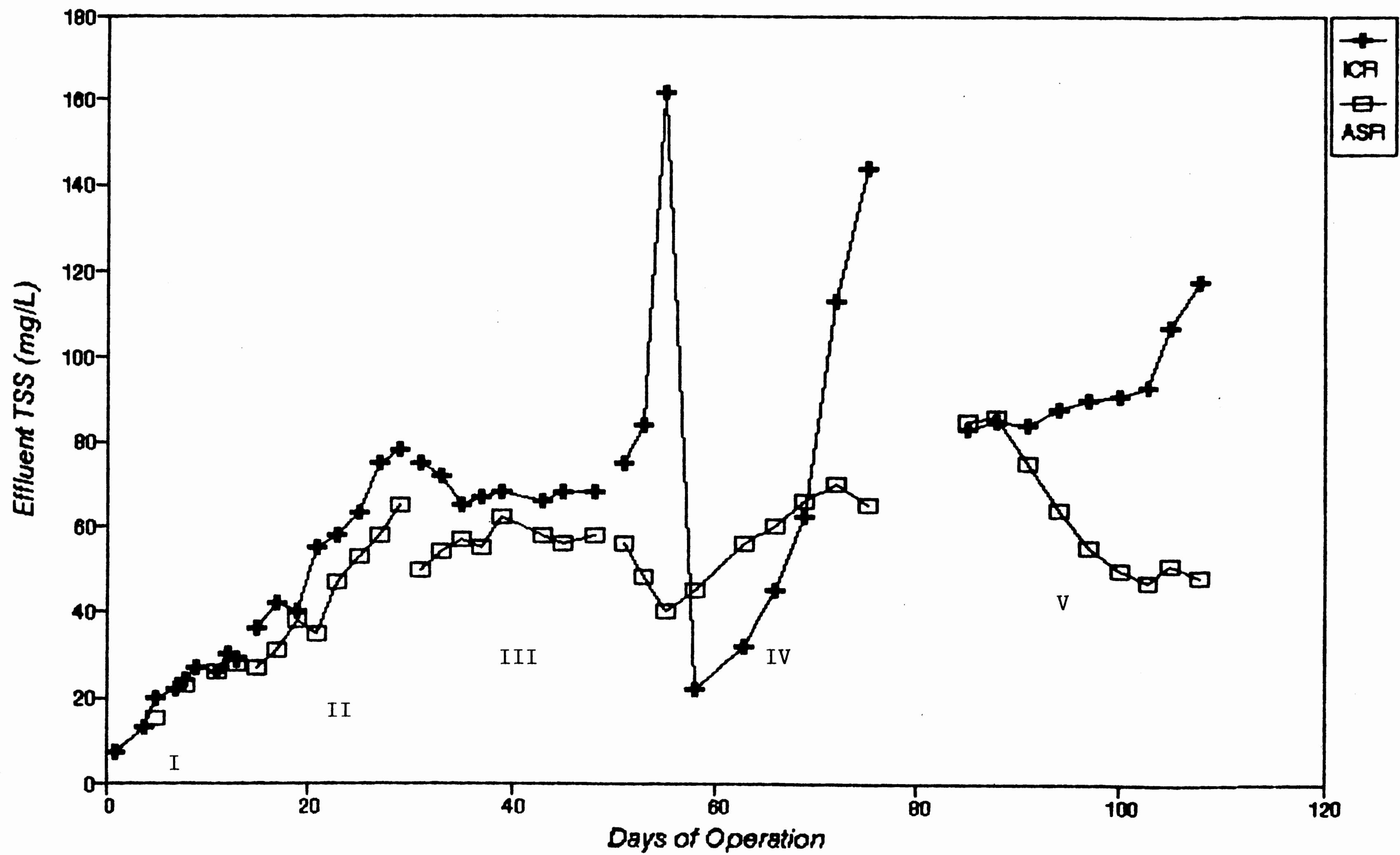


Figure 12. Effluent TSS Profile for Organic Loading Study

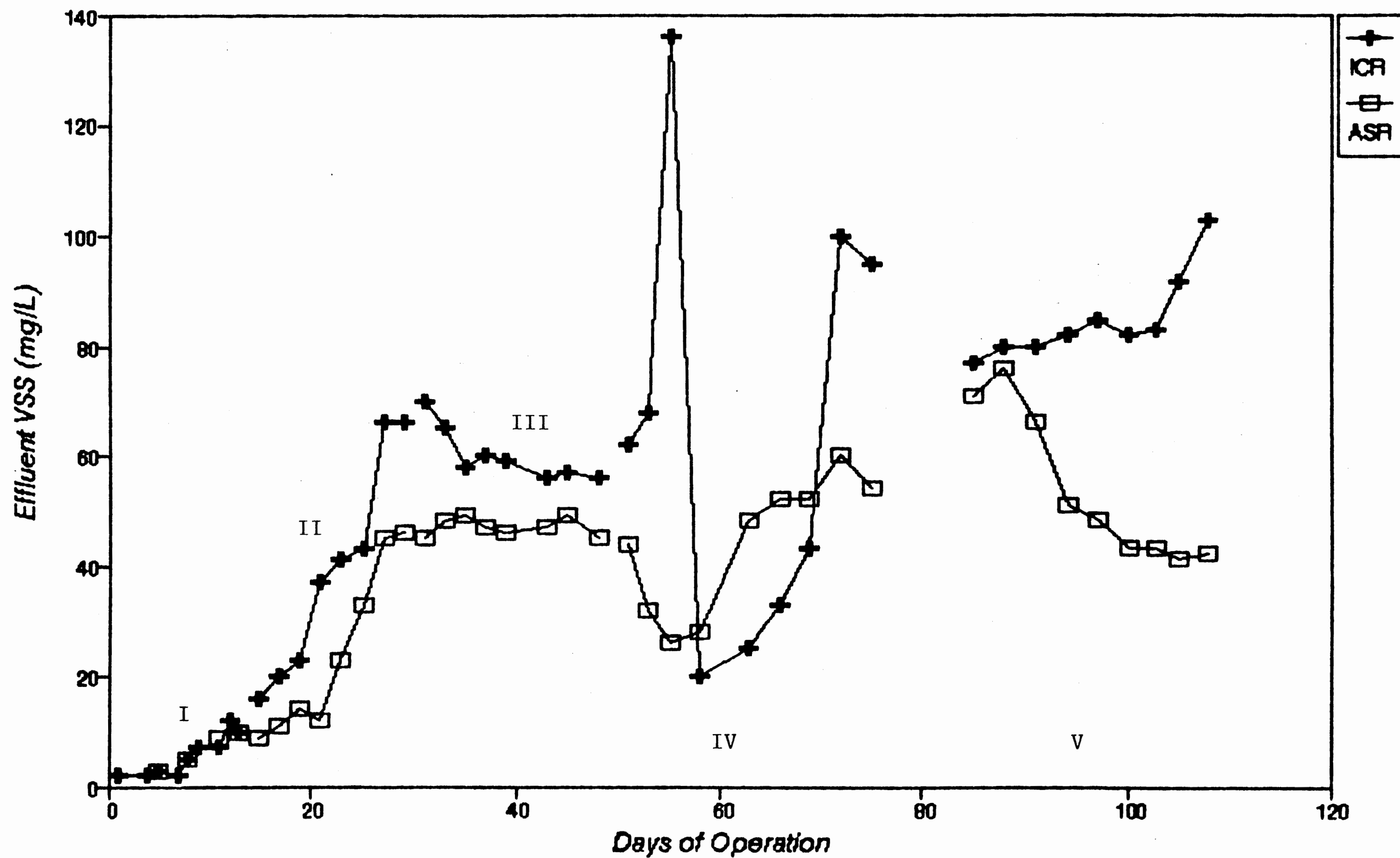


Figure 13. Effluent VSS Profile for Organic Loading Study

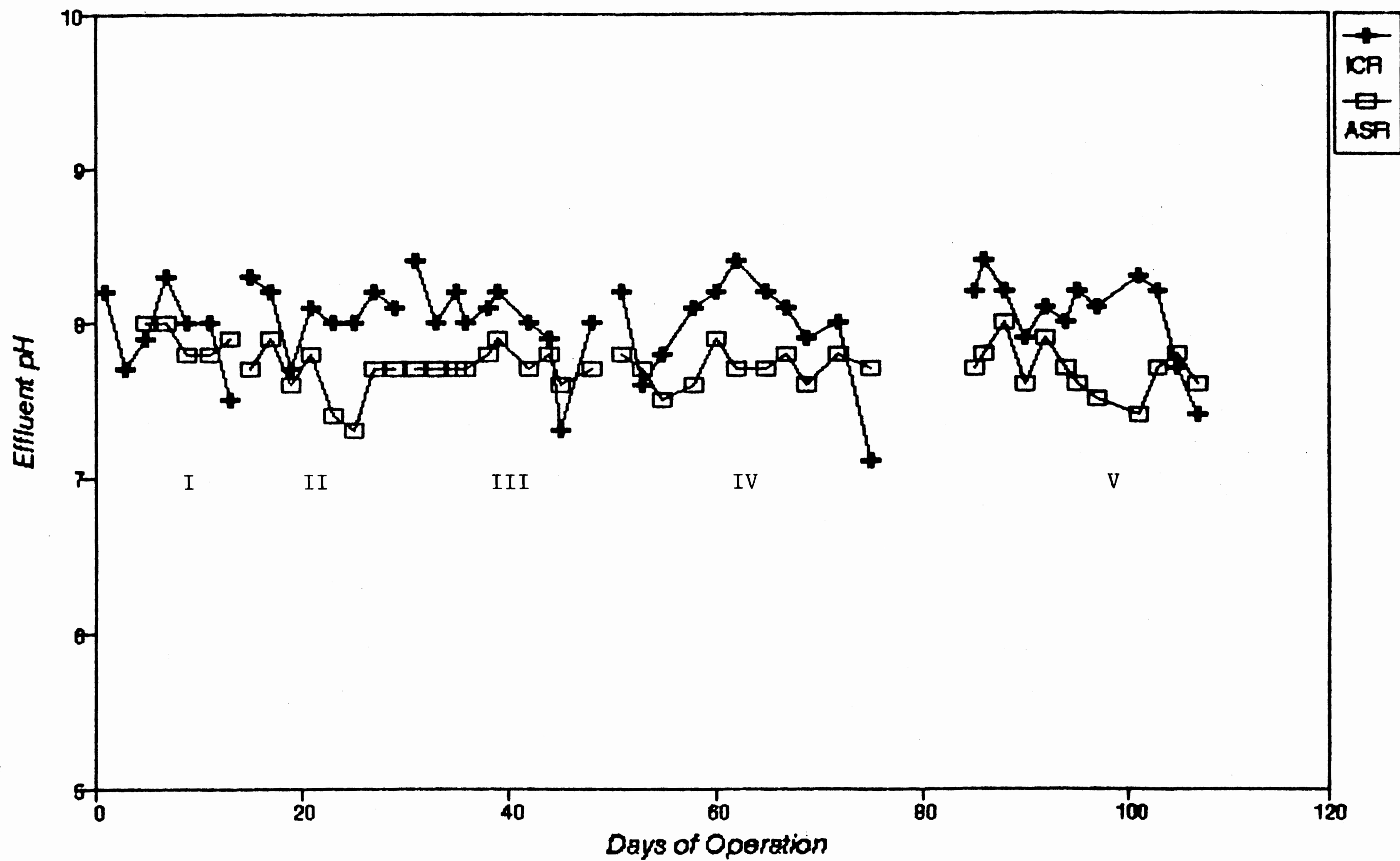


Figure 14. Effluent pH Profile for Organic Loading Study

TABLE X

DO UPTAKE BY CHITOSAN BEADS AND MLSS OF ASR IN ORGANIC LOADING STUDY

Loading No.	Influent TDA Conc. (mg/L)	Total Days of Operation	DO Uptake Rate (mg/L.min.bead)	ASR MLSS (mg/L)	
				MLTSS	MLVSS
I	50	1 - 13	0.18	903	805
II	100	15 - 29	0.07	924	828
III	150	31 - 48	0.19	1770	1640
IV	200	51 - 75	0.18	1375	1222
V	250	85 - 107	0.12	1269	1158

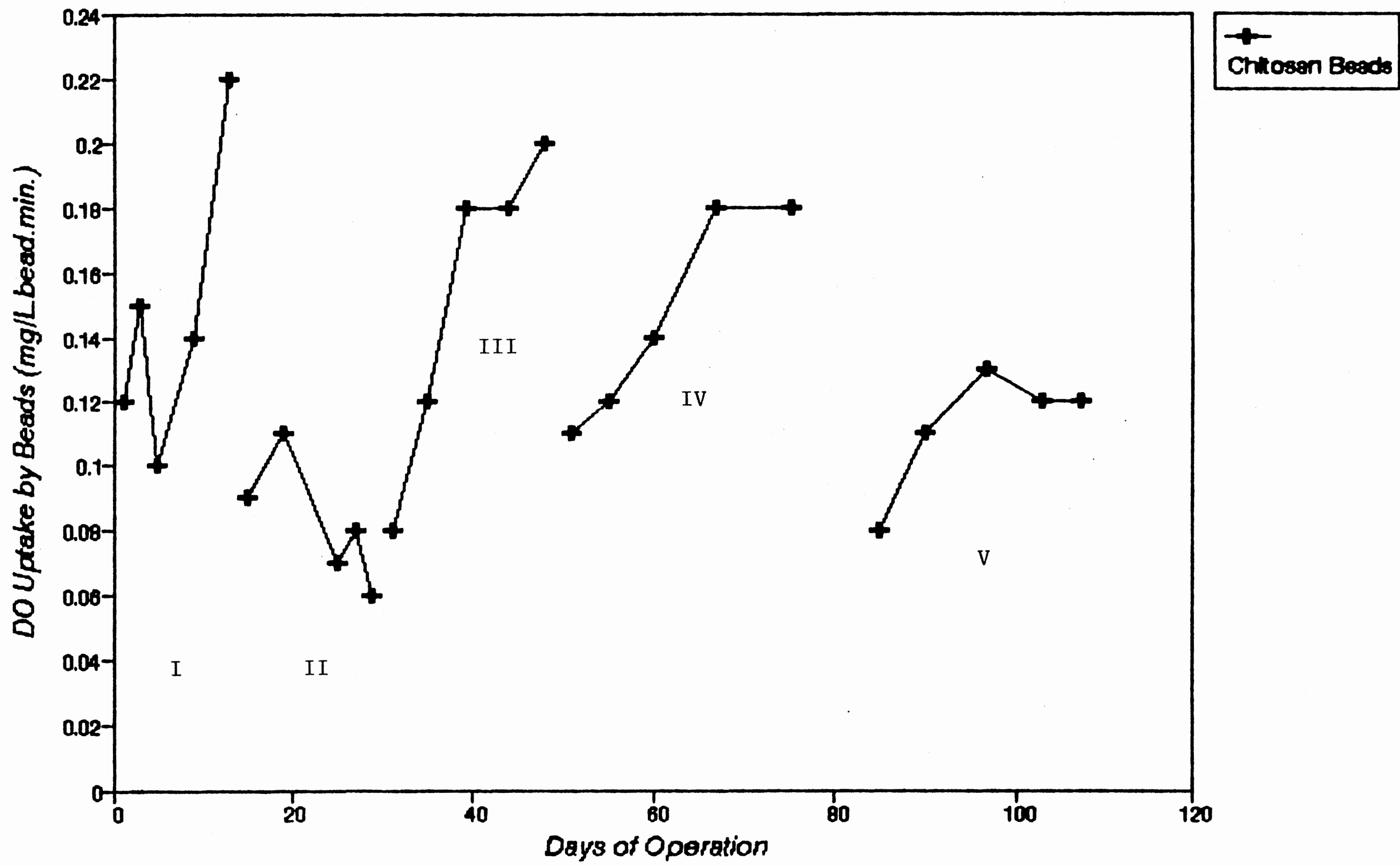


Figure 15. DO Uptake Profile of Beads during Organic Loading Study

researchers to be an indicator of microbial activity (Sofer et al, 1990; Mattiasson, 1983). The average values of DO uptake rates obtained at steady state (based on steady state periods of TDA / SCOD removal profiles) during these runs are shown in Table X. Figure 15 represents the complete DO uptake activity profile for each run. The DO uptake rates were in the range 0.12 - 0.19 mg/L.min.bead throughout this phase except at influent concentration of 100 mg/L TDA where it dropped to 0.07 mg/L.min.bead.

MLSS of ASR

In this phase, the MLVSS fluctuated in the range 0.81 - 1.64 g/L. The biomass exhibited poor settling and agglomerating characteristics. Daily wastage to maintain SRT of six days was done based on the latest MLVSS available. Table VI illustrates the steady-state SRTs achieved in this run. The MLTSS and MLVSS values for the ASR are presented in Table XI and the corresponding plots are given in Figure 16.

Hydraulic Loading Study

At the end of the first phase, the ASR continued to be operated at 250 mg/L TDA concentration until the 8 hour HRT was started. Since the beads in the ICR disintegrated at the end of the organic loading study, fresh beads were installed. The ICR was also run at 250 mg/L TDA concentration for about three days before the start of the next phase. Data procurement for this phase was started two days after the start of the first run i.e. 8 hour HRT.

In the second phase of the study, the hydraulic retention time in the two reactors was decreased from 10 hours through 8, 5 and 3 hours sequentially. At each HRT, the TDA concentration was fixed at 150 mg/L with the same base mix concentration as before (i.e. 4.5 ml carbon stock and 3.0 ml salt stock diluted with tap water to obtain 1 L of feed).

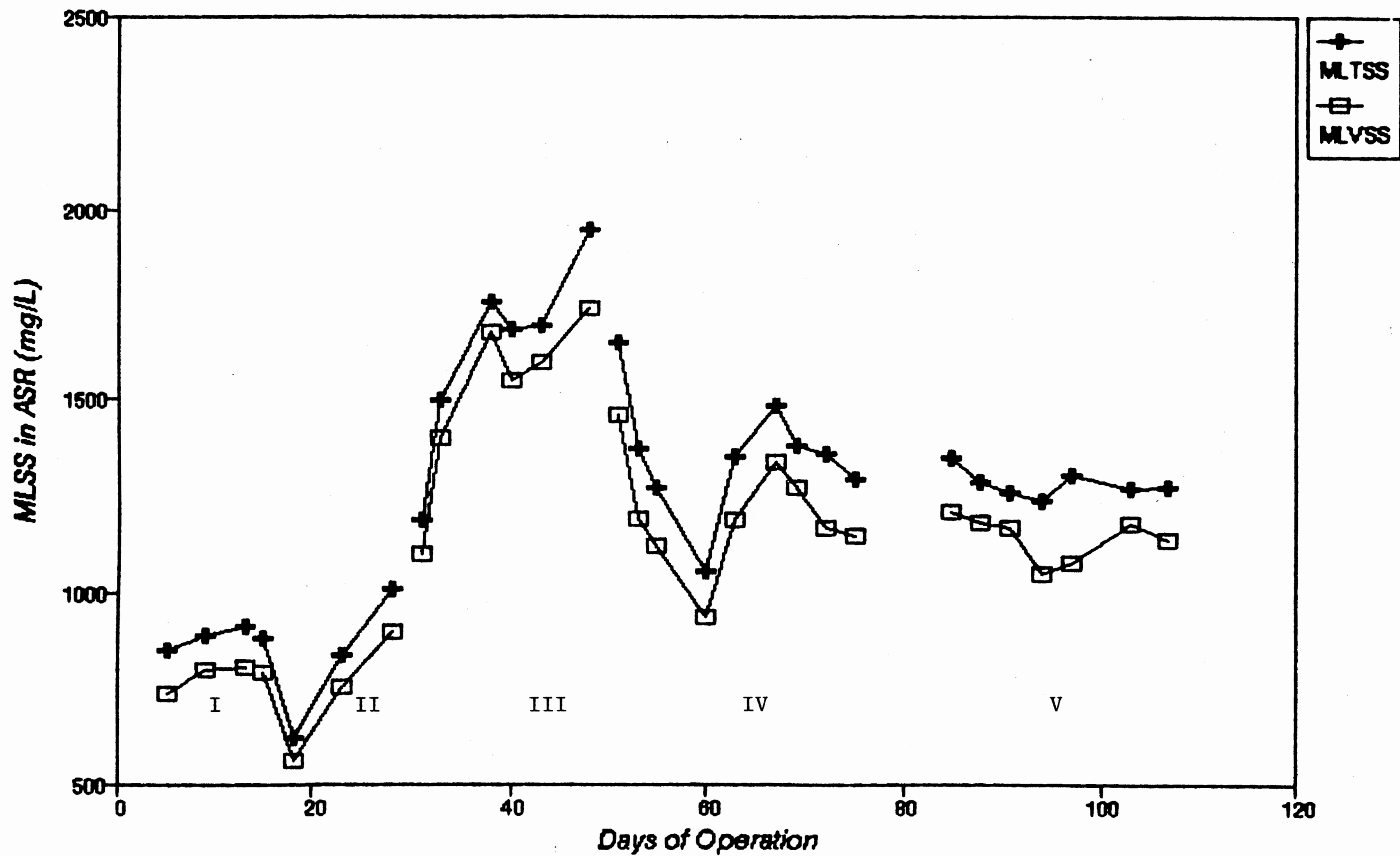


Figure 16. MLSS in ASR during Organic Loading Study

Consequently, the TCOD and SCOD concentrations were in the range 868 - 873 mg/L TCOD and 836 - 851 mg/L SCOD. Since the HRT was changed by increasing the waste flow rate into the reactors, the TCOD and SCOD loading rates increased in the ranges 2.63 - 6.93 g / L.d (TCOD) and 2.56 - 6.76 g / L.d (SCOD). At each hydraulic loading rate, steady-state was allowed to be established before increasing the flow-rate for the next run. A complete summary of operating conditions for this study is presented in Table XI. Data were procured during each run as explained previously in Chapter III.

TDA Removal

The steady state effluent TDA concentrations with an influent TDA concentration of 150 mg/L are given in Table XII. The TDA removal efficiency, in the case of ICR, was initially (at 8 hour HRT) 8%, increased to 14% in the next run, and fell to about 10% at 3 hour HRT. Interestingly, TDA removal efficiency by the ASR increased from 2.8% at 8 hour HRT and stabilized at approximately 8% in the next two runs. The complete effluent TDA and removal efficiency profiles for this phase are depicted in Figures 17 and 18.

TCOD and SCOD removal

Table XIII gives the steady state TCOD and SCOD removal data for this part of the study. It can be seen that, in both reactors, the removal efficiency dropped sharply at HRT of 3 hours. In the ICR, TCOD removal efficiency dropped from 51% (at 8 hour HRT) to about 23% (at 3 hour HRT) while the corresponding drop in SCOD removal efficiencies was from 76% to 39%. The ASR exhibited marginally better TCOD removal but poorer SCOD removal compared to the ICR. TCOD and SCOD removal and removal efficiency plots are illustrated in Figures 19 - 22.

TABLE XI

OPERATING CHARACTERISTICS DURING HYDRAULIC LOADING STUDY

Reactor Type	Reactor Volume (mL)	Loading No.	Total Days of Operation	Influent TDA* (mg/L)	HRT (hours)	Flow Rate (L/d)	Influent COD (mg/L)		SRT (days)	Amt.Biom (g)**/ MLVSS (g)***
							TCOD	SCOD		
ICR	1120	I	1 - 20	150	8	3.4	873	850	-	20
		II	22 - 35	150	5	5.4	868	836	-	20
		III	38 - 58	150	3	8.9	869	851	-	20
ASR	4556	I	1 - 20	150	8	13.7	873	850	5.8	5.8
		II	22 - 35	150	5	21.9	868	836	6.0	7.5
		III	38 - 58	150	3	36.4	869	851	5.6	4.4

* by mass determination; ** mass of biomass immobilized; *** only for ASR

TABLE XII

TDA REMOVAL IN HYDRAULIC LOADING STUDY

Loading No.	Total Days of Operation	Influent TDA* (mg/L)	HRT (hours)	ICR Effluent		ASR Effluent		
				TDA (mg/L)	Rem. Eff. (%)**	TDA (mg/L)	Rem. Eff. (%)**	Remarks
I	1 - 20	150.2	8	138.4	7.9	146.1	2.8	
II	22 - 35	150.6	5	130.3	13.5	138.7	7.9	
III	38 - 58	150.6	3	135.1	10.3	139.0	7.9	Only until Day42

* by HPLC determination; ** removal efficiency of parameter in immediate left column

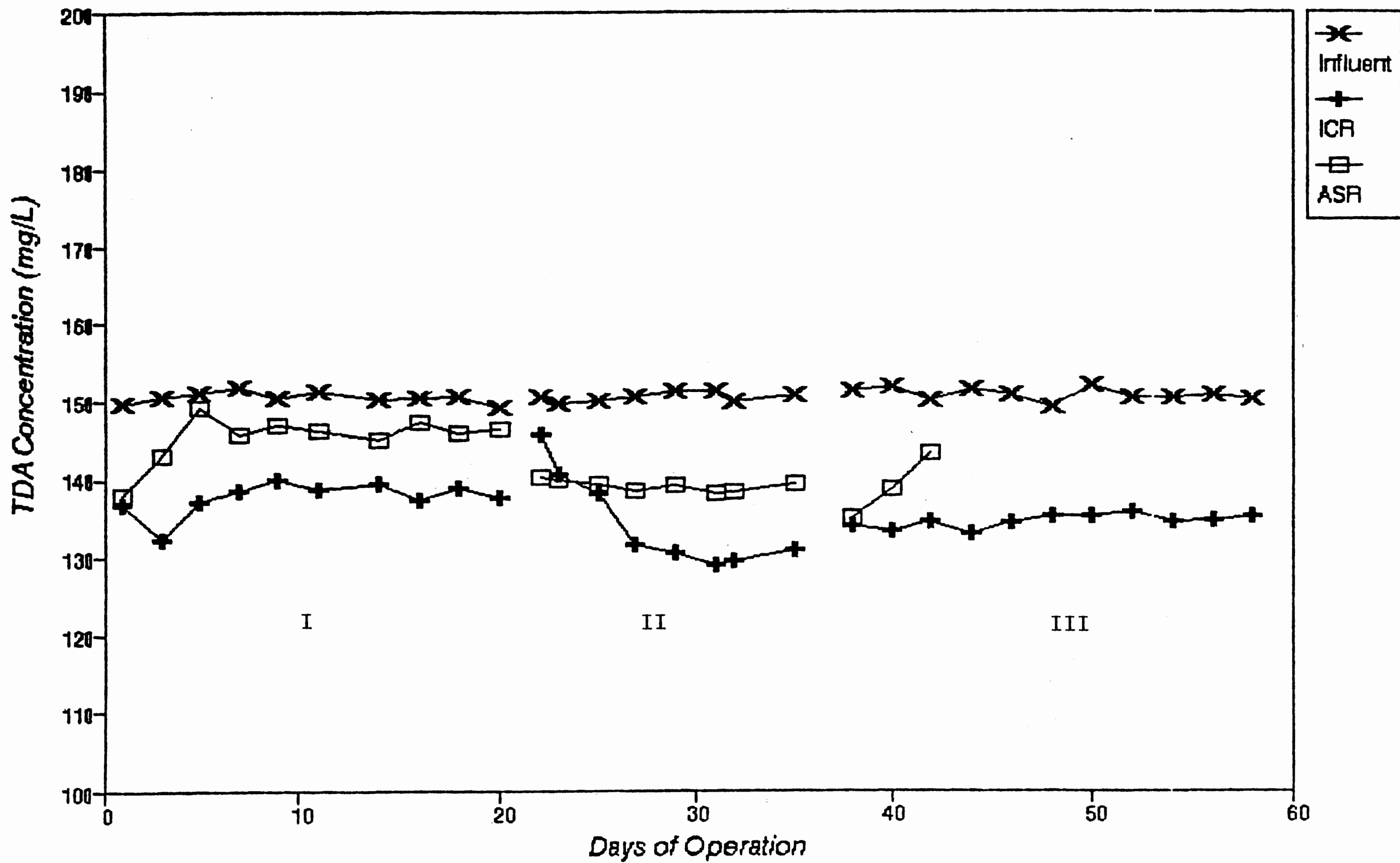


Figure 17. Influent and Effluent TDA Profile for Hydraulic Loading Study

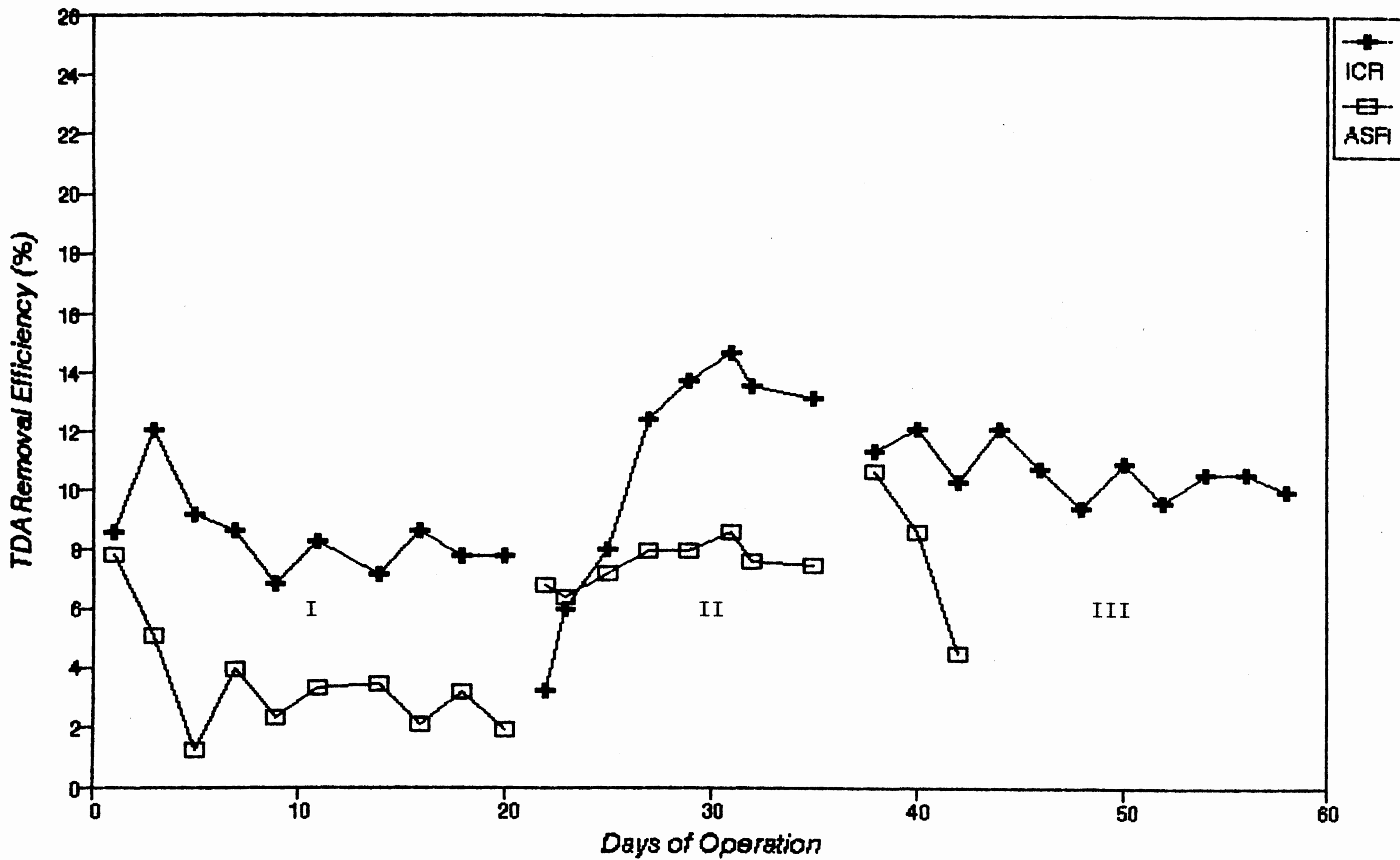


Figure 18. TDA Removal Efficiency Profile for Hydraulic Loading Study

TABLE XIII

TCOD AND SCOD REMOVAL IN HYDRAULIC LOADING STUDY

Loading No.	Total Days of Operation	HRT (hours)	Influent COD (mg/L)		ICR Effluent				ASR Effluent			
			TCOD	SCOD	TCOD (mg/L)	Rem. Eff.* (%)	SCOD (mg/L)	Rem. Eff.* (%)	TCOD (mg/L)	Rem. Eff.* (%)	SCOD (mg/L)	Rem. Eff.* (%)
I	1 - 20	8	873	850	426	51.2	212	75.7	384	56.0	283	67.6
II	22 - 35	5	868	836	384	55.8	235	72.9	375	56.8	298	65.6
II	38 - 58	3	869	851	670	22.9	533	38.7	662 **	23.8**	533**	38.6**

* removal efficiency of parameter in immediate left column; ** data only until day 42

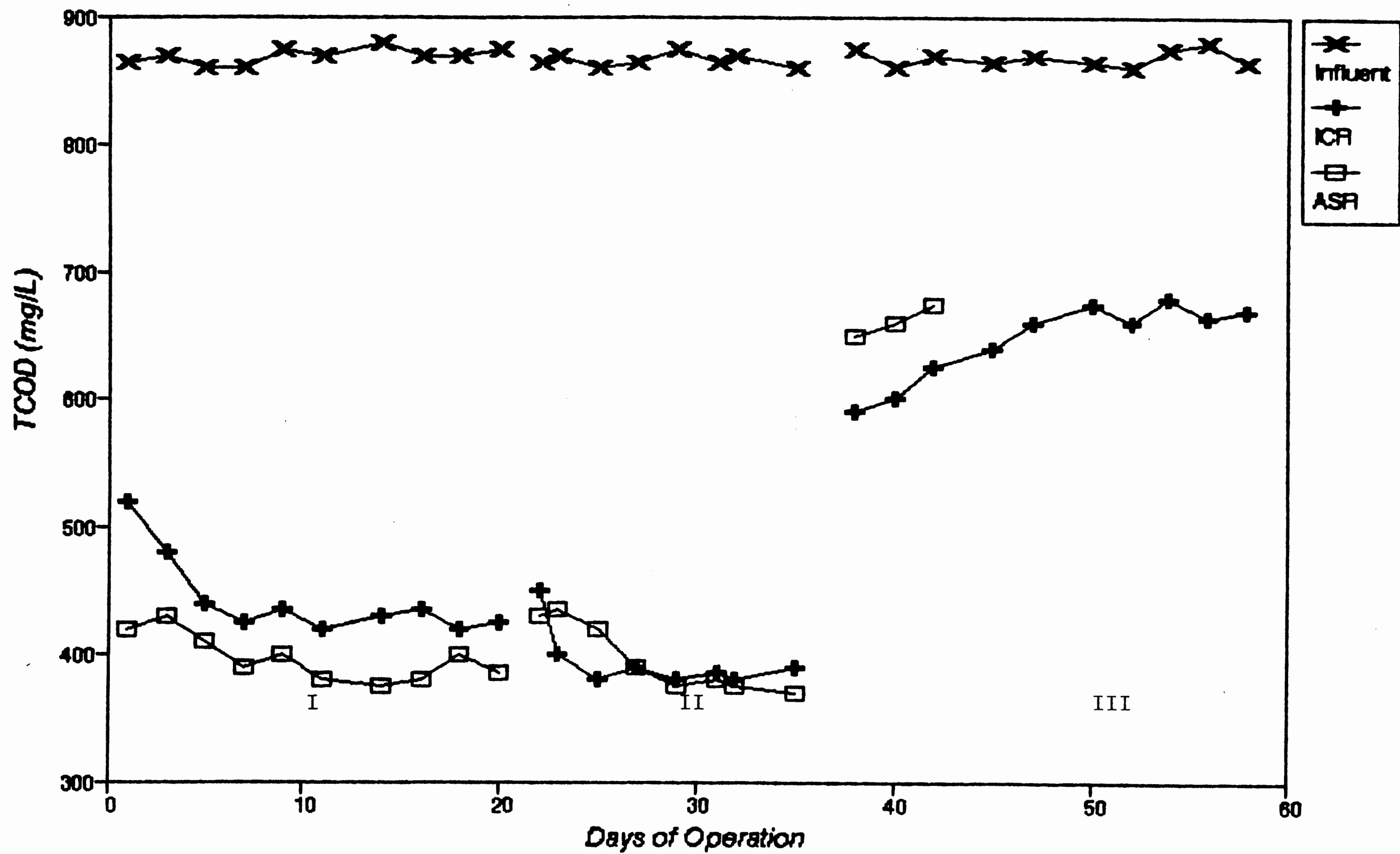


Figure 19. Influent and Effluent TCOD Profile for Hydraulic Loading Study

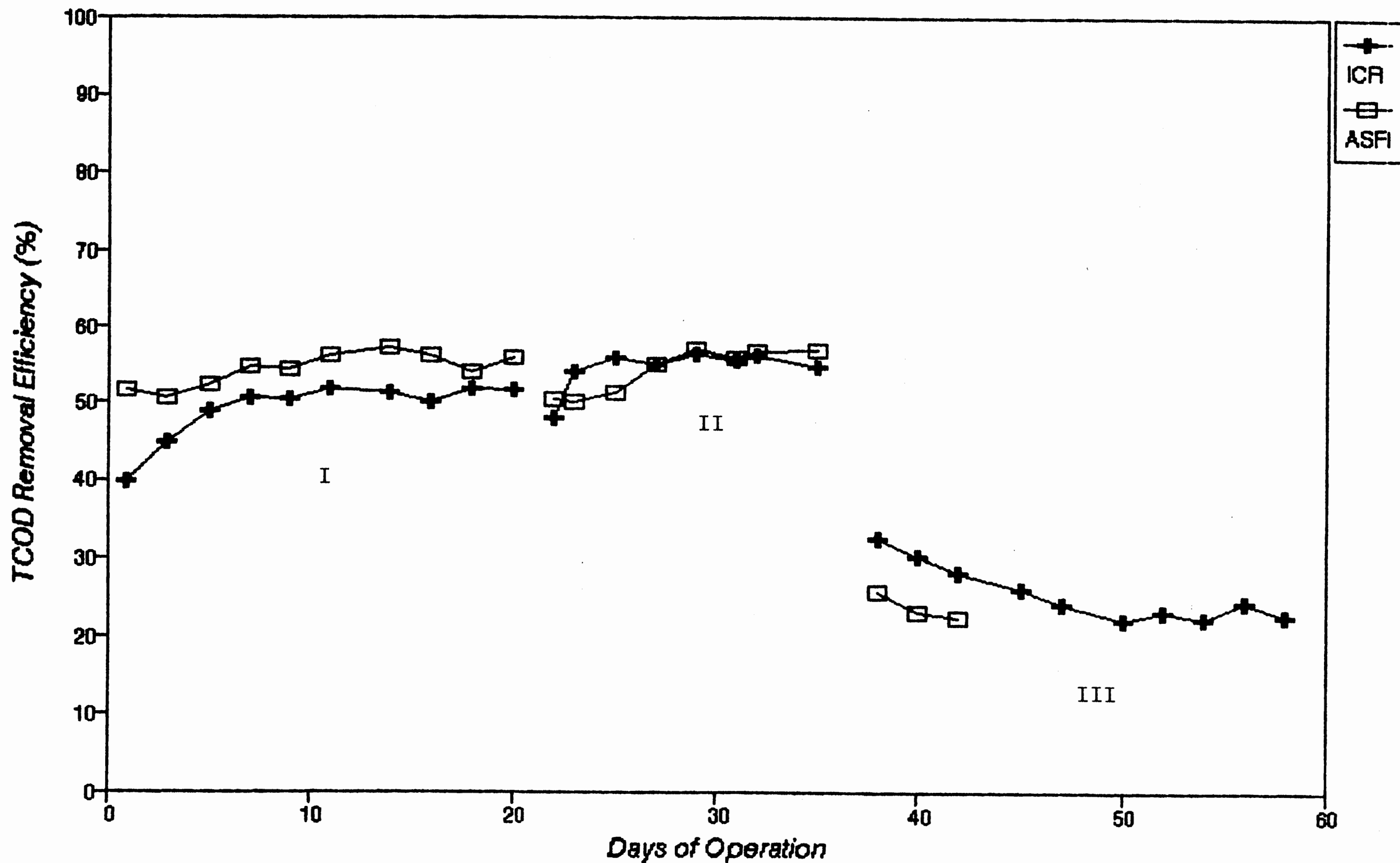


Figure 20. TCOD Removal Efficiency Profile for Hydraulic Loading Study

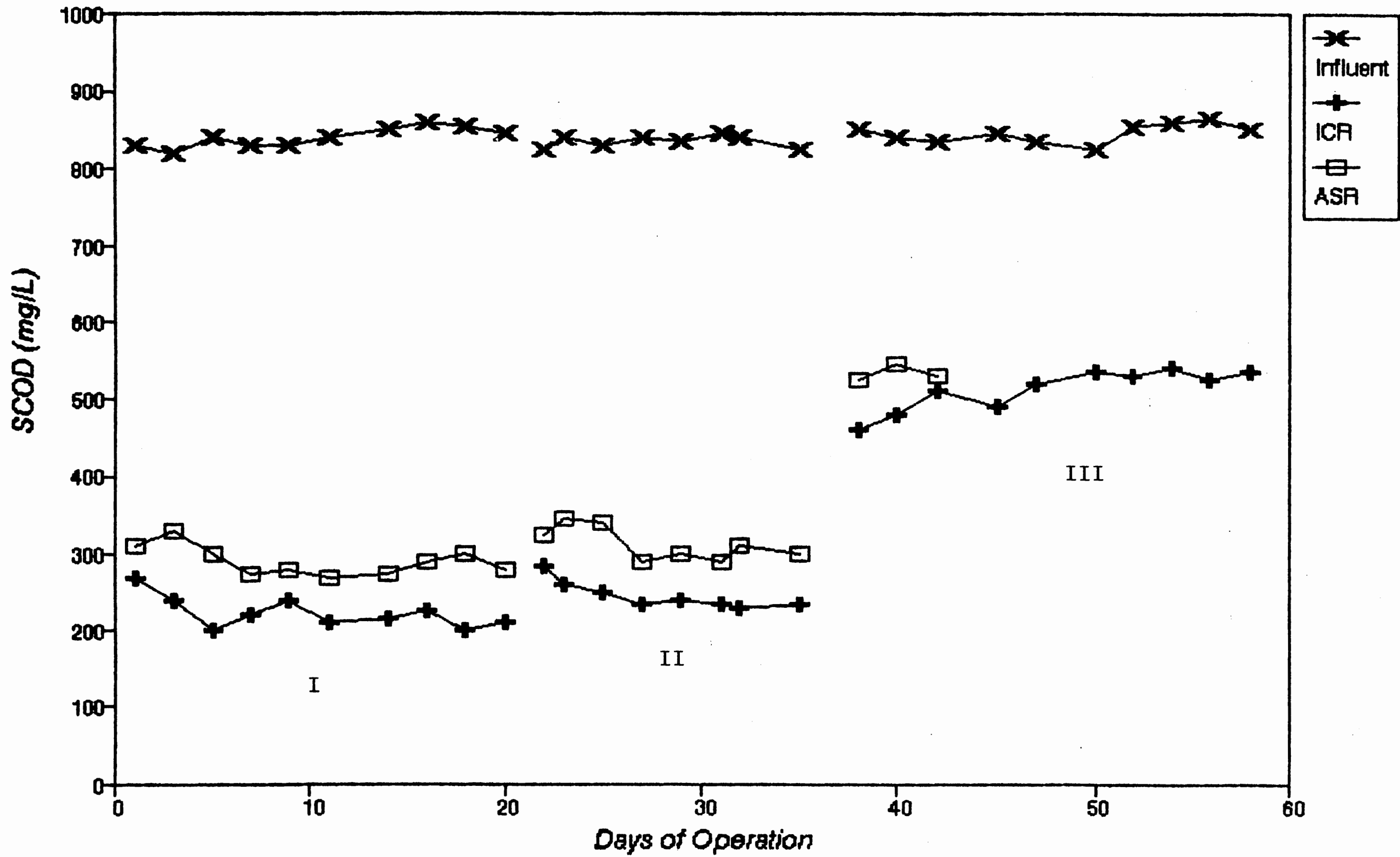


Figure 21. Influent and Effluent SCOD Profile for Hydraulic Loading Study

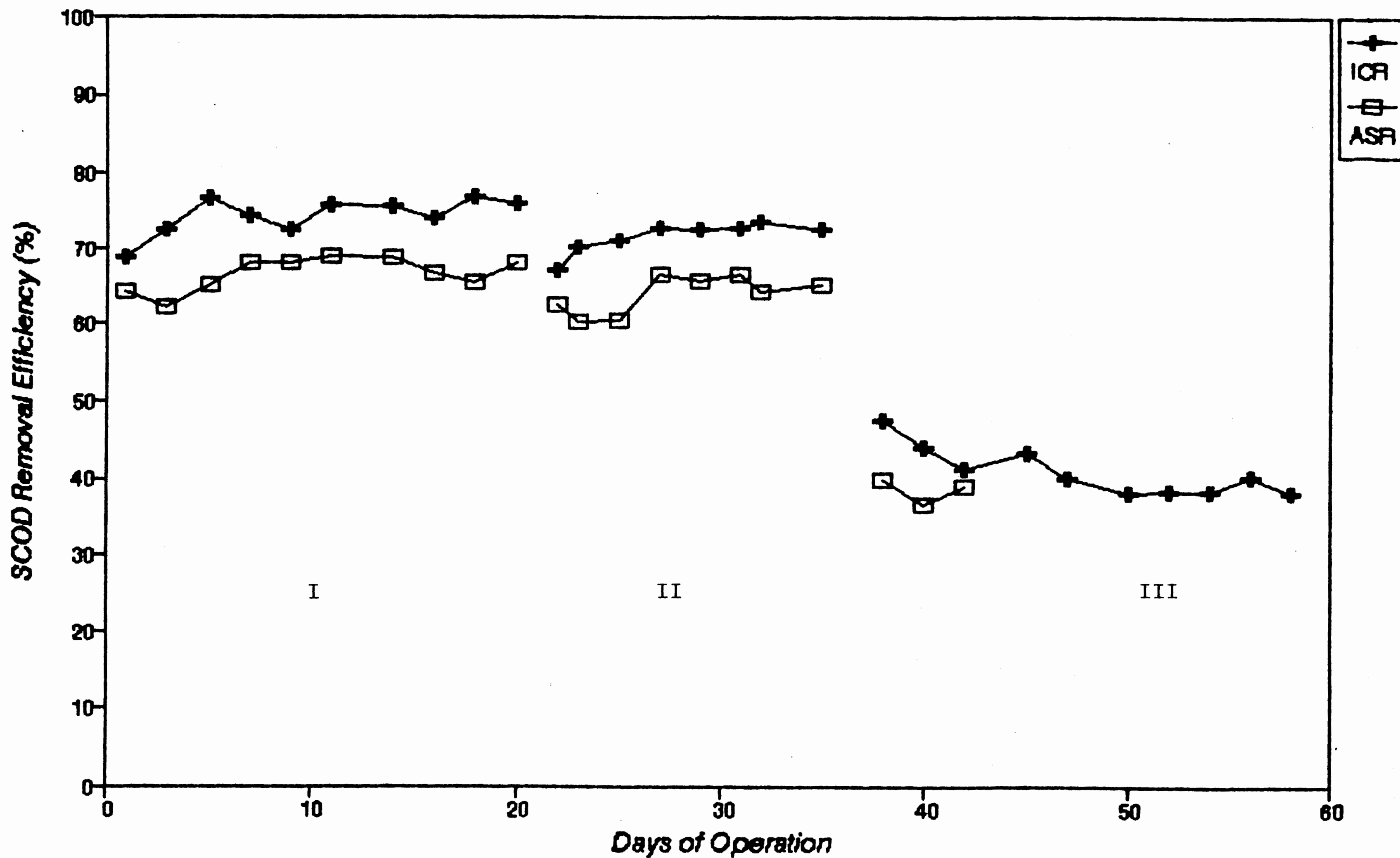


Figure 22. SCOD Removal Efficiency Profile for Hydraulic Loading Study

Effluent Solids and pH

The effluent solids concentrations (TSS and VSS) and effluent pH values for the hydraulic loading study are given in Table XIV. Figures 23 and 24 depict the corresponding profiles. The sharp peak that is seen in the second run for the ASR profile indicates biomass washout that occurred as mentioned in Table XIV. In the case of ICR, matrix debris and other suspended solids affected TSS values significantly. The effluent pH plot is illustrated in Figure 25. Again, instances of bead breakage were clearly indicated by a drop in pH.

In the ICR, solids washout increased due to the high flow rates. A significant observation was that during the 5 hour HRT run there was an apparent increase in the size of chitosan beads due to surface biomass growth. Interestingly, the structural integrity of the biocatalysts was not at all affected in the above stage.

DO Uptake by Chitosan Beads

Table XV shows the DO consumption of beads. The corresponding data is illustrated as a plot in Figure 26. It was observed that DO uptake rate, which had remained almost stable in the range of 0.12 - 0.14 mg/L.min.bead throughout the entire study, dropped to 0.04 mg/L.min.bead at 3 hour HRT.

MLSS of ASR

Table XV and Figure 27 show the MLSS profile in the ASR throughout the second phase.An SRT of approximately 6 days was maintained in the ASR. In this phase, better control over the MLVSS in the reactor was established, compared to the organic loading study. Approximately one week from the start of the 8 hour HRT run, the nature of the

TABLE XIV

EFFLUENT SOLIDS AND pH IN HYDRAULIC LOADING STUDY

Loading No.	Total Days of Operation	HRT (hours)	ICR Effluent				ASR Effluent			
			TSS (mg/L)	VSS (mg/L)	pH Range	Remark	TSS (mg/L)	VSS (mg/L)	pH	Remark
I	1 - 20	8	99	60	7.4 - 8.1	Day18- bead breakage	37	28	7.4 - 7.5	
II	22 - 35	5	94	80	8.0 - 8.2		199	155	7.4 - 7.6	Day 28/30- cell washout
III	38 - 58	3	88	72	7.0 - 7.7	Day42, day56- bead breakage	156	126	7.0 - 7.5	Day 42 - reactor failure

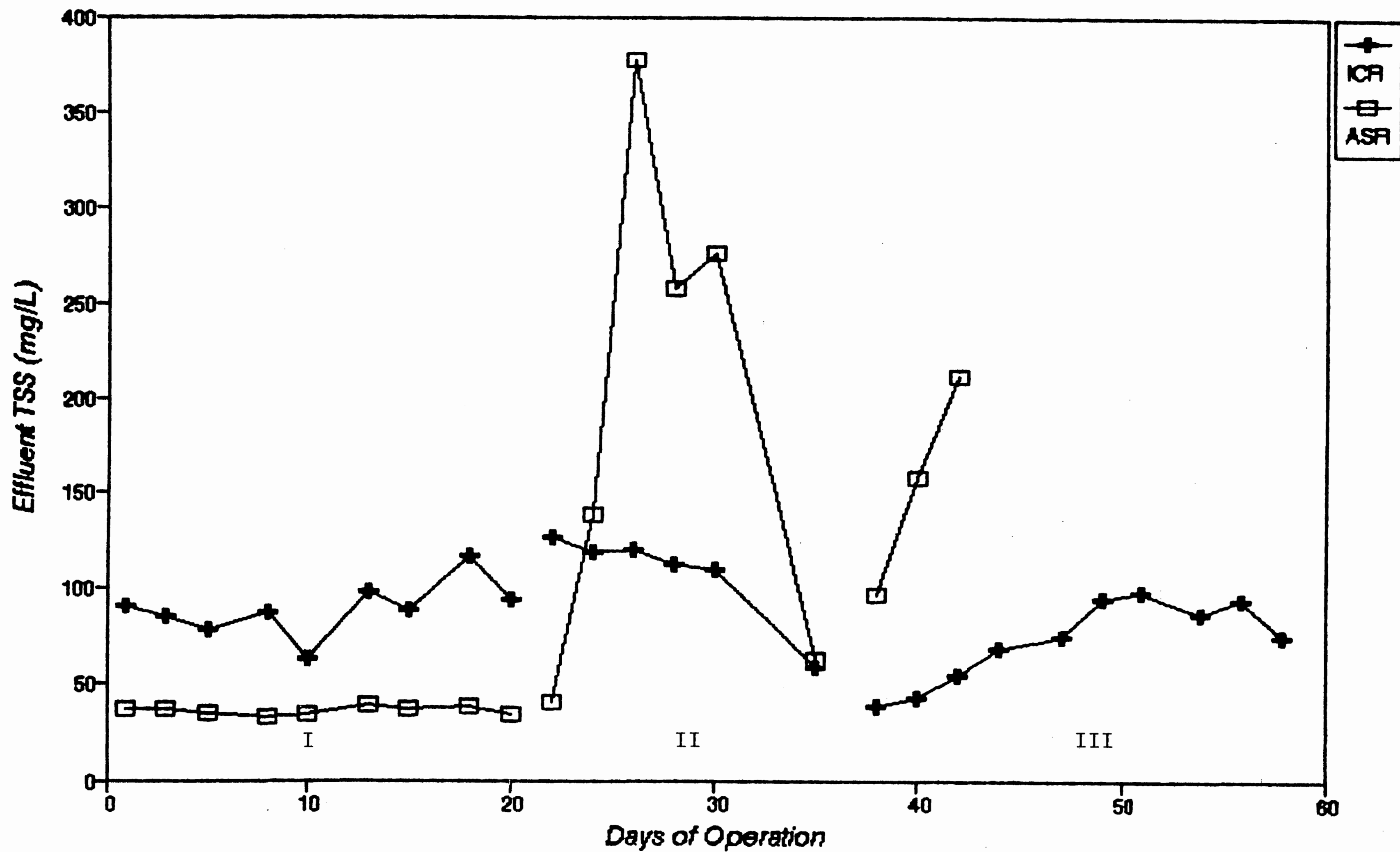


Figure 23. Effluent TSS Profile for Hydraulic Laoding Study

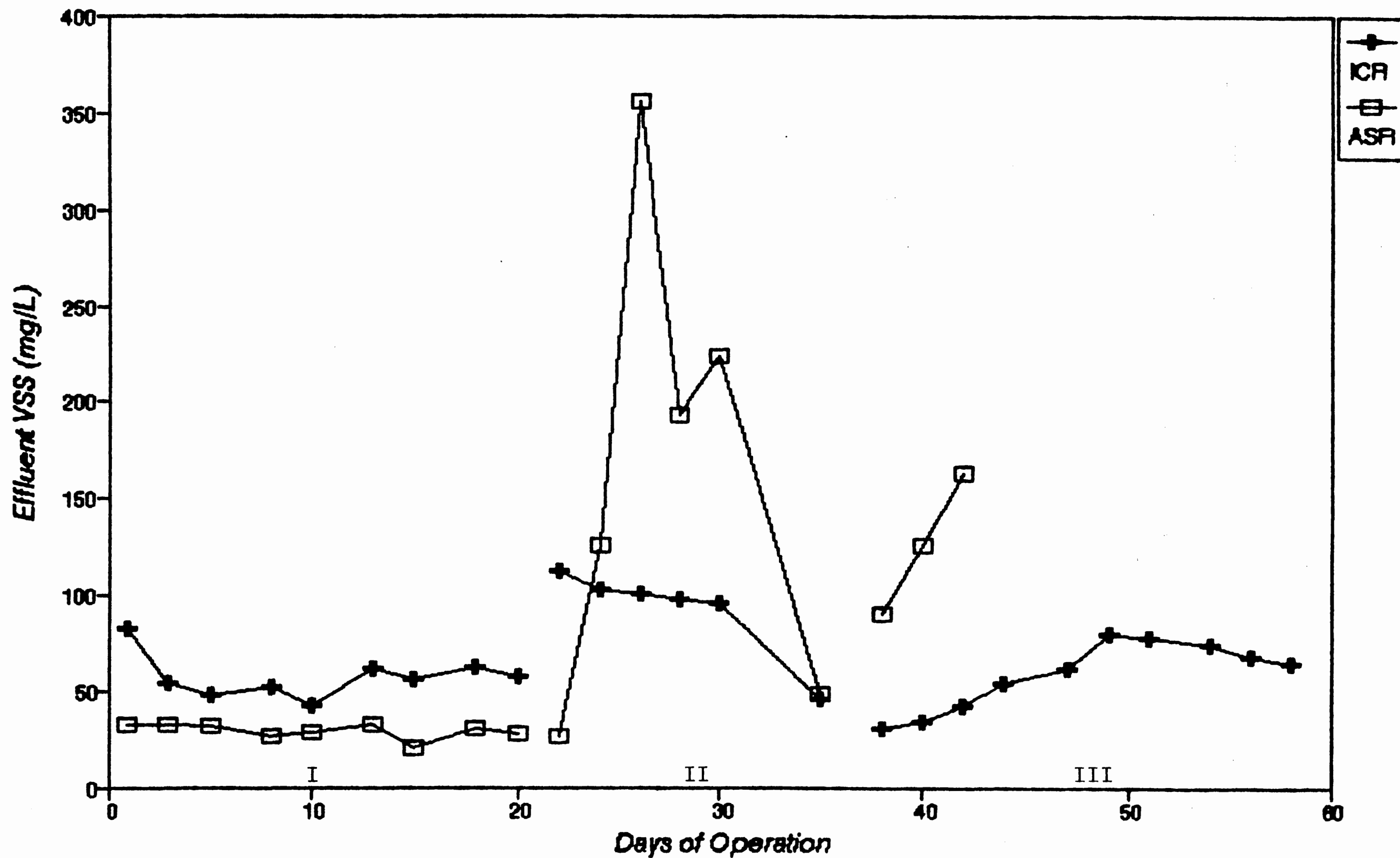


Figure 24. Effluent VSS Profile for Hydraulic Loading Study

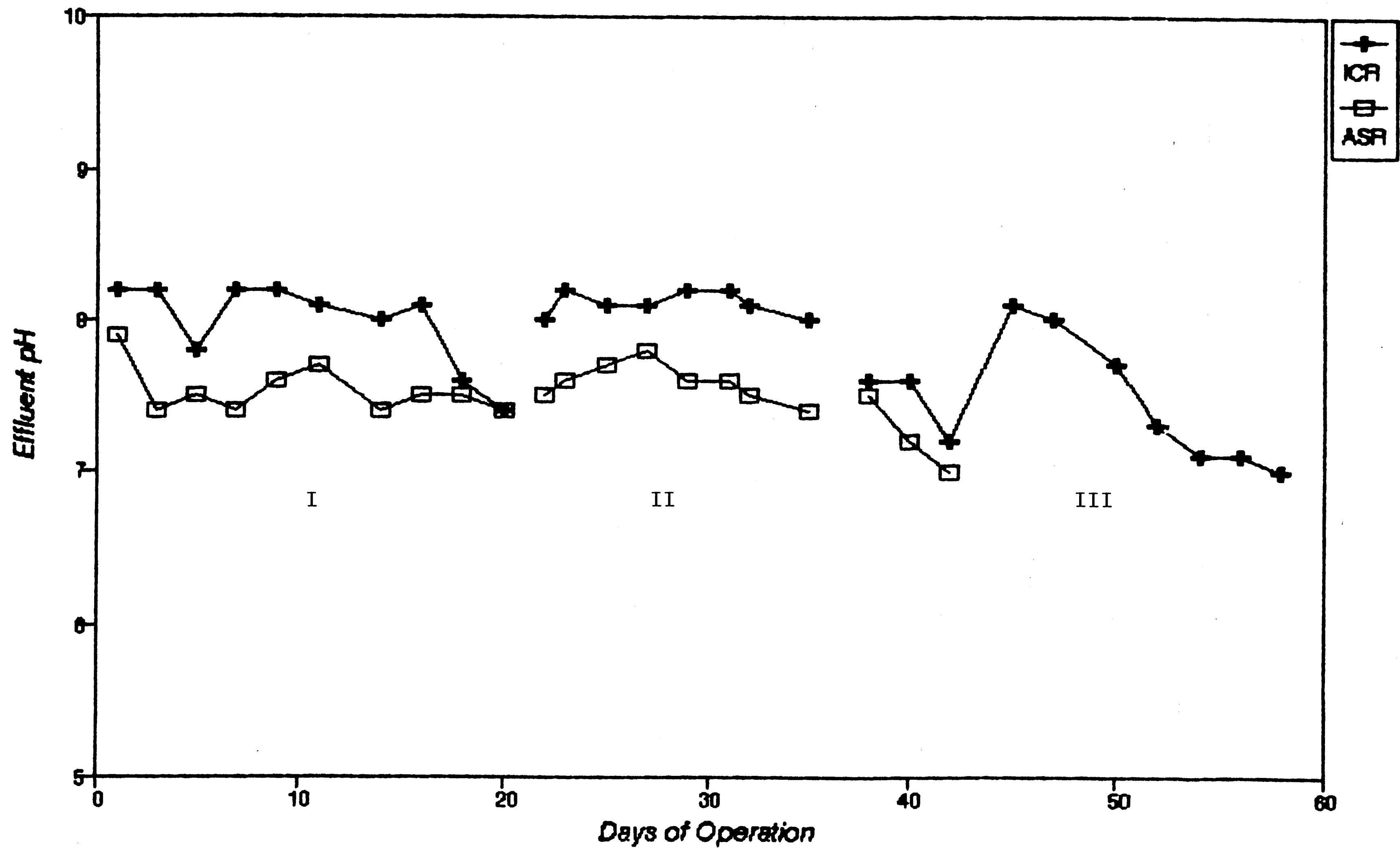


Figure 25. Effluent pH Profile for Hydraulic Loading Study

TABLE XV

DO UPTAKE BY CHITOSAN BEADS AND MLSS OF ASR IN HYDRAULIC LOADING STUDY

Loading No.	Total Days of Operation	HRT (hours)	DO Uptake (mg/L.bead. min)	ASR MLSS (mg/L)		
				MLTSS	MLVSS	Remarks
I	1 - 20	8	0.14	1217	1281	
II	22 - 35	5	0.12	1800	1647	
III	38 - 58	3	0.04	1136	960	until Day 42

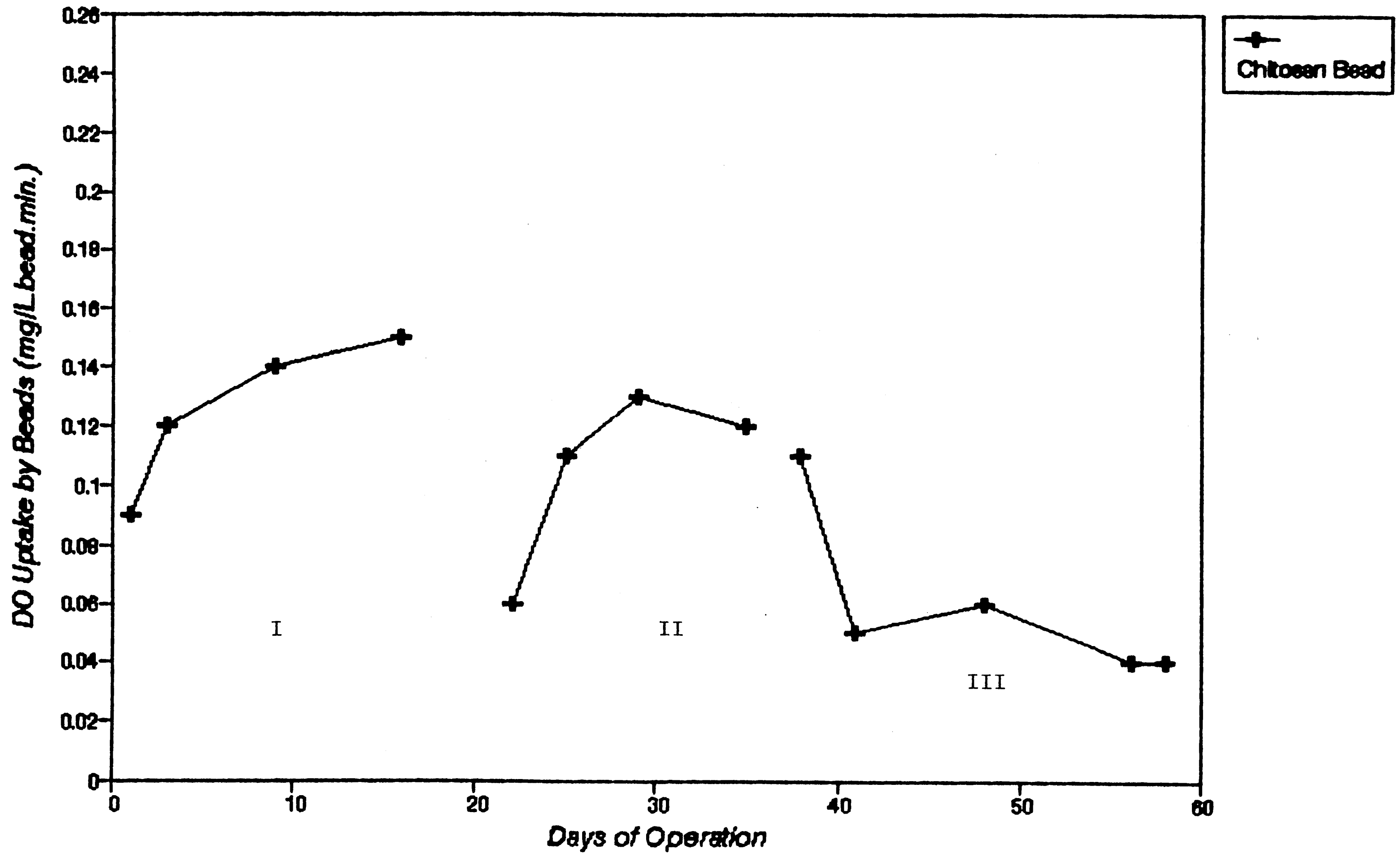


Figure 26. DO Uptake Profile of Beads during Hydraulic Loading Study

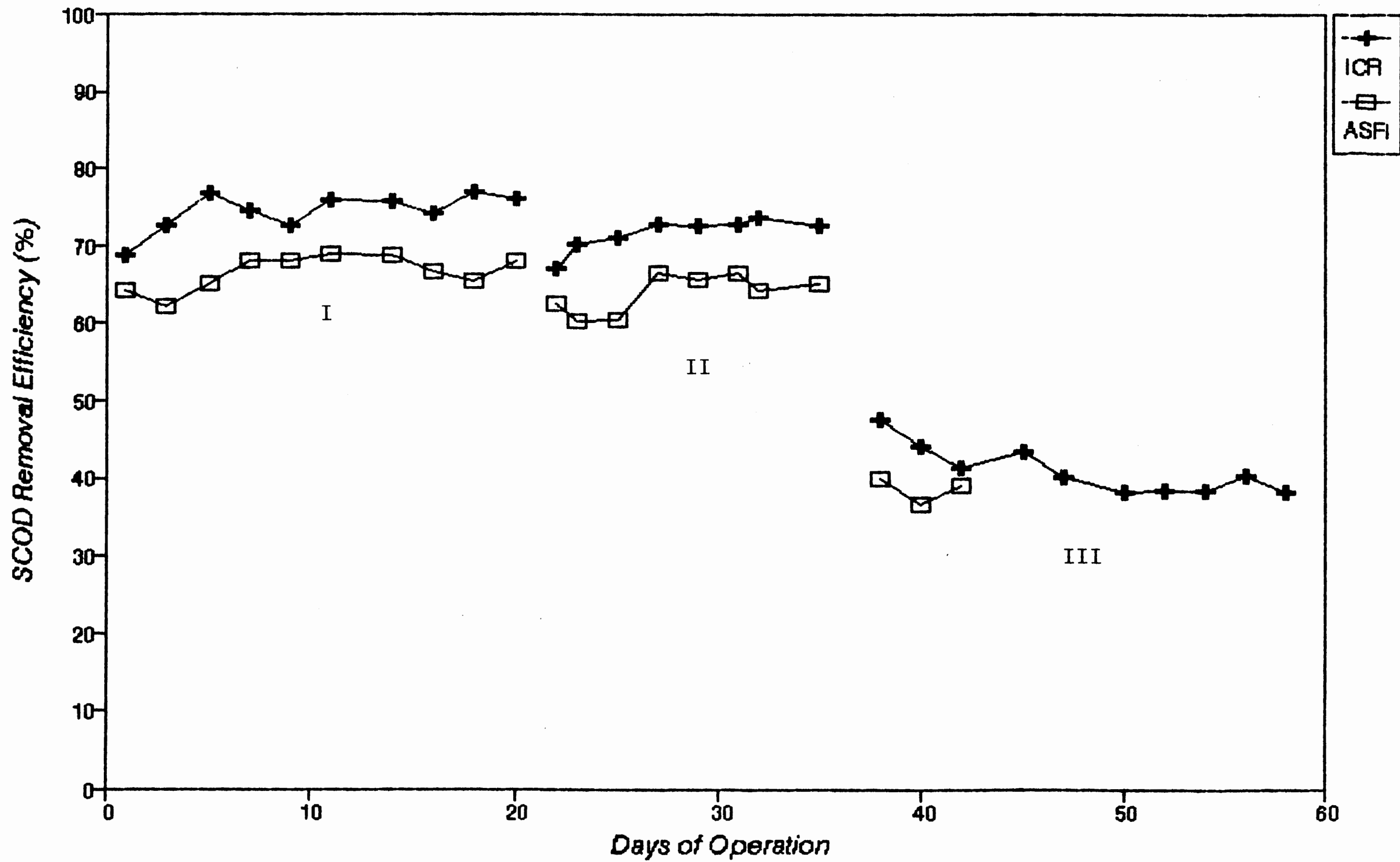


Figure 27. MLSS in ASR during Hydraulic Loading Study

ASR biomass changed once more. The color of the mixed liquor changed to a yellowish-white, even though its nature (poor settleability and agglomeration) essentially remained unchanged. Biomass washout from the reactor increased as the flow rate was increased. In the 3 hour HRT run (days 40 - 42 from the start of phase), due to severe biomass washout and reactor overflow caused by clogging of the effluent port, the reactor operation was discontinued. Since analysis of the available data indicated imminent failure conditions, it was decided that restarting the ASR with a fresh seed was unnecessary.

Supplementary Studies

As a supplement to the main study, a tentative identification of the bacterial species present in the acclimated stock culture and SEM of biomass-free as well as loaded chitosan beads were performed. The results of the identification tests provided useful information on the behavior of the ASR biomass. The micrographs of the bead matrix exemplified the differences between cell-free and loaded biocatalysts.

Bacterial Identification

Based on the constituents of the synthetic wastewater, the bacterial strains typically present in such wastes and the nature of the biopopulation present in the original seed, it was initially suspected that the mixed culture consisted predominantly of Pseudomonas sp. Therefore specific tests for confirmation of these bacteria were done in addition to other more general ones that covered a broad genus range (see Chapter III). A summary of the various tests is given in the Appendix.

The battery of tests conducted produced the following results. Three distinct colonies based on color and morphology were observed in the preliminary pour-plate cultures. These were labelled for convenience as " Yellow Smooth (YS) ", " Yellow Ridged (YR)

"and " White Smooth (WS) " The YS and YR strains were tentatively identified as Pseudomonas sp.Both strains, YS and YR accumulated poly-beta hydoxy butyrate (PHB) storage granules and did not form a fluorescent pigment. YS may belong to RNA group II, whereas YR may belong to RNA group III. YS was also more acid tolerant, therefore it could be P.acidovorcens; YR had denitrifying ability and tested negative for proteolysis, therefore, it could be P.solanacierum.

WS, although a Gram negative, strictly aerobic rod did not appear to be a Pseudomonas sp. considering the other results. It closely resembled the genus Acenitobacter because it was a coccobacilli, was oxidase negative, did not liquefy gelatin (proteolysis), did not act on nitrate, and showed more resistance to penicillin (diameter of inhibition zone was significantly smaller in this case than for the other two strains).

Scanning Electron Microscopy

SEM of 20-day old seeded and unseeded beads was performed on a JEOL 35 U Scanning Electron Microscope at an accelerating voltage of 25 kV in order to elucidate the structure of the chitosan matrix with and without bacteria. Figures 28 - 31 show the micrographs of the unseeded and seeded matrices. The micrographs depict magnifications of 5400X and 10,000X.

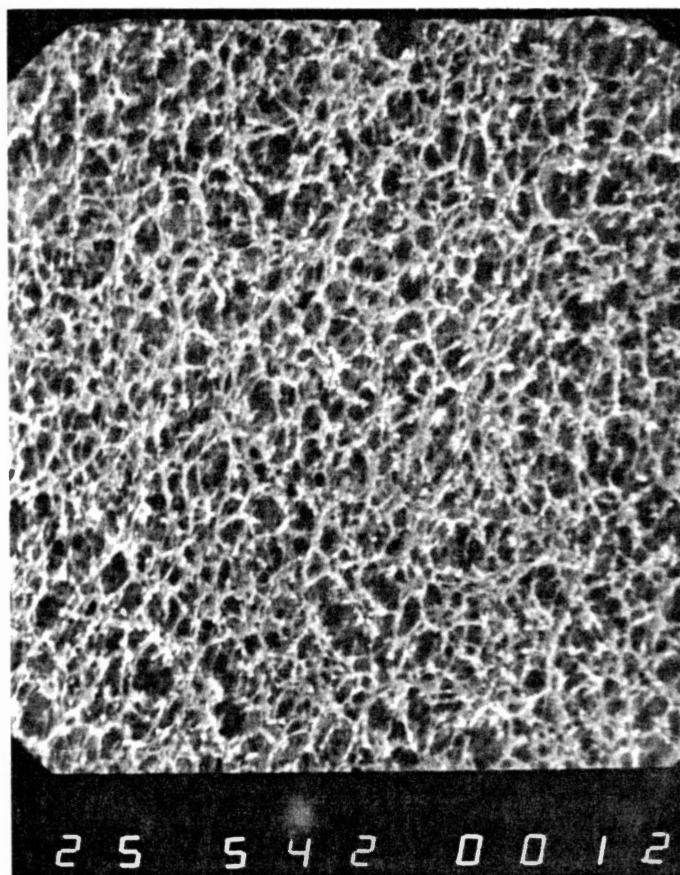


Figure 28. SEM of Unseeded Chitosan Matrix (5400 X)

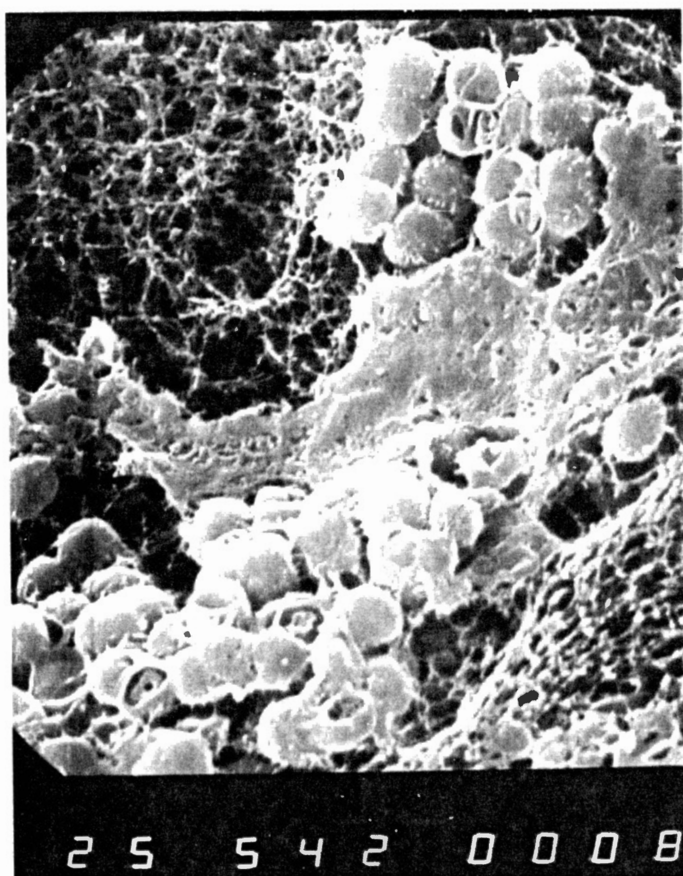


Figure 29. SEM of Seeded Chitosan Matrix (5400 X)

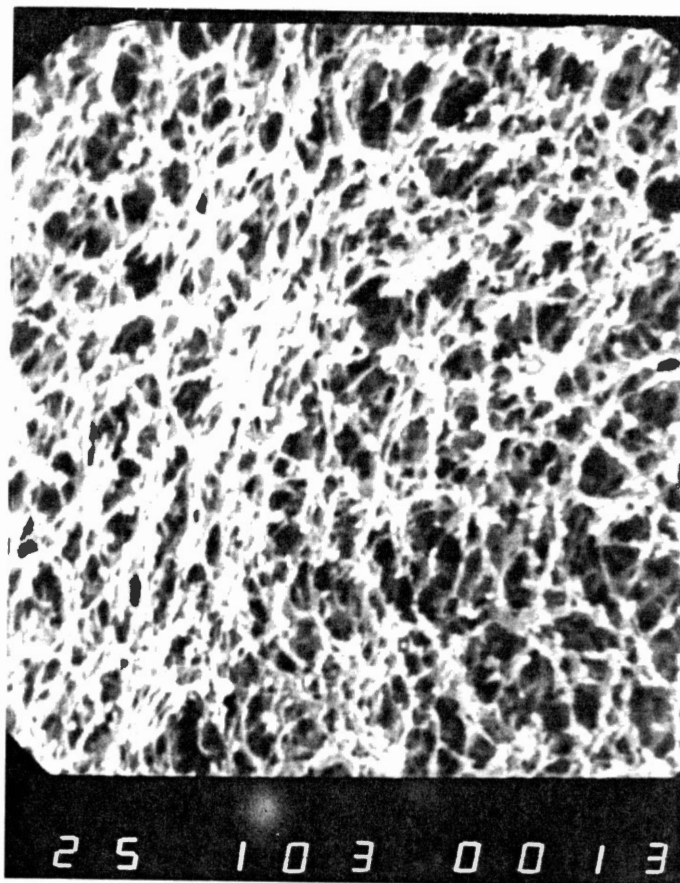


Figure 30. SEM of Unseeded Chitosan Matrix (10,000 X)

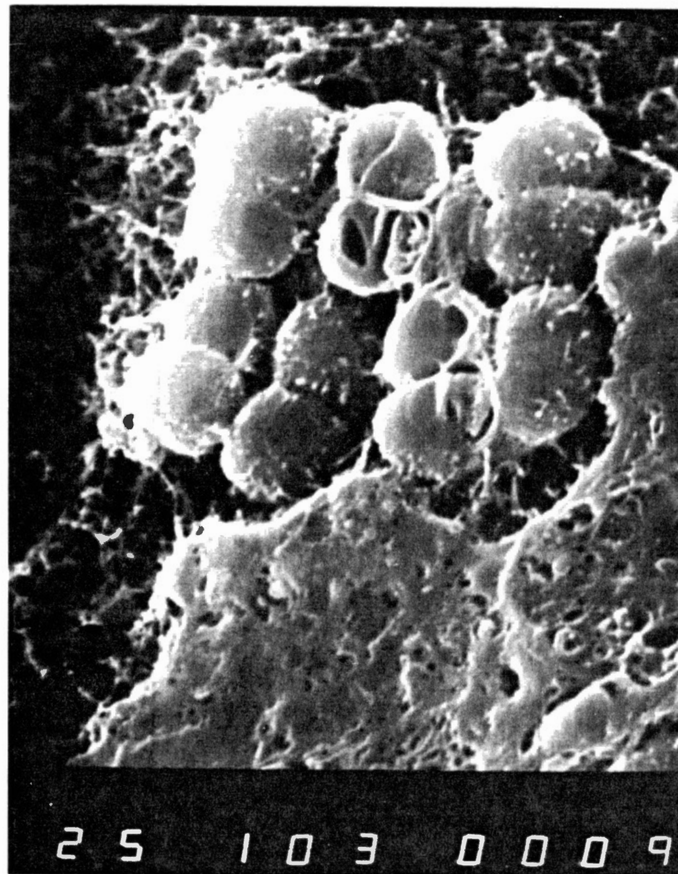


Figure 31. SEM of Seeded Chitosan Matrix (10,000 X)

CHAPTER V

DISCUSSION

Scope

This research was carried out to explore the possibility of applying chitosan biocatalysts in a bench-scale complete mix reactor (ICR) to treat a complex wastewater containing 2,4 TDA. The performance of this reactor was monitored against a control bench-scale ASR operated under similar conditions. The experiments were conducted in two phases.

In the first phase, the HRT was fixed at 10 hours and organic loading was varied by varying the feed TDA concentration. The TDA concentration was increased up to a point at which a significant drop in removal efficiencies of TDA, TCOD and SCOD was noted.

The second series of experiments was carried out at a fixed feed concentration. In this phase, the HRTs were lowered by increasing the feed flow rates. The loading rates were increased upto a point at which substrate removal efficiencies deteriorated. Data for each step of both experimental stages were obtained during the transition period between steps as well as when steady state conditions were established.

The main objective of this chapter is to present the highlights of the study based on the results given in the previous chapter and thereby arrive at some meaningful conclusion regarding the overall performance of, chiefly, the ICR. An attempt is made to draw relationships between results reported by previous researchers (see Chapter II) and those obtained here. The operating conditions for each reactor, sustenance of the reactors at high

TDA / hydraulic loading rates and then susceptibility to failure conditions are discussed in the following sections.

Performance of the Immobilized Cell Reactor

TCOD readings were significantly affected by liquid phase biomass that was continuously washed out directly from the reactor. The occurrence of this problem has been reported earlier by Yang and Wang (1990). Therefore, as far as COD is concerned, it is more appropriate to consider only SCOD removal as a comparison tool with the ASR, instead of both TCOD and SCOD.

In the organic loading study, the ICR' s TDA removal efficiency fluctuated in the range of 21 - 32 %, except at a TDA concentration range of 250 mg/L, where, a significant decrease to about 9 % occurred. The SCOD removal efficiency decreased from 86 % to 74 % as the TDA concentration increased to 250 mg/L. On the other hand, the TDA and SCOD removal efficiencies of the ASR underwent relatively drastic changes. TDA removal efficiency dropped from 35 % to 1 % as feed TDA concentration increased from 50 to 250 mg/L while the SCOD removal efficiency decreased from 80 % (at 50 mg/L TDA) to 30 % (at 250 mg/L TDA). The increased TDA feed concentration affected the ASR more, in terms of SCOD and TDA removal, than it did the ICR. This could be also due to the protection offered the immobilized cells by the chitosan matrix. It may be seen (Table X) that in the above period, the DO activity of beads was mostly 0.18 or 0.19 mg/L.min.bead. A sudden drop to 0.07 mg/L.min.bead (at 100 mg/L influent TDA) could have occurred because, for the first time, the immobilized bacteria previously acclimated to 50 mg/L TDA in the stock culture were subjected to twice the concentration. However, the DO activity at 250 mg/L (0.12 mg/L.min.bead) is also low compared to the other readings; but, this is consistent with the decrease in both SCOD and TDA removal efficiencies. Thus, DO

activity may be related to cell activity and treatment performance. In comparison, DO uptake rates in the range 0.01 - 0.08 mg/L.min.bead were reported by Sofer et al (1990).

The effluent SS and pH readings played a very important role in the daily operation of the reactors. It may be seen from Figures 12 and 13, that an increase in SS preceded or followed bead disintegration. Similarly, a drop in effluent pH indicated imminent dissolution of beads. It was observed that a sharp increase in SS and a decrease in pH occurred about the same time. This may be deduced by comparing the TSS, VSS and pH profiles given in Figures 12 - 14. From Table IX, it can be seen that the chitosan beads remained structurally stable for an average duration of 27 days (Minimum.: 13 days; Maximum.: 36 days).Operation of the ICR was terminated at 250 mg/L influent TDA concentration when TDA removal was almost negligible in the ASR; even though, at this point, the ICR showed 10 times more TDA removal than the control ASR. The color of the liquid phase turned to a deep greenish-brown color at this concentration. This could be due to the production of some by-product.

In the second phase, at constant influent TDA concentration of 150 mg/L and decreasing HRTs, TDA removal efficiency increased from 8 to 14 % as HRT decreased from 8 to 5 hours. However, at the next HRT, it dropped again to 10 %. SCOD removal efficiency decreased from 76 % to only 73 % and then fell to 38 %. In this period, the beads did not show any signs of damage and lasted for 24 days. The average lifespan of the beads in this phase was about 17 days (Maximum: 24 days; Minimum: 10 days). At 5 hour HRT, for the first time in the entire study, the beads clearly exhibited surface biomass growth. It is proposed that the surface biomass helped in reducing toxicity to cells within the matrix as well as protecting the matrix itself; thus increasing the treatment efficiency and structural life of beads. This is evidenced by the increase in TDA removal efficiency at the 5 hour HRT. However, further studies are required to confirm this hypothesis. The possibility of protective surface biomass growth that reduces toxicity to entrapped cells was also suggested by Bettmann and Rehm (1984). The drop in both TDA and SCOD removal

efficiency at 3 hour HRT clearly indicates failure conditions. This is further supported by the sharp drop in DO depletion from 0.12 to 0.04 mg/L.min.bead.

In a concurrent study conducted in this lab, with calcium alginate beads under similar conditions as used in this study, it was demonstrated that the concentration of the liquid phase biomass was negligible compared to the immobilized biomass (Chaubey, 1992). In this test, the alginate matrix was dissolved in sodium hexa-metaphosphate releasing the immobilized cells into suspension (see Chapter II). Then, the TSS and VSS of the suspension were determined. Unlike alginate, chitosan could not be dissolved; precluding the performance of the above test. However, the above finding may be extrapolated for chitosan beads. Thus, the effect of suspended cells on the treatment efficiency of the ICR may be ignored. It was noted by Bettmann and Rehm (1984) that, in practical applications of immobilized cells for wastewater treatment, cell growth in the liquid phase was not important.

The size of the chitosan beads could not be controlled due to the shrinking effect during the curing process. Similarly, the mechanical strength of beads, tested by crushing the beads between two fingers, varied everytime the beads were made. It is evident from Tables IX and XIV and Figures 14 and 25 that bead breakage was preceded by a decrease in pH to below 7.8. This suggests a strong connection between pH conditions in the reactor and bead life; this aspect need to be evaluated further. Throughout the study, beads were sustained in continuous motion by just aeration; the magnetic stirrer was used for short periods only when beads accumulated in a corner of the vessel. Thus, the possibility of bead damage occurring due to prolonged mechanical stirring may be ignored.

The TSS, VSS and pH profiles shown in Table XIV and Figures 23 - 25 indicate bead damage and subsequent debris / cell washout. It is seen that when the new beads were installed, the effluent solids decreased while the effluent pH stabilized at pHs greater than 7.8. There was no significant change in the SCOD and TDA removal efficiencies after the new beads were installed.

Performance of the Activated Sludge Reactor

Even though the TDA removal efficiency of the ASR in the organic loading study started off comparably (to the ICR at 50 mg/L) at 35 %, it began to drop rapidly to 1 % TDA removal efficiency at 250 mg/L at which point this run was stopped. Similarly, with respect to SCOD removal also, a large difference between the removal efficiencies of the two systems started to appear at 150 mg/L TDA influent concentration; SCOD removal efficiency for the ASR decreased from 80 % (at 50 mg/L TDA) to just 30 % at 250 mg/L TDA. From Table VIII, it can be seen that this is significantly less than for the ICR. The effluent solids and pH were fairly stable in this run; this was expected since the dilution rate was very low (HRT = 10 hours) and the settler performed quite efficiently.

In the hydraulic loading study, TDA removal efficiency increased from 3 % (at 8 hour HRT) and stabilized at 8 % for the remaining two runs while the SCOD removal efficiency decreased from 68 % (at 8 hour HRT) through 66 % (at 5 hour HRT) to 37 % (at 3 hour HRT); the last value being almost identical to that of the ICR at 3 hour HRT. TDA removal efficiency in the first run was lower than in the last two runs. This could be due to a residual effect on the bacterial cells caused by the 250 mg/L TDA concentration in the previous phase. The ASR started exhibiting tendency to washout at 5 hour HRT considering that the effluent VSS increased from 28 mg /L at 8 hour HRT to 155 mg/L at 5 hour HRT. Three days after the start of the 3 hour HRT run, the reactor was shut down due to biomass washout and vessel overflow caused by clogging of the effluent port by biomass.

In a study conducted by El Gamal (1985) using the same type of bench-scale ASR unit used in the present research, similar operational problems were reported. A multiple carbon source synthetic wastewater and a mixed culture were used in this study also. It was reported that, at SRT = 6 days, the mixed liquor had changed to a " white milky " color. In this situation, the clarifiers were not able to handle the dispersed and non-flocculated solids

and consequently, effluent solids had increased. Sludge-wasting was stopped and the dose of nitrogen source was increased, to no avail. Controlling the six day SRT was found to be difficult. The mixed liquor further changed in color to a yellowish tinge at SRT = 3 days (El Gamal, 1985). A very similar situation was encountered in the present study also. It is probable that this is typical of a complex waste. Further investigations are required to resolve this problem.

Comparison of ICR and ASR Performances

The performances of the two reactors may be compared using specific substrate removal rates (substrate removed / unit reactor volume . day). Table XVI shows the TDA, TCOD and SCOD loading and removal rates of the two reactors for the entire study. All the loading and removal rates given in Table XVI were calculated based on steady-state data. Corresponding comparitive plots are illustrated in Figures 32 - 34.

It is seen from Table XVI that the ICR consistently performed equal to or better than the ASR at all loading rates with respect to TDA and SCOD removal. The ASR offered higher TCOD removal rates than the ICR at loading rates of 2.65 - 6.90 g/L.d. Obviously, the TCOD removal rates in the ICR at these loading rates were affected by the matrix debris and biomass in the effluent. The specific substrate removal parameter in terms of unit biomass loading (substrate removed / unit time . unit cell mass) was not employed for comparitive purposes since the cell density is constantly changing even under apparent steady-state conditions (Venkatasubramanian et al , 1983).

In summary, comparing the overall performance of both the systems, it appears that the ICR system is equal to or better than the ASR system in terms of TDA, TCOD and SCOD removal.The ASR system is obviously better at high HRTs in controlling effluent solids due to the presence of a clarifier. The ICR performed better than the ASR in the organic loading study. This could mean immobilized cells were able to withstand toxicity

better than free cells in the ASR. Under increased hydraulic loading, taking into account that the ICR does not have a settler, it may be concluded that it has performed better than the ASR; although, there is no significant difference in removal efficiencies achieved. It can also be seen from the results (particularly,figures of treatment profiles) that the ICR achieved steady-state sooner than the ASR did, when loadings, both organic and hydraulic, were stepped up.

Both the ICR and ASR gave the respective highest TDA removal rates at the highest loading rate i.e. 1.20 g/L.d (Table XVI). Due to the high flow rates existing at the above loading rate, product accumulation in the vessel would have been the lowest. This, combined with the fact that TDA removal efficiencies at 250 mg/L TDA loading were extremely low in both units (less than 10 %) could signify that at least one of the byproducts was toxic and therefore detrimental to reactor performance.

In Figures 32 - 34, a large drop in removal rates is seen between loading numbers 4 and 6 (150 - 250 mg/L influent TDA). In this period, both systems appear to be stressed. However, at higher loading rates, the ICR and ASR seem to have recovered. This erratic behavior could be due to toxic conditions in the reactor. The ASR was more severely affected by the adverse conditions than the ICR; especially in terms of SCOD removal. This observation increases the probability of product toxicity especially since both reactors are kinetically complete-mix reactors.

Shear forces in the ICR, as mentioned earlier, are high. This factor could have adversely affected bead stability. Other possible reasons for the short life-span of chitosan beads are, (a) biomass overloading and, (b) internal cell growth - both of which could not be quantified in this study.

Comparing the treatment efficiencies of both reactors with respect to TDA and COD removal, it is obvious that TDA removal was relative low. Apparently, the readily biodegradable constituents of the base-mix were preferred to TDA. It is also possible that TDA biodegradability is limited under aerobic conditions. But, the exact relationship

TABLE XVI

COMPARISON OF SUBSTRATE REMOVAL RATES IN ICR AND ASR

Reactor Type	Loading No.	TDA Loading Rate (g/L.d)	TDA Removal Rate (g/L.d)	COD Loading Rate (g/L.d)		COD Removal Rate (g/L.d)		Remarks
				TCOD	SCOD	TCOD	SCOD	
ICR	1	0.12	0.04	1.50	1.44	1.09	1.24	
	2	0.23	0.06	1.90	1.84	1.43	1.52	
	3	0.37	0.08	2.10	2.06	1.40	1.69	
	4	0.48	0.12	2.22	2.18	1.26	1.72	
	5	0.60	0.05	2.25	2.20	1.07	1.63	Nos. 1-5
	6	0.46	0.04	2.65	2.58	1.36	1.95	correspond
	7	0.72	0.10	4.19	4.03	2.34	2.94	to organic
	8	1.20	0.12	6.90	6.76	1.58	2.62	loading study
								(Table VI).
ASR	1	0.12	0.04	1.50	1.44	1.06	1.15	Nos. 6-8
	2	0.23	0.06	1.90	1.84	1.37	1.43	correspond
	3	0.37	0.04	2.10	2.06	1.34	1.48	to hydraulic
	4	0.48	0.05	2.22	2.18	1.06	1.12	loading study
	5	0.60	0.01	2.25	2.20	0.58	0.66	(Table XI)
	6	0.46	0.01	2.65	2.58	1.47	1.73	
	7	0.72	0.06	4.19	4.03	2.36	2.64	
	8	1.20	0.09 *	6.90	6.76	1.65 *	2.60 *	

* Not steady state data due to reactor failure

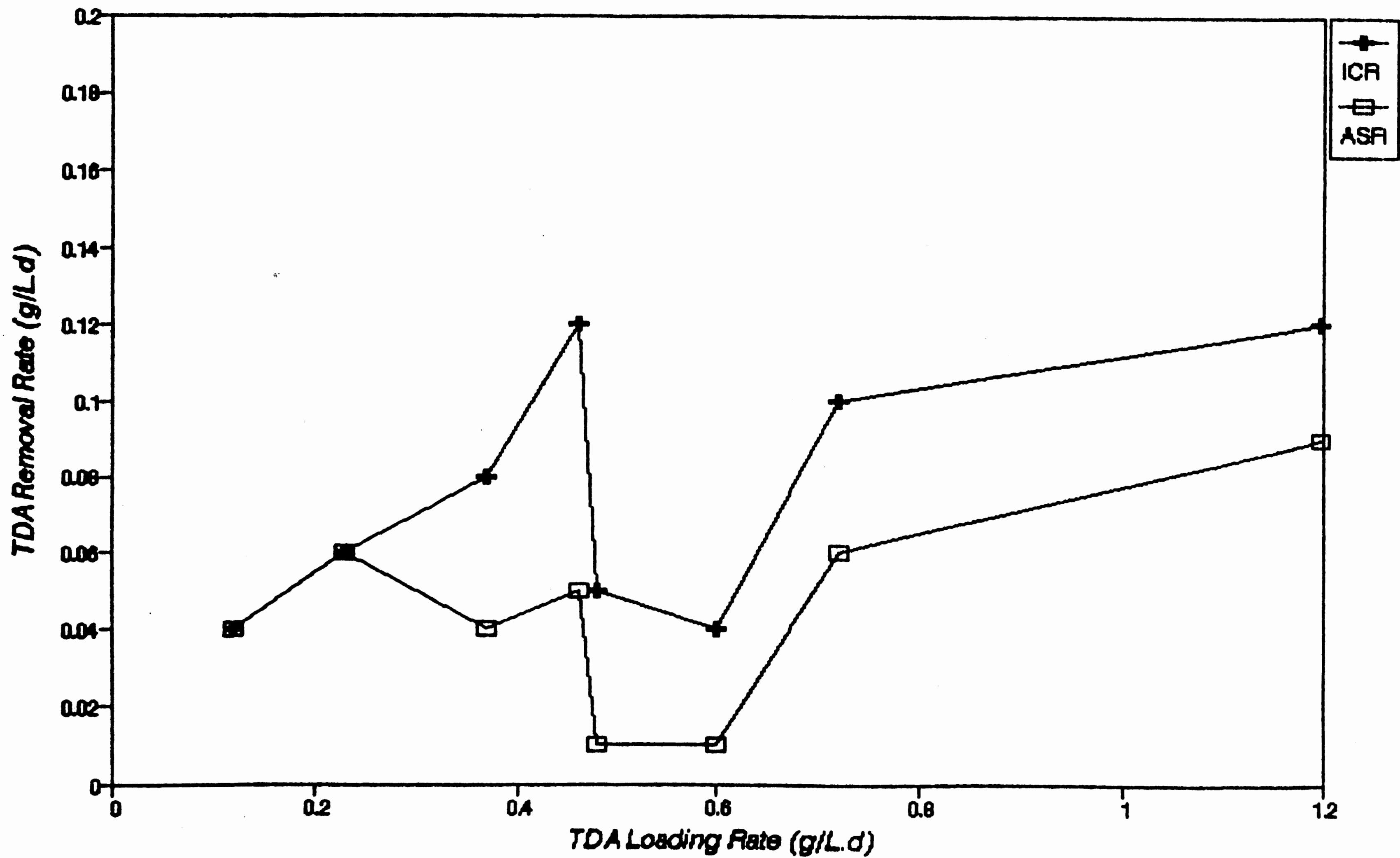


Figure 32. Comparitive Loading and Removal Rates for ICR and ASR

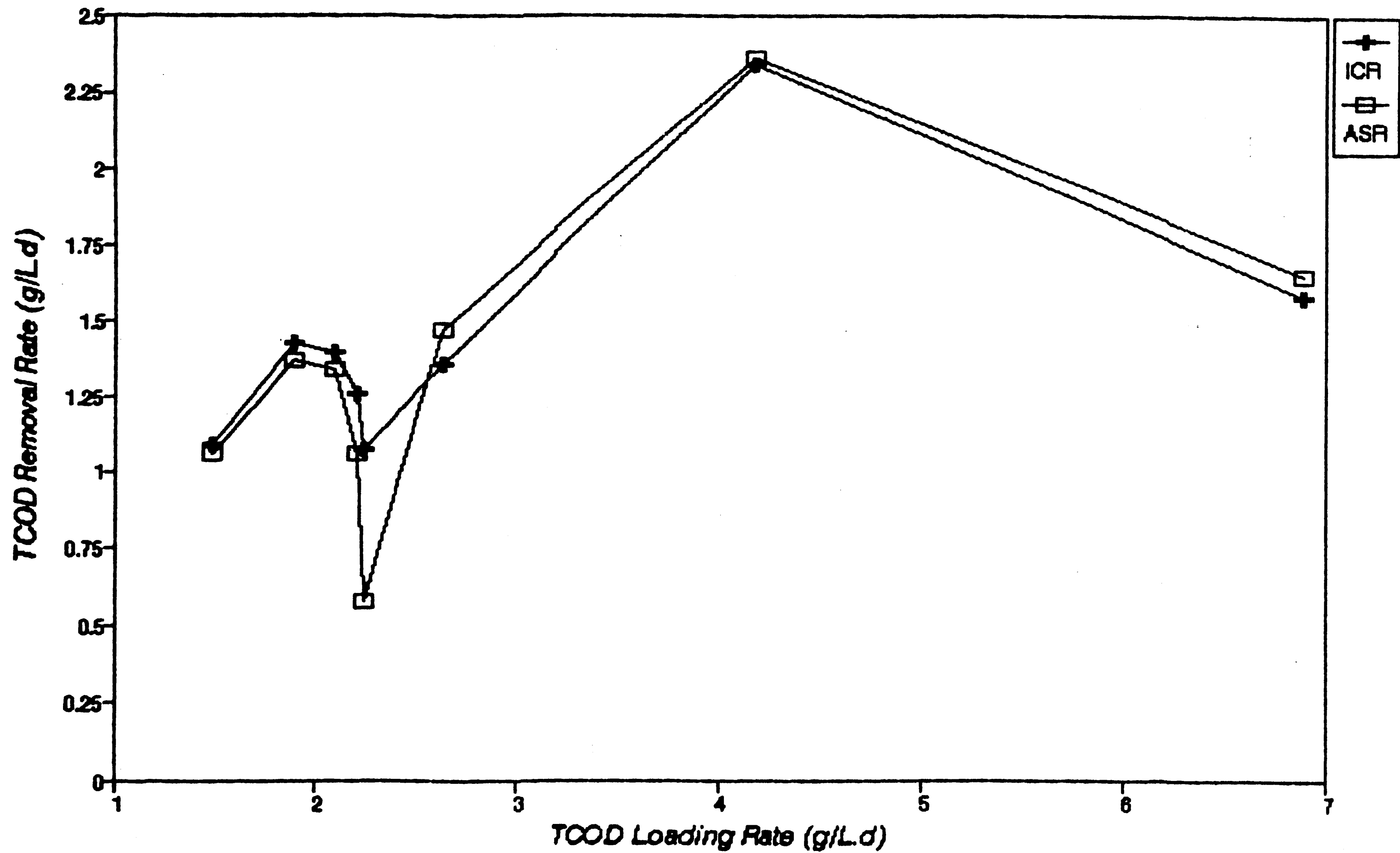


Figure 33. Comparitive TCOD Loading and Removal Rates for ICR and ASR

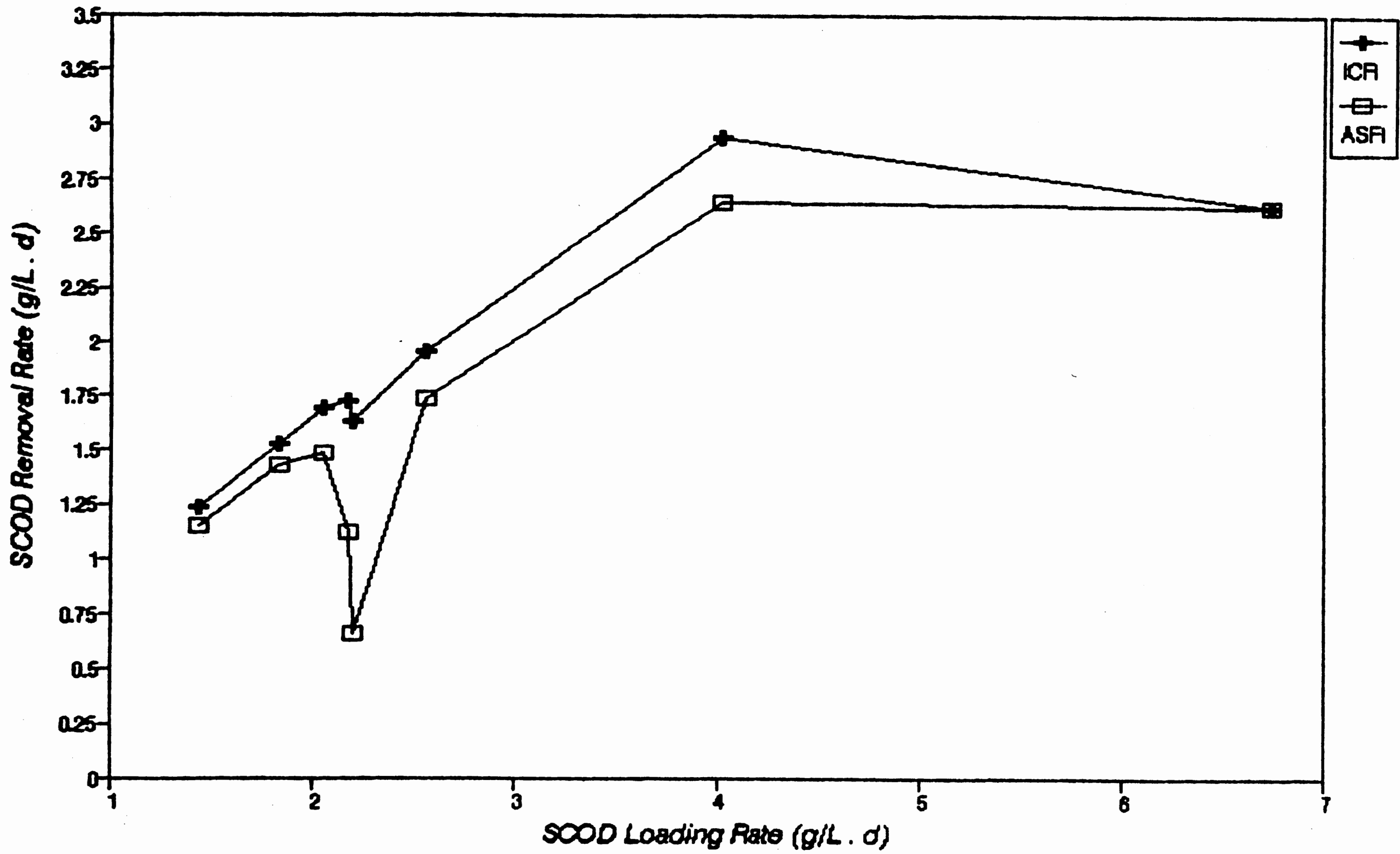


Figure 34. Comparitive SCOD Loading and Removal Rates for ICR and ASR

between TDA and COD removal patterns, were not analyzed and is definitely a basis for future research. Preliminary adsorption studies using chitosan beads without entrapped biomass at 50 and 100 mg/L TDA concentrations showed that adsorption of TDA was negligible. Previous literature on the various properties of 2,4 TDA reports that other types of abiotic removal mechanisms may be ignored (Howard, 1989). Consequently, it is proposed that the dominant TDA removal mechanism was biodegradation. However, more rigorous tests must be conducted in future in order to verify this result.

Discussion on Supplementary Experiments

The micrographs of the chitosan matrix shown in Figures 28 and 30 clearly reveal its highly porous nature. Figures 29 and 30 depict the entrapped cells that were identified previously (see Appendix I). Besides the cells, matrix and biomass debris are also visible in the above micrographs. From these micrographs, it may be seen that the cell size is larger than the pore size; which is a requisite for retaining the cells within the matrix. However, due to the repeated washing and fragmentation procedures involved in preparing the beads for SEM, it is possible that entrapped cells were lost. This possibility may explain the relatively few cells (compared to the amount of biomass originally immobilized) that are seen in the figures. Even though uniform cell distribution is assumed (Venkatasubramanian et al, 1983) for explaining mass transfer within the biocatalyst, it is clear from the figures that the cells are rather unevenly distributed. It is not known whether the preparatory prcodedures had any effect on the cell distribution.

The change in the nature of the mixed liquor in the ASR could be due to the production of extracellular polysaccharides or polymers - a phenomenon typical of many species, including Pseudomonas sp. It is known from the bacterial identification tests that the Pseudomonas sp. present in the culture store PHB, a thermo-plastic polyester. Pseudomonas sp. are also known to produce polysaccharides such as alginate.These

biopolymers are often produced in response to specific enviromental conditions such as excess of carbon sources such as glucose, ethanol, organic acids; under high dilution rates; or nitrogen / phosphorus limitations. It is suspected this production affected the nature of the biomass including its settleability and color (Brierley et al, 1985).

It is a well-documented fact that, dominant species in mixed bacterial cultures are subject to change (Grady and Lim, 1980). In the present study, it should be noted that the identification of species was conducted on the acclimated stock culture. It is possible that the mixed culture underwent species changes during the study since the experiments were conducted in a non-sterile environment. The changes in the nature of the mixed liquour described previously could have been influenced by specific changes in the microbial population.

CHAPTER VI

CONCLUSIONS

In summary, the important findings of this study may be enumerated as follows:

1. The application of a chitosan-immobilized heterogeneous bacterial culture for treating complex synthetic industrial wastewater was successfully demonstrated.

2. The highest TDA removal rate achieved in this study was 0.12 g / L.d with the ICR: once at constant HRT of 10 hours and influent TDA concentration of 200 mg / L and again at HRT of 3 hours and influent TDA concentration of 150 mg/L.

3. The highest SCOD removal rate achieved was 2.94 g / L.d with the ICR at influent TDA of 150 mg / L and HRT of 5 hours.

4. In contrast, the highest TDA removal rate seen in the ASR was 0.09 g / L.d at 150 mg / L influent TDA concentration and HRT of 3 hours; the highest SCOD removal rate achieved with this system was 2.64 g / L.d at influent TDA concentration of 150 mg /L and HRT of 5 hours.

5. The maximum TDA and SCOD removal efficiencies achieved in the ICR were 32 % and 86 %, respectively. The corresponding values for the ASR were 35 % and 80 %, respectively.

6. The highest TDA removal efficiency achieved was only 35 % (in the ASR at 10 hour HRT and 50 mg/L TDA concentration) while a maximum of 86 % SCOD removal was achieved (in the ICR at 10 hour HRT and 50 mg./L TDA concentration). The large difference between the maximum TDA and SCOD removal efficiencies achieved

in this research could mean that TDA removal was inhibited in the presence of the readily-biodegradable constituents in the base-mix.

7. At 250 mg/ L influent TDA concentration, TDA removal efficiency in both reactors was very poor; this could be due to either substrate or product toxicity.
8. Interestingly, at 3 hour HRT in the hydraulic loading study, both the reactors produced the highest TDA removal rates (ICR: 0.12 g/L.d and ASR: 0.09 g/L.d). This fact coupled with the above strongly suggests product toxicity.
9. The longest life span achieved for chitosan beads was 36 days (in the hydraulic loading study during 5 and 3 hour HRT runs) with an average durability of approximately 23 days.
10. The disintegration of chitosan beads is usually preceded or immediately accompanied by a drop in the effluent pH.
11. Operational control of bench-scale ASR at SRT of 6 days using the complex substrate was difficult and resulted in biomass with poor settling and agglomerating properties.
12. The overall treatment efficiency of the ICR in terms of TDA and SCOD removal was equal or superior to that of the ASR.

CHAPTER VII

SIGNIFICANCE OF WORK AND FUTURE RESEARCH NEEDS

Significance of work

This is the first ever study on the application of chitosan-immobilized activated sludge for treatment of a synthetic industrial wastewater. A bench-scale activated sludge reactor of a much larger volume than the immobilized cell unit was used as a control under similar conditions. The raw material for making chitosan i.e. chitin, is a waste product of the sea-food processing industry and is thus easily available. Chitosan is relatively cheap compared to other immobilization matrices such as k-carrageenan, polyacrylamide, polyurethane etc. Therefore, this technique is economically viable. From the results of this study, it is clear that compact and simple reactors may be used for achieving a desired level of treatment efficiency compared to conventional free cell systems.

High TDA / COD loading rates can be obtained by either increasing the feed TDA / COD strength at a fixed HRT or by decreasing the HRTs for a given TDA / COD concentration. Therefore, initial and operating costs of a reactor could depend on feed concentration and HRTs for a given reactor volume. Even though immobilization technology has great potential benefits, it became clear from this study that the durability of the matrix will be an important factor in practical applications. This work also helped in identifying the areas where further study is required in order to develop this technique from an industry point-of-view.

Future Research Needs

Further research should focus on the following aspects to gain a better understanding of the whole process.

1. Design studies for maximizing the stability of chitosan biocatalysts, especially resistance to low pH enviroments. New composite matrices may be developed by combining chitosan with other polymers.
2. Mechanism of TDA biodegradation.
3. Interaction between various constituents of the wastewater and its possible effects on treatment.
4. Batch shaker-flask studies in order to evaluate maximum treatment possible with a given amount of biocatalysts.
5. Means to quantify the internal cell-growth occurring in the beads.
6. Technical solution for limiting or preventing solids washout from the ICR; for instance, reactor modification.
7. MS / GC analyses of effluent to help in identifying the byproducts of treatment.
8. More accurate and continual identification of the microbiology of the reactors to help understand the treatment mechanism and biochemical pathways.
9. Analyses for evolution of potential gaseous products to correlate with the stoichiometry of treatment.
10. Experiments with varying concentrations of base-mix for optimizing the process.
11. Application of TDA as the sole carbon source in batch and in continuous-flow studies to investigate potential substrate and product toxicity problems.
12. Evaluation of the effects of HRT over a wide range of influent TDA concentrations.
13. Need to correlate DO uptake by biocatalysts with viability and rate of activity.

14. Pilot-plant scale studies in order to identify and solve problems with large-scale biocatalyst manufacture, reactor design, external liquid-phase biomass growth and its effects etc.
15. Comparison of capital and O&M costs for ICR and ASR on requirements of biocatalyst manufacture, pumps, reactor volume etc for achieving a given treatment efficiency.

BIBLIOGRAPHY

Bergey's Manual of Determinative Bacteriology, 1980, Breed,R.S., Murray,E.G.D., and Smith,N.R. (Eds.), The Williams and Wilkins Company, Baltimore, Maryland.

Bettmann,H. and Rehm,H.-J., 1984, Degradation of Phenol by Polymer-entrapped Microorganisms, Applied Microbiology and Biotechnology, 20 : 285 - 290.

Brierley,C.L., Kelly,D.P., Seal,K.J. and Best,D.J., 1985, Materials and Biotechnology, In : Biotechnology - Principles and Applications, Higgins,I.J., Best,D.J. and Jones,J.,(Eds),Blackwell Scientific Publications, Boston, Massachussetts.

Brodelius,P. and Vandamme,E.J., 1988, Immobilized Cell Systems, In: Comprehensive Biotechnology, vol. 7a, Rehm, H. -J. and Reed,G., (Eds.), VCH Publishers, New York.

Chaubey, R., 1992, Biological Treatment of Industrial Wastewater using Bacteria Immobilized in Alginate Polymer Matrix, M.S.Thesis, Oklahoma State University, Stillwater.

Cochet,N., Lebeault,J.M. and Vijayalakshmi,M.A., 1990, Physicochemical Aspects of Cell Adsorption, In: Wastewater Treatment by Immobilized Cells, Tyagi,R.D. and Vembu,K., (Eds.), CRC Press, Boca Raton, Florida.

de la Noue,J. and Proulx,D., 1988, Biological Tertiary Treatment of Urban Wastewaters with Chitosan-immobilized Phormidium, Applied Microbiology and Biotechnology, 29 : 292 - 297.

Dolan, J.W. and Snyder,L.R., 1989, Troubleshooting LC Systems - A Comprehensive Approach to Troubleshooting LC Equipment and Separations, The Humana Press, Clifton, New Jersey.

El-Gamal,H.F., 1985, The Effect of Food : Microorganism Ratio and SRT in Operating and Controlling an Activated Sludge Process, M.S. Thesis, Oklahoma State University, Stillwater.

Gaudy,A.F., and Gaudy,E.T., 1988, Elements of Bioenvironmental Engineering, Engineering Press,Inc., San Jose, California.

Gosmann,B. and Rehm,H.-J., 1988, Influence of Growth Behavior and Physiology of Alginate-entrapped Microorganisms on th Oxygen Consumption, Applied Microbiology and Biotechnology, 29 : 554 - 559.

Grady,C.P.L., and Lim,H.C., 1980, Biological Wastewater Treatment, Marcel Dekker,Inc., New York.

HACH Handbook for Waste Analysis, 1991, 2 nd Edition, HACH Co., Loveland, Colorado, 127 - 130.

HACH Products for Analysis Catalog, 1991, HACH Co., Loveland, Colorado, 58 - 59.

Howard,P.H., 1989, Handbook of Environmental Fate and Exposure Data for Organic Chemicals, vol.I (Large Production and Priority Pollutants), Lewis Publishers, Chelsea, Michigan.

Jeuniaux,C., Bussers,J.C., Voss-Foucart,M.F., and Poulicek,M., 1986, Chitin Production by Animals and Natural Communities in Marine Environment, In: Chitin in Nature and Technology, Muzzarelli,R., Jeuniaux,C. and Gooday,G.W., (Eds.), Plenum Press, New York.

Kennedy,J.F. and Cabral,J.M.S., 1983, Methods for the Immobilization of Microbial Cells, In : Immobilized Microbial Cells, vol.4, Chibata,I. and Wingard,L.B.Jr.

Keweloh,H., Heipieper,H. -J. and Rehm,H. -J, 1989, Protection of Bacteria against Toxicity of Phenol by Immobilization in Calcium Alginate, Applied Microbiology and Biotechnology, 31 : 383 - 389.

Klein,J. and Wagner,F., 1983, Methods for the Immobilization of Microbial Cells, In : Immobilized Microbial Cells, vol.4, Chibata,I. and Wingard,L.B.Jr.(Eds.), Applied Biochemistry and Bioengineering, Academic Press,Inc., New York.

Lawrence,A.W. and McCarty,P.L., 1970, Unified Basis for Biological Treatment Design and Operation, J. of the Sanitary Engineering Division, Proceeding of the ASCE, 3 : 757 - 778.

Matsui,S., Murakami,T., Sasaki,T., Hirose,Y. and Iguma,Y., 1975, Activated Sludge Degradability of Organic Substances in the Wastewater of the Kashima Petroleum and Petrochemical Industrial Complex in Japan, Progress in Water Technology, 7 (3/4) : 645 - 659.

Mattiasson, B., 1986, Technological Processes for Biotechnological Utilizations of Microorganisms, In : Biotechnology : Potentials and Limitations, Silver,S. (Ed.), Life Sciences Research Report No.35, Springer-Verlag, Berlin.

Mattiasson,B., 1983, Immobilized Microbial Cells, In: Immobilized Cells and Organelles, vol.II, Mattiasson,B.,(Ed.), CRC Press, Inc., Boca Raton, Florida.

Pennington,J., 1991, Personal Communication with the SEM Laboratory Technician, Department of Veterinary Medicine, Oklahoma State University.

Product Information Material from Vanson Chemical Co.,Inc., Redmond, Washington.

Reynolds,T.D., 1982, Unit Operations and Processes in Environmental Engineering, PWS Publishers, A Division of Wadsworth,Inc., Belmont, California.

Rozich,A.F. and Colvin,R.J., 1986, Effects of Glucose on Phenol Biodegradation by Heterogeneous Populations, Biotechnology and Biengineering, 28 : 965 - 971.

Rozich,A.F., and Gaudy,A.F.Jr., 1985, Response of Phenol-acclimated Activated Sludge Process to Quantitative Shock Loading, J. of Water Pollution Control Federation, 57 (7) : 795 - 804.

Snyder,R.C. and Breder,C.V., 1982, High Performance Liquid Chromatographic Determination of 2,4- and 2,6 - Toluenediamine in Aqueous Extracts, J. of Chromatography, 236 : 429 - 440.

Sofer,S.S., Lewandowski,G.A., Lodaya,M.P., Lakhwala,F.S., Yang,K.C. and Singh,M., 1990, Biodegradation of 2-Chlorophenol using Immobilized Activated Sludge, Research Journal of the Water Pollution Control Federation, 62 (1) : 73 - 80.

Stocklein,W., Eisgruber,A. and Schmidt,H. -L., 1983, Conversion of L-Phenylalanine to L-Tyrosine by Immobilized Bacteria, Biotechnology Letters, 5 (10) : 703 - 708.

Stover,E.L. and Kincannon,D.F., 1981, Proceedings of the 36th Purdue Industrial Waste Conference, May 1981, Ann Arbor Science, West Lafayette, Indiana,1 - 16.

Unger,P.D. and Friedman,M.A., 1979, High-Performance Liquid Chromatography of 2,6- and 2,4- Diaminotoluene and its Application to the Determination of 2,4,Diaminotoluene in Urine and Plasma, J. of Chromatography, 174 : 379 - 384.

Valo,R.J., Haggblom,M.M. and Salkinoja-Salonen,M.S., 1990, Bioremediation of Chlorophenol-containing Simulated Groundwater by Immobilized Bacteria, Water Research, 24 (2) : 253 - 258.

Venkatasubramanian,K. and Karkare,S.B., 1983, Process Engineering Considerations in the Development of Immobilized Living Cell Systems, In : Immobilized Cells and Organelles, vol.II, Mattiasson,B. (Ed.), CRC Press, Boca Raton, Florida.

Venkatasubramanian,K., Karakare,S.B. and Vieth,W.R., 1983, Chemical Engineering Analysis of Immobilized Cell Systems, In: Immobilized Microbial Cells, Chibata,I. and Wingard,L.B.Jr., (Eds.), Applied Biochemistry and Bioengineering, vol.4, Academic Press, Inc., New York.

Vorlop,K. -D. and Klein,J., 1981, Formation of Spherical Chitosan Biocatalysts by Ionotropic Gelation, Biotechnology Letters, 3 (1) : 9 - 14.

Vorlop,K. -D. and Klein,J., 1987, Entrapment of Microbial Cells in Chitosan, Methods in Enzymology, 135 : 259 - 268.

Wagner,F., Lang,S., Bang,W. -G., Vorlop,K. -D. and Klein,J.,1982, Production of L-Tryptophan with Immobilized Cells, Enzyme Engineering, 6 : 251 - 257.

Yang,P.Y. and Wang,W.L., 1990, Entrapment of Microbial Cells for Wastewater Treatment, In: Wastewater Treatment by Immobilized Cells, Tyagi, R.D. and Vembu,K., (Eds.), CRC Press, Boca Raton, Florida.

APPENDIX

RESULTS OF BACTERIAL IDENTIFICATION TESTS

S.No.	Test	Type		
		" WS "	" YS "	" YR "
1.	Gram	negative	negative	negative
2.	Shape	coccus	rod	rod
3.	Motility	non-motile	motile	motile
4.	Aerobic / Anaerobic	aerobic	aerobic	aerobic
5.	Catalase	positive	positive	positive
6.	Oxidase	negative	positive	positive
7.	Gel Liquefaction	negative	positive	positive
8.	Denitrification	none	reduces NO_3 to NO_2	denitrifies NO_3 to N_2
9.	Indole	negative	negative	negative
10.	Glucose Utilization	negative	positive	positive
11.	King's pH 7.2	negligible growth	excellent growth	excellent growth
12.	King's pH 4.5	negligible growth	good growth	negligible growth
13.	PHB Storage Granules	negative	positive	positive
14.	Growth at 30°C	positive	positive	positive

APPENDIX (Continued)

15.	Growth at 37°C	negative	negligible	positive
16.	Flourescence	negative	negative	negative
17.	Resistance to Penicillin	high	low	low

VITA

Suresh Subramanian

Candidate for the Degree of

Master of Science

Thesis: BIOLOGICAL TREATMENT OF A SIMULATED INDUSTRIAL WASTEWATER USING CHITOSAN - IMMOBILIZED ACTIVATED SLUDGE

Major Field: Environmental Engineering

Biographical:

Personal Data: Born in Calicut, Kerala State, India, March 26, 1966, the son of M. Subramanian and Kamala Subramanian.

Education: Graduated from St. Mary's Higher Secondary School, Madras, Tamil Nadu State, India, in 1984; received Bachelor of Engineering (Hons.) Degree in Civil Engineering from Birla Institute of Technology and Science, Pilani, Rajasthan State, India, in 1988; completed requirements for the Master of Science degree at Oklahoma State University in July, 1992.

Professional Experience: Engineer Trainee, Hindustan Motors Private Ltd., Madras, India, from May, 1986, to July, 1986; Design Engineer, Development Consultants Private Ltd., Calcutta, India, from January, 1988, to July, 1988; Site Engineer, M/s Kotaiah and Co., Engineers and Contractors, Madras, India, from August, 1988, to June 1989; Teaching Assistant, Department of Civil Engineering, Oklahoma State University, from August, 1989, to May, 1990; Research Assistant, Department of Civil Engineering, Oklahoma State University, from August, 1990, to December, 1991.