Wastewater Treatment by Immobilized Cells

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PREFACE

Biological wastewater treatment has been practiced in many forms since the early part of the 20th century. However, immobilized cell processes have only recently been intensively studied and applied by water pollution abatement researchers and engineers. Because of recognized attributes over suspended growth process, today there is greater interest in immobilized cell treatment processes.

There exists a need to provide up-to-date and pertinent scientific information concerning immobilized cell processes for the treatment of wastewater to the worldwide community of engineers, planners, academicians, scientists, researchers, consultants, students, and sewage treatment plant operators. This publication is an attempt to fulfill the need and an outgrowth of literature dedicated to the state-of-knowledge in wastewater treatment. We have tried in this book to penetrate as broad a perspective as possible. In the construction of the chapters, we have also left much up to the individual contributors. Some chapters have been written as essentially the reviews of an application of immobilized cell technology while others have used data obtained in the author's own laboratory to illustrate the use of immobilized cell technology. Yet others have concentrated on specific topics and cited a few key ways in which immobilized system could be exploited.

Essential information on the feasibility of various immobilization methods has been reviewed and examined with special reference to wastewater treatment. Included are the stability of various supports (inorganic and organic), techniques used for microbial attachment (involving experimental procedures), factors affecting affinity for the support, strength of fixation, nature of polymers, role of radical groups, properties of attached microorganisms, effects of carriers on settling properties of biomass, characteristics of biofilm on carriers, and changes in cell metabolism as a result of immobilization.

The morphologies for the identification of immobilized cells, the methods of identification of structure and composition of microbial aggregates, and analytical methods for the estimate of biomass in the presence of carriers are discussed. Applications of immobilized cells to toxic wastes, anaerobic and aerobic systems, and operational criteria for different wastes are specified. The results of immobilized microalgae and cyanobacteria for wastewater treatment are reported and their future prospects are highlighted.

Various immobilized cell bioreactor configurations have been critically reviewed with respect to design and granulation process: biomass retention, resistance to washout, diffusional resistances, response to toxic shocks, theoretical aspects of hydrodynamic characteristics, start-up and steady-state process, selection of support particles, particle size and active biomass, head loss considerations, surface area, reactor liquid velocity, hydraulic retention time, aerobic vs. anaerobic systems, temperature and substrate concentration effects, metabolic interspecies transfer, stability, suspended solids and microbial film interactions, SRT requirements, and operational issues.

We wish to thank sincerely the authors of the chapters which comprise this publication. Any statement or views presented here are totally those of the authors.

R. D. Tyagi
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THE EDITORS

R. D. Tyagi, Ph.D., is professor of Biochemical Engineering at INRS-Eau, Université du Québec, Sainte Foy. Dr. Tyagi was graduated from the University of Delhi receiving a B.Sc. degree in 1971 and a B.Sc. (chemical technology) from Kanpur University in 1974. He received his Ph.D. (biochemical engineering) in 1979 from Indian Institute of Technology, Delhi. He served as a lecturer at the Indian Institute of Technology, Delhi, from 1979 to 1980 and 1982 to 1984 and as a scientist at the Université de Technologie de Compiegne, Compiegne (France) from 1980 to 1982. It was in 1984 that he assumed his present position.

Dr. Tyagi is a member of Chemical Institute of Canada, Canadian Society of Chemical Engineers, Canadian Association on Water Pollution Research and Control and Canadian Society of Microbiologists. He has received a merit award for outstanding research accomplishment from the Natural Sciences and Engineering Research Council of Canada (Civil Engineering) in 1988.

Dr. Tyagi's research interests revolve around wastewater treatment, bioreactor design and analysis, continuous fermentation, and bioconversion by immobilized cells.

Kannan Vembu, Ph.D. is Senior Consultant in the Biochemical Engineering Unit at Arthur D. Little, Inc., Cambridge, MA. He specializes in biotechnology and biological wastewater treatment processes.

Dr. Vembu received his Ph.D. in biochemical engineering in 1979 from I.I.T. Delhi, India. He has a B.S. in chemical engineering and an M.E. in chemical plant design. He joined the Technology Department of Hindustan Dorr-Oliver Ltd., Bombay, India in 1979. His area of specialization was biological wastewater treatment and design of solid-liquid separation systems. In 1984 he was appointed as Senior Development Engineer/Biotechnology Industry Specialist at Dorr-Oliver Inc., Stamford, CT. He was involved in the design of fixed film (fluidized bed) systems for wastewater treatment and development of new products for biotechnological applications. Prior to joining Dorr-Oliver Inc. he did postdoctoral research in biochemical engineering at Drexel University, Philadelphia.

Dr. Vembu has extensive experience in biological wastewater treatment, development of bioreactors for microbial, mammalian, and plant cell culturing, and design of solid-liquid separation systems. Currently, Dr. Vembu is consulting for biotechnology and environmental industries. His present interests include bioremediation of hazardous wastes, groundwater decontamination, bioleaching, and conversion of MSW to useful products. In biotechnology he is involved in the evaluation of process technologies, engineering feasibility, downstream processing systems, and market assessment.

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Chapter 1

PHYSICOCHEMICAL ASPECTS OF CELL ADSORPTION

N. Cochet, J. M. Lebeault, and M. A. Vijayalakshmi

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I. WHOLE CELL IMMOBILIZATION, A NATURAL PHENOMENON

The adsorption and immobilization of cells onto solid surfaces is a phenomenon omnipresent in nature, e.g., the fixation of microorganisms, on soil particles,[1] on sand particles or rocks, etc. As Marshall et al.[2] reported, this phenomenon has important ecological implications in terms of soil erosion, corrosion of metal, and pollution of water. In fact, Silverman and Munoz[3] have shown the acidic solubilization of the mineral supports by the adsorbed cellular layers. This solubilization is a mechanism through which the flora can make use of the inorganic salts.

Others have shown the presence of microbial films on the vegetable leaves and roots.[4] Dental plaque is another example of this type of microbial film formation.[5] One of the positive outcomes of microbial films is the depollution of s elts heavily polluted with petrol.

II. WHAT IS THE BASIC MECHANISM OF THE ADSORPTION?

At the outset, we can say that the adsorption of the cells depends on the surface properties of both the cell surface and the support to which these cells adhere.[5] Moreover, the two important characteristics involved are surface charge and surface energy.[6]

Many hypotheses concerning these properties are presented. Jones et al.[7] have shown that bacterial adhesion to a solid surface is based on extracellular

polysaccharides without any other special structural properties. Corpe[8] confirms that this adsorption is due to the charges on the bacterial cell walls. In 1971, Meadows,[9] based on experiments using different proteins as additives during the cell adsorption studies, has shown that the isoelectric point of the macromolecules, mainly proteins of the cell walls, play an important role in the adsorption to solid surfaces.

A similar mechanism based on the mucopolysaccharide layer of the animal cells is known for the animal cell aggregation.[10]

III. IMMOBILIZED CELLS AND THEIR POTENTIAL USES

The immobilized or adsorbed cell systems are used in certain traditional processes such as the production of vinegar. With the use of reactors containing immobilized cells, e.g., for the continuous production of alcohol, amino acids are already known.[11]

However, in the past 10 years, an interdisciplinary approach making use of physicochemical properties of the surfaces has allowed a better understanding of the mechanism of microbial cell immobilization.

The immobilized cell system has the following interesting traits:

- It allows the recycling of the biological catalyzers.
- It allows the reactor to function at very high cell concentration, without rheological or mass transfer limitations.
- There is a decrease in the metabolic regulation effect due to product accumulation.
- A better utilization of the substrate even at low concentration, thanks to the localized concentration of nutrients and hydrolytic coenzymes at the support-substrate/interface.
- The possibility of using the cells in their stationary phase where only the metabolic chains are active.

Compared to the immobilized enzymes, the immobilized cells present an advantage of the improved stability of the multienzymatic systems, added to the fact that no additional step of extraction and purification is needed.

Nevertheless, there are a few disadvantages of this system:[12]

- The undesirable side reactions.
- Inhibition of certain metabolic activities due either to product accumulation or some toxic substances accumulation.
- The diffusional limitation of the substrates, mainly those of high molecular weight. This is one of the major limitations in the case of entrapped cells.
- The cell leaking from the solid support.

Some of these limitations can be overcome, especially in the case of immobilization of nonviable cells, where the cells are used mainly as sources of catalysts in the bioconversion. The permeabilization of the cells using organic solvents (toluene or dimethyl sulfoxide) is a well-known phenomenon to improve the diffusion of substrates into these cells.[13]

IV. THE SOLID SUPPORTS IN WHOLE CELL IMMOBILIZATION

As mentioned earlier, the cell retention on a solid support depends to a great extent on the nature of the solid support, mainly the available surface, porosity, and its charge.

The stability of the cell retention depends on the same properties as cell leakage is often noticed while varying the pH, ionic strength, or even the dynamics of the ion exchange conditions by varying the flow of the liquid medium.

A. Inorganic Carriers

Although inorganic support materials have less reactive groups on their surfaces than organic supports, historically, they were most widely used for microbial attachment. They can be subdivided into ungrafted and grafted supports (inorganic materials with specific organic groups attached by various coupling agents to their surfaces). The techniques used for microbial attachment to inorganic carriers are adsorption and coupling. There are three major mechanisms responsible for cell attachment to inorganic support:

1. Electrostatic interactions between charged cells and charged carriers.
2. Partial covalent bond formed via replacement of hydroxyl groups on inorganic surfaces with amino or carboxyl groups on cell surface.
3. Covalent bond formation between ligands on the cell surface and specific organic groups, grafted to an inorganic surface. For this reaction, the inorganic surface should first be treated with a special coupling agent.

A great variety of inorganic supports such as sand, brick, glass, ceramics, mineral silicates, metal oxides, and magnetic particles were utilized as supports for microbial attachment. In spite of the fact that adsorption to an inorganic support represents the simplest and quickest procedure, the strength of cell attachment greatly depends on microbial cell wall composition as well as on carrier surface properties, and is affected by the pH and the ionic strength of the solution, cell age, surface charges, surface area of the support, and the carrier composition. Each of these factors will be discussed in detail.

However, according to Navarro and Durand[14] in their work on the immobilization of the *Saccharomyces uvarum* on brick particles, cell immobilization is mainly due to the compatibility of the pore sizes. They demonstrated

with the help of a mathematical model that the cells are preferentially fixed inside the pores having the same diameter as the strain.

1. pH Effect

The effect of pH on microbial attachment was studied by several authors. Yeast immobilization *(S. carlsbergensis)* onto three various supports (Kieselguhr, bentonite-H^+ and amino bentonite) were investigated. Navarro and Durand[14] found that Kieselguhr and bentonite-H^+ significantly adsorbed at pH = 3, but amino bentonite did the same at pH 5.0. These data suggest that a simple modification in pH induces an important variation of the carrier retention properties as a consequence of the particle microenvironment modification.

Marcipar et al.[15] studied adsorption of four various microorganisms (*Trichosporon* sp., *Rhodotorula* sp., *S. cerevisiase,* and *Candida tropicalis*) on the inorganic ceramic support as a function of pH 4 and 6. The cells of different microbial strains were adsorbed to the support from a standardized microbial suspension with defined microbial concentration. The results demonstrate that, although the rate of adsorption is rather specific for each microbial strain tested, the percentage of cell adsorbed was higher at pH 4.0. An almost 40% decrease in the amount of cell adsorbed was noticed at pH 6.0.

2. Effect of Ionic Strength

Ionic strength plays an important role in microbial adsorption. At equal pH values, an increase in ionic strength of the medium results in an increase in the percentage of cells adsorbed. At pH 4.0, in the absence of NaCl, the percentage of cells immobilized was 8.86%. With addition of NaCl to a final concentration of 2.0 *M*, the percentage of cells immobilized increased to 16.03%. An increase in microbial binding due to ionic strength was also observed at pH 6.0. These data confirmed that microbial attachment by adsorption depends both on pH as well as ionic strength of the solution. Even small modifications could result in microenvironmental changes which would affect charges on cell or support surface, ion-ion interaction, or support cell partial covalent bond formation, thereby modifying the adsorption.

3. Role of Support Surface Charges

The systematic exploration of the fixation of a number of microorganisms on the ion exchange resins by Rotman[16] in 1960 has shown that the chemical composition of the cell envelope plays an important role in the specific retention of the microorganism on a given resin. The differences in the composition of peptides, diamino acetic acid, and the hexosamines are the major constituents contributing to this selectivity. In fact, this author has used these resins for the cell separation as well as cell enrichment.

It is obvious that negatively charged bacterial or yeast cells could readily attach to the surfaces of positively charged supports such as DEAE-Sephadex® A-50, DEAE-Sephadex® A-25, Amberlite® IR-45 or Biorad® AG-21K, due to electrostatic interactions. However, it appears that those very negatively charged cells can be successfully attached to negatively charged inorganic carriers such as glass or ceramics. In order to understand the mechanisms involved in cell-carrier interactions, it is necessary to know the charge on the support surface.[17] This was done by studying the electrophoretic mobility of various supports using an electrophoretic mass transport analyzer. The basis of this method is based on the migration of the charged carrier particles into or out of the particles chamber, depending on the carrier charge and on the polarity of the chamber electrode. The change in the support weight in the chamber can be determined gravimetrically. From it, the electrophoretic mobility and ζ potential on the support surface can be calculated. It appeared that five inorganic carriers under investigation were negatively charged. However, the degree of those charges was quite different. The smallest charge was observed on the surface of fritted glass. Inorganic carriers designed as cordierite had a ζ potential almost equal to the natural support. However, zirconia-coated ceramic had almost six-times higher negative charge on its surface compared with other carriers. This carrier also exhibited the highest biomass accumulation for *Penicillium chrysogenum* and the biocatalyst formed was found to be stable during a long period of continuous column operation. These data are in correlation with high lactase enzyme loading on the zirconia-coated porous glass support, as was earlier observed.[18]

The high biomass accumulation of negatively charged cells on negatively charged supports suggests that charge-charge interactions cannot be the only mechanism involved in microbial attachment to an inorganic carrier.

4. Direct Bridge Formation between Inorganic Carrier and Cell

Nordin et al.[19] have studied the adhesion of *Chlorella* cells onto glass surfaces. They attribute the phenomenon mainly to electrostatic forces. However, Sulkowski[20] has shown in his systematic investigation that the combined hydrophobic and electrostatic forces are responsible for cell adhesion to glass surfaces.

One of the possible explanations for the attachment of negatively charged cells to negatively charged support may be partial covalent bond formation during immobilization. The formation of direct linkage between hydroxides of the transition metals (titanium, zirconium, iron, etc.) and microbial cells via chelation process was described elsewhere. Metallic hydroxides in aqueous solutions usually undergo hydrolysis and polymerization, with reduction of the charges on the surface. The degree of polymerization is inversely proportional to the pH. It was postulated that the mechanism of microbial immobilization on metallic hydroxides involved the replacement of hydroxyl groups on the surface of these compounds by carboxyl, hydroxyl, or amino

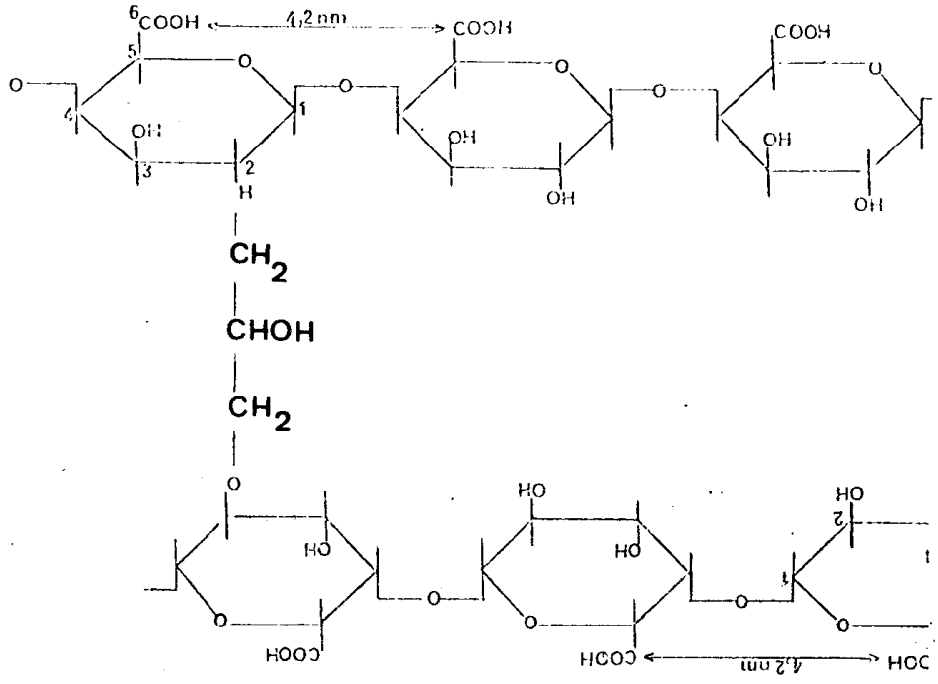

FIGURE 1. Cross-linked pectate.

groups on the cell surface. That suggests a binding through the mechanism of charge transfer between transition metal and microbial cell wall. The projected structure[21] of the complexes of metal hydroxide with amino groups is presented in Figure 1.

Escherichia coli, *S. cerevisiae*, *Serratia marcescens*, *Lactobacillus*, and *Acetobacter* cells were immobilized on metallic hydroxides.[22] Microbial cells, suspended in 0.9% (w/v) saline (A_{600} = 0.126) were mixed with a freshly prepared sample of metal hydroxide and stirred for 5 min at room temperature. The mixture was then allowed to stand and the suspension settled out, leaving clear supernatant (A_{600} = 0.222). Immobilized cell preparation was harvested by centrifugation. Attached cells were alive, which was confirmed by measurement of cell respiration. Kennedy et al.[22] proved that the microorganisms were attached to the surface of the hydroxide and not just trapped in the matrix. If microbial cells, so immobilized, were intended to be used in a continuous process at a pH range of 2.0 to 5.0, these authors recommended the use of titanium instead of zirconium hydroxide. Use of zirconium hydroxide was recommended for a pH range higher than 5.0.

5. *Effect of Support Composition*

Formation of a partial covalent bond as one of the mechanisms responsible for microbial attachment suggests the importance of support composition. Inorganic carriers are usually oxides, e.g., alumina, silica, magnesium, and titania.

TABLE 1
Physicochemical Characteristics of the Ceramic Carrier

Chemical composition (%)		Physical characteristics	
SiO_2	(57.7)	Real density	(2620 kg/m^3)
AlO_3	(38.1)	Water absorption	(3—5%)
FeO_3	(1.5)	Open porosity	(7—11%)
TiO_2	(1.4)	Porosity of the bed	(40—45%)
K_2O	(0.5)	Granulometry	(2—5 mm)
Na_2O	(0.1)		
CaO	(0.4)		
MgO	(0.1)		

Chemical composition is one of the ceramic supports, commercialized in France and used for yeast immobilization is presented in Table 1. Ceramics as well as glass are viewed as hard liquids; therefore, when placed into aqueous solution, the ion exchange usually occurs on their surfaces. Since microbial immobilization is performed in buffer solution on the surfaces of inorganic supports, instead of metal oxides, metal hydroxides are formed. The hydroxyl groups of metallic hydroxides can be replaced by suitable amino or carboxyl groups on the cell surface, just as it was previously described for the zirconia complex. As a result, a partial covalent bond is formed between cell and inorganic carrier.

Penicillium chrysogenum and *Streptomyces olivochromogenes* fungi were grown on a variety of inorganic supports, including silica, glass, cordierite, and zirconia ceramic.[17] Mycelial bioaccumulation on the supports was recorded after 24 and 48 h of growth. It appeared that *P. chrysogenum* preferred cordierite support over fritted glass. In contrast with that, *S. olivochromogenes* mycelium seemed to prefer fritted glass over cordierite after 24 h of incubation. Both fungi under investigation grew equally well on zirconia-coated ceramic. These data also suggest the great importance of support composition for mycelial bioaccumulation.

The logical question would be whether it is possible to increase the retention capacity of the carrier as well as long-term biocatalyst activity by incorporating specific metals into the carrier matrices.

B. Organic Carriers

Vijayalakshmi et al.[21] used cross-linked pectate as the polysaccharide backbone. Pectate particles were suspended in a 0.5 *M* $FeCl_3$ solution at pH = 5.0 for 24 h. Then the gel was washed free of excess transition metal. The Fe^{+3} content in the coupled support was found to be 500 μM/g dry gel.

Cross-linked pectate with and without a coupling agent (imino-diacetic acid) was used for derivatizing with iron. It was established that the backbone polysaccharide, with or without chelating agent and without Fe^{+3}, does not bind the yeast cells at all. However, the carrier with Fe^{+3} retains almost 50%