IMMOBILIZED MICROBIAL CELLS

K. Venkatsubramanian

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Immobilized Microbial Cells

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FOREWORD

The ACS SYMPOSIUM SERIES was founded in 1974 to provide a medium for publishing symposia quickly in book form. The format of the Series parallels that of the continuing ADVANCES IN CHEMISTRY SERIES except that in order to save time the papers are not typeset but are reproduced as they are submitted by the authors in camera-ready form. Papers are reviewed under the supervision of the Editors with the assistance of the Series Advisory Board and are selected to maintain the integrity of the symposia; however, verbatim reproductions of previously published papers are not accepted. Both reviews and reports of research are acceptable since symposia may embrace both types of presentation.

PREFACE

Biochemical processing with immobilized microbial cells represents a novel approach to biocatalysis. Such a system offers a number of unique advantages over traditional fermentation processes as well as the more recent immobilized enzyme processes. Although this concept is still relatively new, a few immobilized cell systems have already been commercialized. This, in turn, has triggered a surge of research activity in this exciting and rapidly growing field. Numerous conferences and symposia have been held on the subject of enzyme engineering in recent years. Although they contain a few papers on the subject of immobilized microbial cells, no single conference was devoted to covering this subject matter exclusively. Therefore, we organized a symposium on immobilized microbial cells as part of the 176th Annual Meeting of the American Chemical Society held at Miami Beach in September 1978.

This volume contains most of the papers presented at the symposium. In addition, several chapters written by leading experts in the field have also been included. Several important aspects of immobilized microbial cell technology are discussed here: carriers for immobilization, methods of cell attachment, biophysical and biochemical properties, reactor design, and process engineering of bound cell systems. A number of applications in the food, pharmaceutical, and medical areas—including those commercialized already—have been described. In essence, this is a comprehensive single volume state-of-the-art presentation of immobilized microbial cell systems.

The first chapter by Vieth and Venkatsubramanian provides a broad overview of the subject matter including the rationale for immobilizing microbial cells, the advantages and disadvantages of such an approach, and the overall prospects and problems of a technological development based on bound cell systems. The chapter by Messing and associates discusses the critical pore dimensions needed for fixing microorganisms inside various inorganic matrices. This is followed by an interesting discussion on the adhesive forces that come into play in fixed microbial systems.

A series of biochemical processes mediated by fixed cells are described next. They vary in complexity in terms of the number of individual enzymatic reactions, and coenzymes involved. Included in this section are descriptions of immobilized cell systems for producing coenzyme A, pantothenic acid, antibiotics, and extracellular enzymes. In

addition, waste treatment applications such as phenol degradation and denitrification are outlined.

Several important industrial applications are discussed next, starting with two commercial processes for the conversion of dextrose to fructose. The chapters by Bungard and co-workers and Roels and his associates describe two different approaches to this interesting commercial problem. Because of the commercial importance of this process, we have also included a paper by Goldberg on the use of glucose isomerase enzyme (as opposed to the whole organism containing the enzyme immobilized on a porous polymeric matrix). Chibata discusses several industrial applications of immobilized microbial cells as practiced in Japan. The chapters by Mattiasson and Suzuki and his associates discuss many interesting analytical applications of immobilized cell systems. The final chapter by Kastl describes a process for immobilizing isolated organelles and use of such a system in detoxifying drugs.

I am indebted to all the authors for preparing the manuscripts to meet a tight publication schedule, and to the reviewers for their prompt responses. Many thanks are due to Charles Cooney and George Charalambous of the Microbial and Biochemicl Technology Division and the Agricultural and Food Chemistry Division, respectively, for encouraging me to organize this symposium, and to John Whittaker for serving as cochairman of the symposium. I am thankful to the ACS Books Department for its assistance. Finally, the impeccable secretarial help of Diane Otto is gratefully acknowledged.

H. J. Heinz Company
Pittsburgh, Pennsylvania
April 10, 1979

K. VENKATSUBRAMANIAN

CONTENTS

Immobilized Microbial Cells in Complex Biocatalysis

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Continuous heterogeneous catalysis by fixed microbial cells represents a new approach to established fermentation processes. Immobilization of isolated (and purified) enzymes and microbial cells mediating simple, monoenzyme reactions has already been reduced to industrial practice. However, the development of immobilized cell systems to carry out complex fermentation processes--characterized by multiple reactions and complete reaction pathways involving coenzymes--is still in its infancy. Drawing upon our rather concerted effort in this area over the past several years, we are appraising the prospects and problems of such a technological advancement in this brief communication.

The Approach

In earlier papers from this laboratory, we have proposed the terms "Controlled Catalytic Biomass" and "Structured Bed Fermentation" to describe immobilized cell systems effecting complex biocatalysis (1,2). The meaning of these terms is obvious when one considers the biocatalyst in relation to its microstructure, predesigned catalytic reactor design, and controlled catalytic activity vis-a-vis cellular reproduction. Some of the potential advantages of such a catalytic system are summarized in Table I.

Examining the character of microbial cells in classical fermentation, it is clear that they possess the desired catalytic machinery in a highly structured form. The controlled conditions of fermentation permit retention of this meticulous structural

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TABLE 1

POTENTIAL ADVANTAGES OF IMMOBILIZED WHOLE CELL SYSTEMS OVER CONTROLLED FERMENTATIONS

1. Placement of Fermentation on Heterogeneous Catalysis Design Basis
2. Higher Product Yields
3. Ability to Conduct Continuous Operations As Opposed to Traditional Batch Fermentation
4. Operation at High Dilution Rates Without Washout
5. Ability to Recharge System by Inducing Growth and Reproduction of Resting Cells
6. Decrease or Elimination of Lag and Growth Phases for Product Accumulation Associated With the Non-Growth Phase of the Fermentation
7. Possibility of Accelerated Reaction Rates Due to Increased Cell Density

integrity but the resulting cellular suspensions are usually at low concentration. Considering free enzymes derived from these cells, it is possible to concentrate them by extraction processes, but lacking the ancillary structure which stabilizes them in the cell, they are relatively unstable. Some structural reconstitution is possible by immobilization, leading to higher concentration and better stability but one is then restrained to consideration of single step or two-step reactions. With immobilized cells, one has the concentrated form, there is structural preservation and stability together with the possibility of improved reactor design, based upon the characteristics of the carrier. Thus, immobilized cell systems constitute an important option within the framework of biochemical technologies (Table 2). The overall rationale for whole cell immobilization is outlined in Table 3.

In all our work, we have used reconstituted bovine hide collagen as the carrier matrix of choice. The biomaterial, collagen, offers a number of unique advantages as a support for microbial cell immobilization. Other publications from our laboratory describe these advantages as well as the procedures to prepare fixed cells in detail (3, 4). We have attached many different microorganisms in this manner; some of the complex reactions mediated by such fixed cell preparations are shown in Table 4.

Process Variables

Several important considerations in the preparation and use of collagen-bound cell systems are adumbrated here with citric acid production by immobilized Aspergillus niger as an example. The collagen membrane must be crosslinked to make it structurally strong enough to withstand the shear forces in reactor operation. It was found that post-tanning the collagen-cell membrane by exposing it to a 5% glutaraldehyde solution for one minute resulted in an optimal retention of catalytic activity which was a linear function of the cell loading. We can load the structure up to 70% cells (by dry weight) and the amount of expressed activity in batch assay increases proportionately. However, the mechanical strength drops off too drastically, and a good compromise is 50% cells on a dry weight basis. In the course of these studies, we came to realize that the dehydration of cells is deleterious; even under refrigerated conditions cell activity could reduce significantly. This has led us to new dispersion techniques and/or drying or solidification techniques to preserve these fragile structures which can so easily denature (6).

Maximal catalytic activity of the cells is retained upon immobilization when the cells are in the proper physiological state. This corresponds to an optimal induction of enzyme activities participating in the desired reaction sequence;

TABLE 2

BIOCONVERSION NETWORK

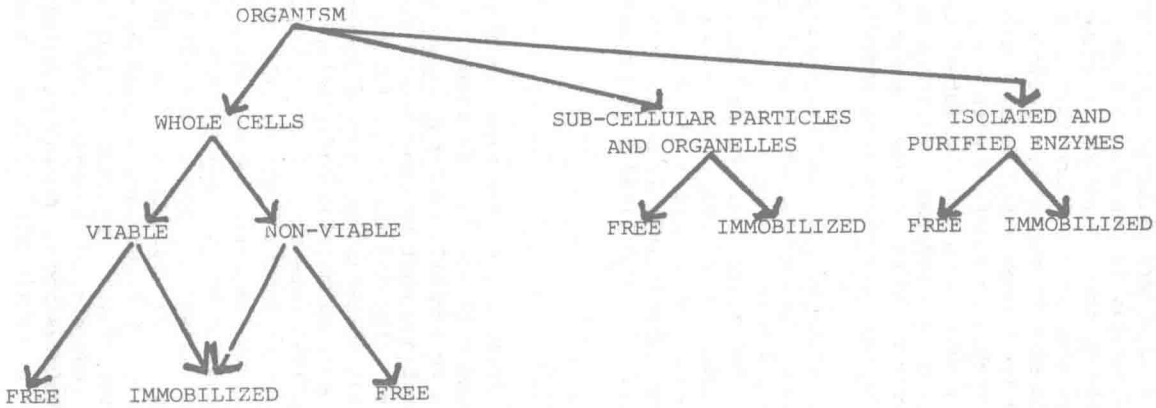

TABLE 3

RATIONALE FOR WHOLE CELL IMMOBILIZATION

1. Obviates Enzyme Extraction/Purification
2. Generally Higher Operational Stability
3. Lower Effective Enzyme Cost
4. High Yield of Enzyme Activity on Immobilization
5. Cofactor Regeneration
6. Retention of Structural and Conformational Integrity
7. Greater Potential for Multi-Step Processes
8. Greater Resistance to Environtal Perturbations

TABLE 4

COLLAGEN-IMMOBILIZED CELL SYSTEMS

	Microorganism	Substrate	Product	Comments
1.	Serratia marcescens	Glucose	2-Keto gluconic acid	Multi-enzyme
2.	Acetobacter sp.	Ethanol	Acetic acid	Multi-enzyme; cofactor
3.	Corynebacterium lilium	Glucose	Glutamic acid	Pathway (primary metabolite)
4.	Aspergillus niger	Sucrose	Citric acid	Primary metabolite
5.	Chloroplast	Water	Oxygen	Immobilized organelle; first step in biophotolysis of water
6.	Anacystis nidulans	Water	Oxygen	Immobilized algal cells
7.	Anacystis nidulans	Nitrate	Ammonia	Biological nitrogen fixation
8.	Streptomyces griseus	Glucose	Candicidin	Antibiotic synthesis; secondary metabolite
9.	Pseudomonas aeruginosa	---	---	Concentration of plutonium from waste waters (bioadsorption)
10.	Klebsciella pneumoniae	Nitrogen	Ammonia	Microbial fixation of atmospheric nitrogen
11.	Mammalian erythrocyte	---	---	Model studies of *in vivo* enzyme action

it is manifested in peak product synthesis rate in the fermentation. For citric acid production with A. niger, it turns out to be 72 to 96 hours in batch fermentations. Of course, in a typical fermentation process one has to repeat this pattern each time. A better alternative, it would seem, would be to harvest the cells at their peak activity, followed by their immobilization so as to retain them in a viable state for reuse until their stability has decreased to an uneconomical point.

Once immobilized, the cells must be kept in a viable state in the membrane without further excessive reproduction. This is necessary to channel the substrate into the desired product rather than to additional cell mass. Besides, it would minimize cell elution from the carrier matrix as well as preserve the mechanical integrity of the carrier. We have found that one way to accomplish this is by limiting the concentration of one of the essential nutrients in the medium; for example, nitrogen concentration. An indirect benefit of this approach is lowering the growth of contaminating organisms.

Ease of reactor scale-up is an important process engineering consideration; maximizing the efficiency of contact between the catalyst and its substrate is an equally critical issue. We have determined that where the bound-cell membrane can be rolled into a spiral wound reactor configuration (6), it provides excellent contact efficiency. The collagen membrane is wound together with a polyolefin Vexar spacer material. The resulting open multichannel system promotes plug flow contact with very low pressure drop even when operating with particulate substrate matter which would cause plugging problems in the conventional type of fixed bed operation. Fermentation substrates are often characterized by precisely this type of substrates; so this is a large plus factor in favor of this type of design. Furthermore, it is possible to design-in high activity per unit volume, as a result of the coiling of a large amount of membrane into a confined volume. The basis for scale-up becomes then simply the membrane surface area.

Presented in Figs. 1 and 2 are data relating to external and internal mass transfer for the case of citric acid synthesis. The effect of linear velocity on the observed reaction rate (Fig. 1) shows, for this case, the presence of a significant boundary layer resistance below a flow rate of 235 ml/min. The existence of non-negligible pore diffusional resistance is deducible from Fig. 2, in which the dependence of observed reaction rate on film thickness is depicted. Overall the immobilized cells exhibited about 50% of the specific activity of the free cells (in fermentation) toward the production of citric acid.

With regard to other significant factors, oxygen transfer can be singled out as of paramount importance. To enhance this transport step, we operated the spiral wound reactor countercurrently. In other words, a special provision was incorporated into the reactor design to allow flow of pure oxygen countercurrent

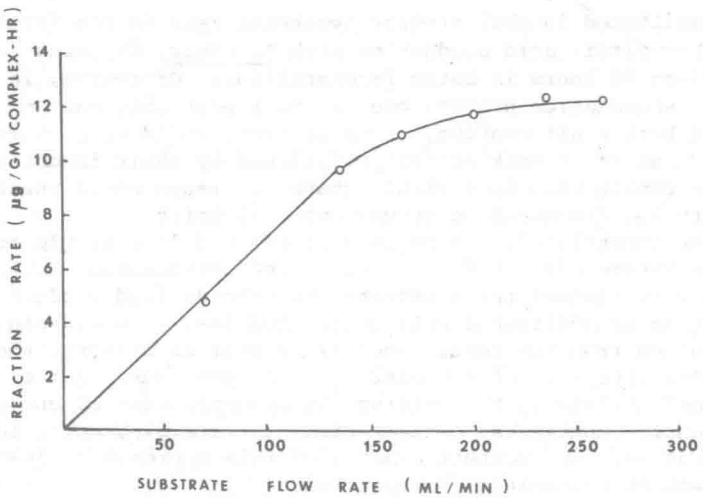

Figure 1. Dependence of reaction rate on linear velocity

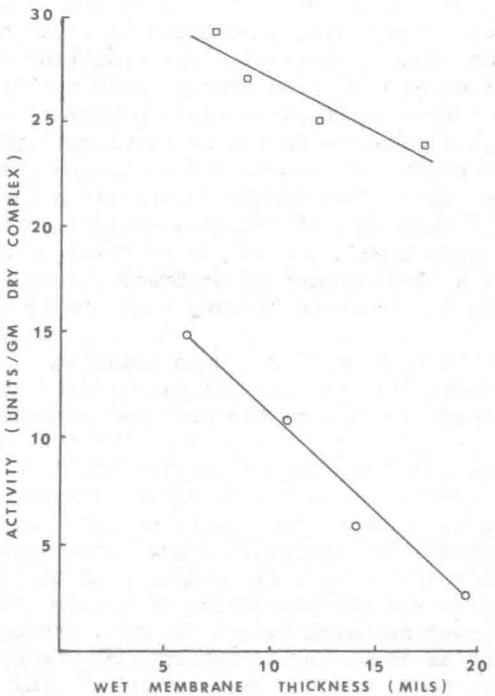

Figure 2. Effect of membrane thickness on citric acid production rate. (○) Shake flask, (□) reactor.