WOLF R. VIETH

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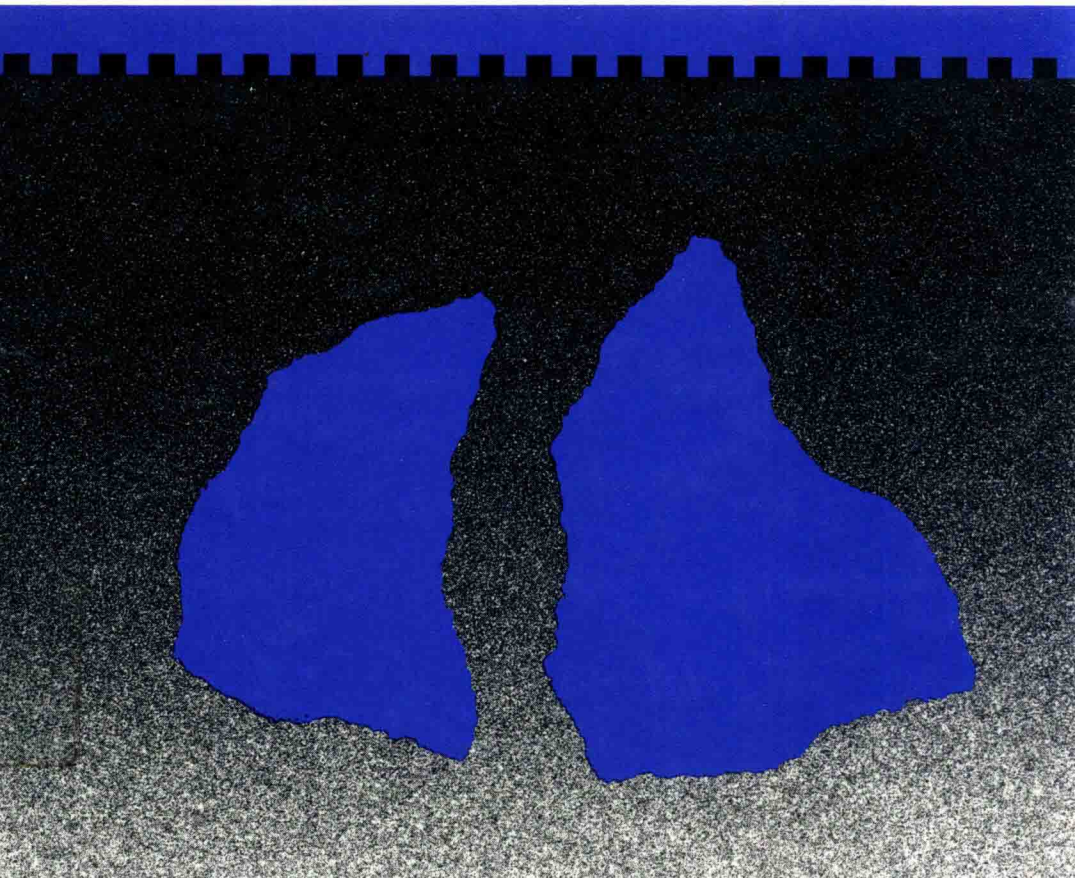
Kinetics, Mass Transport, Reactors and Gene Expression



BIOPROCESS ENGINEERING

Kinetics, Mass Transport, Reactors and Gene Expression

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A Wiley-Interscience Publication

JOHN WILEY & SONS, INC.

New York • Chichester • Brisbane • Toronto • Singapore

This text is printed on acid-free paper.

Library of Congress Cataloging in Publication Data:

Vieth, W. R. (Wolf R.)

Bioprocess engineering: kinetics, mass transport, reactors and gene expression / Wolf R. Vieth.

p. cm.

"A Wiley-Interscience publication."

Includes bibliographical references and index.

ISBN 0-471-03534-3 (cloth)

1. Biochemical engineering. 2. Biotechnology. 3. Genetic engineering. 4. Bioreactors. I. Title

TP248.3.V54 1994 93-44655

660'.63--dc20

Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

BIOPROCESS ENGINEERING

The author dedicates this book
to his faculty colleagues at Rutgers University
and to his research colleagues of the
Engineering Foundation Conference on Biochemical Engineering

FOREWORD

"In the practice of Chemical Engineering, the premium is on the man who can best apply a theoretical background and research aptitude to the formulation and solution of real problems." Much has changed since the time this statement first appeared.* At this stage, the author would like to offer an amended version, as follows:

"In the practice of Chemical and Biochemical Engineering, the premium is on the hybrid individual who can best systematize and apply a synthesis of chemical and engineering fundamentals, principles of biosciences, and a research aptitude, to the formulation and solution of real problems."

K. Venkatasubramanian in his Ph.D. thesis called attention to a quotation from Goethe, "Ein Gedanke kann nicht erwachen ohne andere zu wecken (A thought cannot arise without awakening others)." That is precisely the type of environment we have all been striving to create at Rutgers and to carry over into the Engineering Foundation Conference on Biochemical Engineering.

The appearance of this book is timed to coincide with the 25th anniversary of the biochemical engineering research program in the Rutgers Department of Chemical and Biochemical Engineering, as well as the gathering of research colleagues for Biochemical Engineering VII, inaugurated some fifteen years ago. Members of the Rutgers group chaired the first three biennial conferences and have maintained a lively interest and commitment ever since.

One of my students said a very kind thing recently, that reading my book, he could hear me speaking. With ongoing book projects, I have not had much time for attendance of technical meetings, other than the Biochemical Engineering Conference, for awhile. I hope my colleagues, especially those who read these books, will agree with my student, and understand my preoccupations.

*C. Selvidge and W. R. Vieth, Graduate Study at the School of Chemical Engineering Practice. MIT Publication Office (August, 1967).

PREFACE

As a microcosm, the evolution of the Rutgers program in Biochemical Engineering has parallelled the accelerated dynamics of the field globally.

In 1970, to focus our efforts, we embarked upon a long range research program in collagen technology for developing generalized biocatalyst/bioreactor systems. In the late 1970s we diversified and committed ourselves along the lines of alternate carriers (e.g., alginates) and reactors (e.g., fluidized beds) and, most importantly, to the use of recombinant cells.

We also made a commitment then to the Engineering Foundation biennial biochemical engineering conferences as a vehicle and as a testing ground for introducing our work along with that from many newly emerging laboratories. From these combined activities, I have enjoyed a rare opportunity to share and receive the best insights from my professional colleagues in participating in the development of the field.

The present work draws naturally on the results of research in our Rutgers laboratories, but even more so on the published results of seven Engineering Foundation conferences offered since 1978 and on many professional journal sources. In addition, it incorporates a state-of-the-art review and synthesis of a large body of the mushrooming current literature. I've tried to provide a lucid treatment of biokinetics and biocatalysis, and to show their integration with reactor concepts in bioprocesses, thereby tracing the rapid, recent evolution of biotechnology.

My goal is to provide the reader with a coherent synthesis of biochemical process systems. The book begins with simple enzyme and cell-based process kinetic models and then moves on to stress the kinetics of gene expression and product formation, with a unifying emphasis on operon concepts.

The book is intended for advanced undergraduate and graduate level students and professionals engaged in the field of biotechnology. Although written from an engineering standpoint, it should appeal to biochemists, microbiologists and food scientists as well.

In conjunction with my first two volumes* on membrane systems and on diffusion in and through polymers, this work completes and interrelates the trilogy. Increasingly we are reminded of the need to stimulate original thinking and dialogue across disciplinary lines. So most especially I have worked hard to ease the line separating large numbers of current chemical engineering practitioners from biochemical engineers.

Wolf Vieth

Piscataway, New Jersey
September, 1992

*W. R. Vieth, "Membrane Systems: Analysis and Design," John Wiley and Sons: New York, 360 pp. (1988).

W. R. Vieth, "Diffusion In and Through Polymers," Hanser Publishers: Munich; Dist. in U. S. by Oxford University Press: New York, 322 pp. (1991).

ACKNOWLEDGEMENTS

Publication of this manuscript marks completion of the fourth joint project by the author and his partner and wife, Peggy. (Actually, the eighth, counting our four children.) The first of these projects was the manuscript of the author's doctoral thesis, completed in 1961. She set a standard of cooperative effort at that time that has been unequaled in any work outside our partnership. In other words, I became a bit spoiled. But we enjoyed the activity so much that we resolved to resume it after our children had grown up, and did so in the form of these books.

Beginning about 1985, Peggy began mastering the uses of the computer and getting after me to begin, especially on a book dealing with bioengineering. This is no easy task, however, and I felt I had to work up to it a little more gradually, starting closer to my own technical foundations (the first two books). Meanwhile, Peggy began to wonder if this book would ever appear and began referring to it as "The Phantom of the Operon." At that point I had no choice but to proceed.

I am grateful to Peter Prescott and Dr. Ed Immergut, Hanser Publishers, who originated the project, for their assistance in the preparation of this book and to Professors Henrik Pedersen and Takishi Matsuura for their collaboration in research activities, which had an impact on the subject matter.

I wish to acknowledge the permission of the authors and publishers of the following journal and book to reproduce the portions of text specified: Ch. 10, pp. 303-318 and App. B. pp. 348-376, excerpts from pp. 245-298, J. M. Howell and W. R. Vieth, *J. Mol. Catal.*, Elsevier Sequoia S. A; and Ch. 2, pp. 24-29, excerpts from pp. 235-266, "Annual Reports of Fermentation Processes," Academic Press Inc.

BIOPROCESS ENGINEERING

CONTENTS

1

CLASSICAL BIOTECHNOLOGY

1.0 HISTORICAL PERSPECTIVE

Biotechnology had its origins when mankind first began to domesticate microorganisms by fermenting them in batch culture. The Congress of the United States, in 1984, defined Biotechnology as follows: "Commercial techniques that use living organisms, or substances from those organisms, to make or modify a product, including techniques used for the improvement of the characteristics of economically important plants and animals, and for the development of microorganisms to act on the environment." As Lee (1992) correctly points out, the area cannot be considered new. Indeed, fermentation processes have been practiced since early civilization and have been continually perfected with time, largely on an empirical basis. Historically, the technology evolved to the production of increasingly more valuable products such as antibiotics. More recently, the advent of recombinant DNA technology has dictated the development of novel processes for the production of totally synthetic products, such as monoclonal antibodies, as well as the scaling up of these processes, to such an extent that the incorporation of modern engineering concepts and methods in the development, design, and control of fermentation operations is becoming universal. Thus, biotechnological process developments are now parelleling and even fusing with developments in chemical technology (e.g., membrane separations in downstream processing).

"Fermentation" is no longer used in its original classic sense, but rather it is taken to mean "chemical reactions catalyzed by enzyme systems, which in turn are produced during the growth of microorganisms" (Gaden, 1955). The growth of a microorganism is a complex process and the transformation of a nutrient into a metabolic end product usually involves a large number of individual chemical reactions. This complexity of biological systems has, until the recent knowledge explosion brought about by systematic studies in molecular biology, contributed to delays in development in the areas of process modeling and optimization of fermentation operations (Rai, 1973).

As an outgrowth of earlier developments, the overall spectrum of activity which now exists can be conveniently represented in a bioconversion network, as shown in Fig. 1.1, which rather strikingly demonstrates the evolution of the field as it pushes outward from fermentation technology.

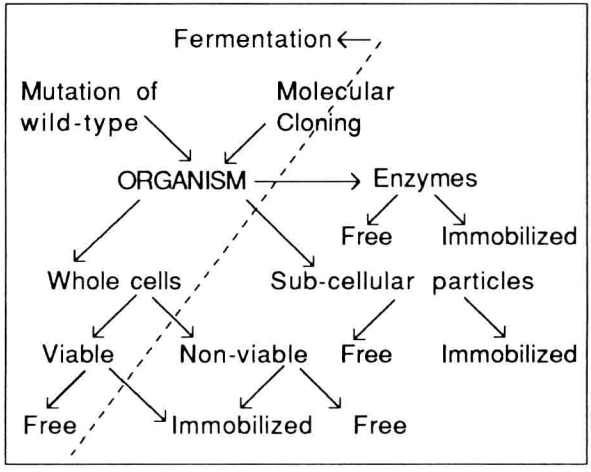

Figure 1.1 Network of bioconversion.

For instance, although only recently appearing on the scene, enzymatic processes *per se* have already found large scale industrial application in the food and pharmaceutical industries (Chibata et al., 1986) and a wide variety of smaller scale applications in medical diagnostics and therapeutics (Pedersen and Horvath, 1981; Bernath et al., 1976). Barker and Petch (1985) elaborate on the enzymatic process for high fructose corn syrup, while Tanaka and Fukui (1985) describe the bioconversion of lipophilic compounds by immobilized biocatalysts in the presence of organic solvents.

Chibata et al. (1986) list some examples of current large scale processes as follows:

- Production of L-amino acids from acetyl-DL-amino acids using immobilized aminoacylase.
- Production of 6-aminopenicillanic acid from penicillin G using immobilized penicillin amidase.
- Production of high fructose syrup using immobilized glucose isomerase.

- Production of L-aspartic acid using immobilized microbial cells.
- Production of L-malic acid using immobilized microbial cells.
- Production of L-alanine using immobilized microbial cells.
- Hydrolysis of lactose in milk using immobilized β-galactosidase.

Recently, Tosa et al. (1988) described an economically attractive slurry bioreactor-crystallizer scheme for the large-scale production of L-malic acid. In the crystallizer, a supersaturated product leaves solution, while remaining unreacted solid substrates dissolve. In this way, a substrate in slurry form is completely converted to a product in slurry form.

In scaled-down operations, Pedersen and Horvath (1981) point out that the most widespread early use of immobilized enzymes in analytical applications (e.g., medical diagnostics) is the employment of enzyme tubes in continuous-flow analyzers of the Technicon type. Highly active enzymic layers are deposited on the inner walls of plastic tubes, which are coiled into modular forms of open tubular heterogeneous enzyme reactors (dubbed "others"). Under the conditions of operation, the reactors are diffusion controlled, displaying "linear chemistry;" i.e., the pseudo first order kinetic conditions assure chemical analytical linearity.

Intermediate between fermentation and enzyme technology lies the immobilized whole cell regime. Examining the character of cells employed in fermentation, it is clear that they possess the desired enzymatic machinery in a highly structured form. The controlled conditions of fermentation permit retention of this structural integrity, but the resulting cellular suspensions are usually at low concentrations. It is possible to concentrate the free enzymes derived from these cells by extraction processes, but without the ancillary structure that stabilizes them in the cell, they are relatively unstable. Some structural reconstitution is possible by immobilization, which leads to higher concentration and better stability, but one is then constrained by economics to consider chiefly single step or two step reactions. With immobilized cells, one has the concentrated form: entire enzymatic pathways remain interconnected and viable, cellular microstructural characteristics are preserved and stabilized and the possibility of improved reactor design is opened up, based upon the characteristics of the carrier.

1.1 ROLE OF KREBS CYCLE IN PRIMARY METABOLITE SYNTHESIS

Salient examples of the above are afforded by careful examination of the tricarboxylic acid (TCA) cycle, when it is functioning in the following ways:

i. microbial respiration;
ii. production of monosodium glutamate;
iii. production of citric acid.

Aerobic cells obtain most of their energy by respiration, the transfer of electrons from organic fuel molecules to molecular oxygen (Fig. 1.2). The final common pathway into which all the fuel molecules - carbohydrates, fatty acids and amino acids - are ultimately degraded in catabolism is the Krebs tricarboxylic acid cycle. A large fraction of the energy released is through this pathway. The overall reaction catalyzed by the cycle may be considered as:

$$CH_3COOH + 2\,H_2O \rightarrow 2\,CO_2 + 8\,H \qquad [1.1]$$

Acetic acid enters by condensation with four-carbon oxaloacetic acid to form citric acid. For each complete turn of the cycle, two molecules of CO_2 are produced with regeneration of the consumed oxaloacetic acid. The oxidations in the cycle are carried out by certain nucleotides (NAD^+, $NADP^+$ and FAD^{++}). The electrons derived from intermediates of the cycle flow down a multimembered chain of electron-carrier enzymes of successively lower energy level until they reduce molecular oxygen, the ultimate electron acceptor in respiration. During this process, much of the free energy of these electrons is conserved in the form of the phosphate-bond energy of ATP, by the process known as oxidative phosphorylation. All the enzymes or enzyme-complexes catalyzing reactions of the tricarboxylic acid cycle, electron transfer and oxidative phosphorylation are located within the mitochondrion, a respiratory organelle in some microbial cells.

It has been known for some time that the process of oxidative phosphorylation can be uncoupled from electron transfer by addition of specific uncoupling agents. Some examples of these compounds are 2,4 dinitrophenol, dicumarol and carbonylcyanide phenylhydrazone. Carbonylcyanide phenylhydrazone is the most powerful compound and completely stops cells' metabolic activities. The mechanism of action of uncoupling agents is not known exactly but it is believed that uncouplers cause structural damage to the inner membrane of the mitochondrion, bringing about alteration of the proton gradient.

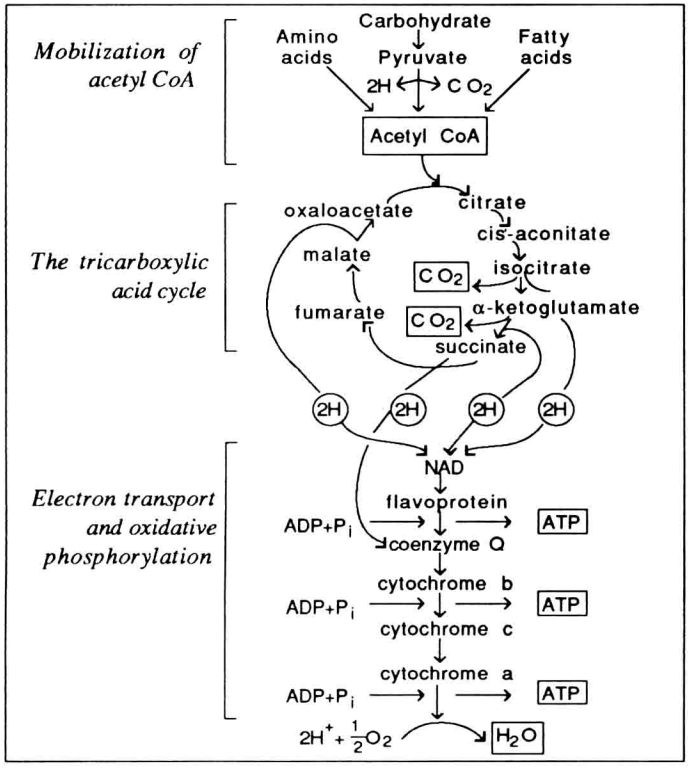

Figure 1.2 Flowsheet of respiration.

Induced microbial respiration refers to the increase in the rate of respiration of cells (i.e., oxygen uptake rate) when oxidative phosphorylation (energy conservation as ATP) is uncoupled from the process of electron transfer. Additionally, reactions involving ATP as energy source - such as synthesis of cellular material - are inhibited to a large extent.

Theoretically, one expects an optimum level of ATP (or uncoupler concentration) to exist just sufficient to carry out the catabolic reactions of the substrate without inhibition. Higher concentrations of ATP would cause feedback inhibition, and lower concentrations would result in exhaustion of the cells by not providing energy to consume the substrate. This phenomenon has been observed by Poe and Estabrook (1968) while measuring enthalpy of oxidations of succinate by the rat liver mitochondrion, and also analyzed in our laboratory (Fig. 1.3) in studies with intact yeast cells (Shah, 1975; Vieth, 1978).