

BIOTECHNOLOGY IN INDUSTRY

*Selected Applications
and Unit Operations*



BIOTECHNOLOGY IN INDUSTRY

*Selected Applications
and Unit Operations*

By

RAJANI JOGLEKAR

ROBERT J. CLERMAN

ROBERT P. OUELLETTE

PAUL N. CHEREMISINOFF



ANN ARBOR SCIENCE
THE BUTTERWORTH GROUP

1 5 -
B
1
Copyright © 1983 by Ann Arbor Science Publishers
230 Collingwood, P.O. Box 1425, Ann Arbor, Michigan 48106

Library of Congress Catalog Card Number 82-48642
ISBN 0-250-40605-5

Manufactured in the United States of America
All Rights Reserved

Butterworths, Ltd., Borough Green, Sevenoaks
Kent TN15 8PH, England

PREFACE

This book examines selected industrial applications of biotechnology in detail, and surveys research and development (R&D) in process design and equipment. Commercial-scale processes are described in three generic application areas: (1) proteins and chemicals from pulp and paper industry wastes, (2) single-cell protein (SCP) for human consumption from organic waste materials, and (3) high-fructose corn syrups using immobilized enzyme technology. In addition to technical, product and cost information, each process is evaluated in terms of potential improvement or modernization. A survey of unit operations (defined as process steps resulting in chemical, biological or physical changes) provides a basis for evaluating future R&D trends such as continuous operation, enzyme and cell immobilization, and reduced energy requirements for fermentation and product separation.

Biotechnology involves a wide range of industrial applications of biological processes and materials. Throughout history, fermentation has been used in producing and processing foods and alcoholic beverages. It was not until the 19th century, however, that the role of microorganisms in fermentation processes was demonstrated. Although the post-World War II growth of petroleum-based processes displaced some applications, fermentation has remained an important component of the food and pharmaceutical industries.

Today biotechnology is undergoing a resurgence in a wide range of industries. A combination of factors has led to this renewed interest in biological processes, including:

- the opportunity to replace petroleum-based feedstocks with low-cost organic substrates;
- lower operating temperatures and pressures; and
- reduced environmental hazard.

In addition, recent developments in molecular genetics (e.g., recombinant DNA technology) and process design (e.g., continuous reaction, use of immobilized enzymes) may form the basis for the growth of new markets

through the introduction of new and better products and the improvement of process economics for existing products.

The MITRE Corporation has been studying the growth of biotechnology in industry. This book, the second in a series of studies sponsored by Electricité de France, examines in detail the following three process areas:

- proteins and chemicals from pulp and paper industry wastes;
- single-cell protein (SCP) for human consumption from organic waste materials; and
- high-fructose corn syrups (HFCS) using immobilized enzymes.

The first book on this subject preceding this study was *Biotechnology and Energy Use* presented by the authors and published by Ann Arbor Science in 1981.

Information is presented on current, commercial-scale facilities, as well as potential future improvements likely to result from research and development. To provide a basis for evaluating these and other processes, biotechnology is defined in terms of a set of "unit operations," and recent technical developments are surveyed.

The authors would like to express their appreciation to consultants Murray Moo-Young and C. W. Robinson of the Biochemical Engineering Group, University of Waterloo, Waterloo, Ontario, Canada. From the MITRE Corporation, we thank Irwin Frankel for his review comments, Bob Atkins and the Graphics Department for preparing the illustrations, the library staff for assistance with literature collection, Barbara Dougherty for help with document preparation, and Virginia Gaughan for her patience and skill in typing.

We acknowledge Electricité de France for sponsorship, support and encouragement of this project.

Rajani Joglekar
Robert J. Clerman
Robert P. Ouellette
Paul N. Cheremisinoff



Joglekar

Clerman

Ouellette

Cheremisinoff

Rajani Joglekar received her BSc in Biology from the University of Bombay, and an MS in Biology and in Environmental Sciences from Northeastern University and George Washington University, respectively. Her research interests and publications are in the areas of plant growth and development, and development of biological tests for detecting toxic chemicals in the environment. Since 1978, working as a member of the technical staff in the Environment Division of the MITRE Corporation, she has been studying the health and environmental effects of energy technologies. These studies have included the health effects of coal and oil shale, health and environmental effects of synthetic fuels, oil and gas end use, and development of research plans for coal liquefaction technologies. Currently, Ms. Joglekar is involved in evaluating the industrial applications of biotechnology.

Robert J. Clerman is a group leader in the Environment Division of the MITRE Corporation. He received his BS in Biology from the State University of New York at Fredonia, and has an MS in Environmental Sciences from the University of Virginia. His research interests and publications have covered a wide range of environmental issues. Since joining MITRE, Mr. Clerman has been involved in studies relating to the fate and effects of chemicals in the environment. Projects have included development of a tiered testing system for new chemicals evaluation, design of a residuals monitoring program (both for the Federal Republic of Germany), and evaluation of organic pollutants in water (for the Department of Energy and the National Cancer Institute). Currently, he is managing studies of industrial applications of biotechnology.

Robert P. Ouellette is Technical Director, Environment Division, of the MITRE Corporation. Dr. Ouellette has been associated with MITRE in varying capacities since 1969. Earlier, he was with TRW Systems, Hazelton

Labs Inc. and Massachusetts General Hospital. A graduate of the University of Montreal, he received his PhD from the University of Ottawa. Dr. Ouellette is a member of the American Statistical Association, Biometrics Society, Atomic Industrial Forum and the National Science Foundation Technical Advisory Panel on Hazardous Substances. He has published numerous technical papers and books on energy and the environment and is co-editor/co-author of the Electrotechnology series published by Ann Arbor Science.

Paul N. Cheremisinoff is Associate Professor of Environmental Engineering at New Jersey Institute of Technology. He is a consulting engineer and has consulted on environmental/energy/resources projects for the MITRE Corporation. He has more than 30 years of practical design, research and development and engineering experience in a wide range of industries including pollution control, waste treatment, and chemical and process industries. He is author/editor of many Ann Arbor Science publications, including: *Pollution Engineering Practice Handbook*, *Carbon Adsorption Handbook* and *Environmental Impact Data Book*.

CONTENTS

1. Introduction	1
Background and Purpose	1
Scope and Approach	2
Organization of This Book	3
2. Alcohol Production from Spent Sulfite Liquor	5
Evolution of Technology	5
Process Description	6
Liquor Recovery and Preparation	10
Yeast Fermentation and Fermented Liquor Recovery	15
Distillation	16
Process Control	19
Other Processes	20
Product Uses	21
Cost Estimation	22
Evaluation	22
3. Torula Yeast from Spent Sulfite Liquor	29
Evolution of Technology	30
Process Description	30
Liquor Recovery and Stripping	32
Fermentation/Yeast Propagation	37
Separation and Recovery	41
Product Characteristics and Uses	44
Cost Estimation	46
Evaluation	46
4. Single-Cell Protein from Organic Wastes	49
Evolution of Technology	49
Organic Wastes as Substrates	50

SCP for Human Consumption	56
SCP Production Processes	57
Waterloo SCP Bioconversion Process	61
Pretreatment	62
Fermentation	64
Product Recovery	65
Removal of Nucleic Acids in SCP	65
Product Uses	67
Cost Estimation	67
Cost Analysis	68
Waterloo SCP Cost Estimates	69
Evaluation	70
 5. High-Fructose Corn Syrup	 73
Evolution of Technology	74
Process Description	75
Corn Wet Milling	76
Corn Refining (Starch Conversion)	80
Isomerization of Glucose to HFCS	82
Process Control	83
Product Characteristics and Use Patterns	88
HFCS Uses	90
HFCS Supply and Consumption	91
Cost Estimation	92
HFCS in Europe	93
Evaluation	95
 6. Unit Operations in Biotechnology	 99
Scope and Approach	100
Summary of Findings	101
Preparation of Biological Catalyst	101
Preparation of Substrate	101
Reaction	106
Product/Catalyst Separation and Purification	108
 7. Unit Operations Survey	 111
Cell Culture	111
Suspended Cell Culture Apparatus	111
Immobilized Cell Culture Method	112

Enzyme Immobilization	113
Enzyme Immobilization Using Activated Carbon	113
Multilayer Enzyme Immobilization	114
Immobilization of Enzymes on Magnetic Materials	115
Preparation of Substrate	116
Twin-Screw Extruder	116
Attrition Bioreactor	119
Sterilization	121
Continuous Sterilization	121
Reaction	122
Tower Fermenter	122
Rotofermenter	124
Scraped Tubular Fermenter	126
All-Glass Fermenter	128
Monitoring	129
Continuous-Flow Viscometer	129
Continuous Monitoring of Optical Density	131
In-Tank Turbidity Measurement Device	134
Cell Mass Sensor	135
Microcalorimeters	135
Oxygen Electrode	139
Yeast Biochemical Oxygen Demand Sensor	141
Carbon Dioxide Sensor	141
Coulter Counter Method for Measurement of Microbial Growth	142
Microorganism Activity Measurement	145
Measurement of Cell Concentration Based on Electrical Impedance	145
Affinity Sensors for Metabolite Measurements	148
Gas Control	150
Introducing Gas into Fermentation Liquids	150
Gas Collection Apparatus	151
Energy Recovery	152
Offgas Energy Recovery	152
Membrane Separation	154
Reverse Osmosis for Process Stream Treatment	154
Biopolymer Separation from Fermentation Broths	155
Suspended Solids Removal	159
Two-Second Separator	159
Cell Recovery	160
Separators for Cell Harvesting	160
Cell Recovery via Coagulation	161

Distillation	162
Alcohol Recovery System	162
Distillation with Vapor Recompression	164
Sorption	165
Sorption Method of Separating Water-Ethanol Mixtures	165
8. Summary	169
Proteins and Chemicals from Pulp and Paper Industry	
Wastes	170
Alcohol Production from Spent Sulfite Liquor	170
SCP from Pulp and Paper Wastes	170
SCP from Organic Wastes	171
High-Fructose Corn Syrup	172
Unit Operations in Biotechnology	173
Index	175

FIGURES

2-1.	Schematic of alcohol production process	7
2-2.	Comprehensive flow diagram for alcohol production	8
2-3.	Liquor recovery and preparation for fermentation	10
2-4.	OC/CIP spent liquor recovery system. Five-stage continuous countercurrent wash line operating under vacuum and plus flow conditions	13
2-5.	Yeast fermentation and fermented liquor recovery	15
2-6.	Distillation unit	18
2-7.	Georgia Pacific's integrated pulp, paper and chemical production plant	23
3-1.	Schematic of torula yeast production process	31
3-2.	Comprehensive diagram of torula yeast production process	34
3-3.	Liquor recovery and stripping process	36
3-4.	Fermentation and yeast generation	38
3-5.	Waldhof fermenter	39
3-6.	Product separation and recovery	43
4-1.	Simplified diagram of the Pekilo process	58
4-2.	SCP from sulfite waste liquors	59
4-3.	Domestic waste recycle system	59
4-4.	Animal recycle feedlot wastes system	60
4-5.	Schematic of the Waterloo Bioconversion Process	61
4-6.	Comprehensive process diagram: Waterloo process	62
5-1.	Corn wet milling process	77
5-2.	Comprehensive diagram: corn wet milling process	78
5-3.	Comprehensive diagram: corn refining process	84
5-4.	Isomerization of glucose to fructose process	86
5-5.	Second-generation HFCS processing	88
6-1.	Process steps in biotechnology	104
7-1.	Cell culture apparatus	112
7-2.	Ultrafiltration cell	115

7-3.	Enzyme reactor	116
7-4.	Twin-screw extruder	117
7-5.	Schematic diagram of the attrition bioreactor	120
7-6.	Continuous sterilization plant flow diagram	121
7-7.	Diagrammatic representation of tower fermenter	123
7-8.	Schematic diagram of the rotofermenter assembly	125
7-9.	Mechanically scraped tubular plug-flow fermenter	127
7-10.	Pneumatically scraped tubular plug-flow fermenter	128
7-11.	Details of constant-temperature chamber for continuous- flow capillary viscometer	130
7-12.	Apparatus for measurement of cell suspension viscosity	131
7-13.	Schematic diagram of effective dilution device	132
7-14.	Calibration curve showing linear relationships between cell density and OD	133
7-15.	Light pipe for turbidity measurement	134
7-16.	Cell mass sensor	136
7-17.	Experimental assembly for batch culture	137
7-18.	Experimental setup for pH-regulated continuous culture ...	138
7-19.	Dissolved oxygen sensor	140
7-20.	Mechanical structure of carbon dioxide sensor	143
7-21.	Diagram of a Coulter counter for measuring cell number and size distribution	144
7-22.	Two-electrolytic-electrode system	146
7-23.	Electrical circuit for measurement of cell concentration	147
7-24.	Schematic diagram of a fiber-optic affinity sensor	149
7-25.	Gas collection apparatus	152
7-26.	Energy recovery system	153
7-27.	Modified Gelman cell	155
7-28.	Reverse osmosis system	156
7-29.	Axial filter	157
7-30.	Pleated filter cartridge	158
7-31.	Microscreen flow pattern	158
7-32.	Flowsheet of the magnetic separation process	159
7-33.	Alcohol recovery	163
7-34.	Flowsheet of the distillation plant with a heat pump	164

TABLES

1-1.	Processes Described	4
2-1.	U.S. Industrial Ethanol Production by Material Used	21
2-2.	Potential Improvements to Alcohol Production from SSL ..	24
3-1.	Nutritional Values of Torula Yeast	44
3-2.	Typical Amino Acid Composition of Dried Torula Yeast ...	45
3-3.	Typical Elemental Analysis of Yeast	45
4-1.	SCP Recovery Using Organic Wastes	52
4-2.	Nitrogen and Protein Contents of Microbial Cells and Con- ventional Foods	57
4-3.	Summary of Methods Available for RNA Reduction in SCP	66
4-4.	Profile of Essential Amino Acids in Waterloo Fungal SCP and Other Protein Products	68
5-1.	U.S. HFCS Producers, Plant Sizes and Production Capacities	75
5-2.	HFCS Composition	89
5-3.	HFCS: Estimated U.S. Supply and Use, 1970-1980	91
5-4.	HFCS Production and Capacity in Europe, 1976	95
6-1.	Summary of Unit Operations Survey	102

CHAPTER 1

INTRODUCTION

BACKGROUND AND PURPOSE

The use of biological processes and materials for industrial purposes dates back thousands of years. The Babylonians and Sumerians, as early as 6000 B.C., used yeast to produce alcohol in the form of beer. By about 4000 B.C., the Egyptians were using the carbon dioxide produced by brewer's yeast to leaven bread. Over the centuries, fermentation processes were developed for production of various food products, such as vinegar, pickles and cheeses. It was not until the seventeenth century, however, that the existence of microorganisms was recognized, and another 200 years before Pasteur demonstrated in the 1870s that the products of fermentation were created by these microorganisms. The following 100 years saw the emergence of biochemistry, the development of industrial processes to produce solvents such as acetone and butanol, and the discovery of penicillin and other antibiotics. Although some fermentation processes were displaced after World War II by processes based on petroleum, fermentation has remained an important component of the food and pharmaceutical industries.

From this rich historical context, a new term, "biotechnology," has emerged to describe modern applications of biology (principally microbiology) in industry. Although much of the current excitement over biotechnology centers on recombinant DNA (a powerful tool for genetic engineering), this is only one factor contributing to modern biotechnology. Research is accelerating on both the biological elements (e.g., enzyme immobilization, new strains and fermentation kinetics) and the engineering elements (e.g., pretreatment of feedstocks, reactor design and product separation).

The advantages in using any microbial process in industry can be traced to a fundamental characteristic shared by all microorganisms: small

2 BIOTECHNOLOGY IN INDUSTRY

size and correspondingly high surface-to-volume ratio. This facilitates rapid transport of nutrients into the cell, thereby supporting a high metabolic rate. The commonly used analogy is highly appropriate: each cell operates as a miniature chemical factory. The diversity of microbial life is also an advantageous characteristic from an industrial use standpoint. Microorganisms can be found that exist under the widest possible range of conditions and that metabolize a variety of substrates. This makes it possible to design industrial fermentation processes that rely on inexpensive nutrients and operate under ambient conditions.

Aside from improved understanding of the biological and engineering principles, other, external factors have led to the recent emergence of biotechnology. These include:

- opportunities to use low-cost organic substrates (e.g., forestry residues or agricultural wastes) in favor of petroleum-based feedstocks;
- reduced energy consumption and increased safety due to lower operating temperatures and pressures; and
- reduced environmental hazard.

The above incentives, combined with new technological developments, have led to a consensus that biotechnology will be the most dynamic area of industrial innovation in the 1980s, analogous to the emergence of microelectronics in the 1960s. World markets for biotechnology products are projected to be on the order of hundreds of billions of dollars by the end of the century.

This book provides an evaluation of current commercial-scale processes and the new generation of technologies anticipated over the coming decade. More specifically, detailed process descriptions are provided in three generic application areas:

1. proteins and chemicals from pulp and paper industry wastes;
2. single-cell protein (SCP) for human consumption from organic waste materials; and
3. high-fructose corn syrups (HFCS) using immobilized enzyme technology.

Commercial facilities are operating or under construction for each of these applications. To adequately appreciate the new generation of technologies emerging from research and development, biotechnology is defined in terms of a set of unit operations, and representative examples are provided of state-of-the-art technology available at each process step.

SCOPE AND APPROACH

Detailed process descriptions for the three application areas stated above are based on the leading commercial facilities in North America. Unlike our earlier reference (*Biotechnology and Energy Use*, Clerman et al., Ann

Arbor Science Publishers, Inc., Ann Arbor, MI 1981), the descriptions are an in-depth examination of current state-of-the-art rather than a survey of research and development. The four processes selected are identified in Table 1-1 along with the general application areas they represent.

Information on the processes was obtained from the following sources:

- scientific and technical literature,
- patents,
- process, product and equipment brochures, and
- experts in industry and academia.

Because of the commercial potentials of these processes, there was some difficulty in obtaining detailed information. These proprietary restrictions varied as indicated in Table 1.1. Data on the HFCS process were the most closely protected, due to the highly competitive nature of this industry. Information was available on the demonstration plant for the Waterloo process for SCP production; however, descriptions of commercial facilities currently under construction in North America and Europe were held proprietary. To provide the best possible descriptions within the above constraints, we defined hypothetical plants closely modeled after actual commercial facilities and determined operating ranges, wherever possible, in lieu of precise figures. Even with this approach, some gaps remained that could not be filled by literature searches or consultation with experts.

ORGANIZATION OF THIS BOOK

Chapters 2 through 5 contain process descriptions. Each description is organized into the following general subsections:

- Evolution of Technology,
- Process Description,
- Product Uses,
- Cost Estimation, and
- Evaluation.

The content of most of these chapters is self-evident. The evaluation section is reserved for an assessment of the process in its current configuration and suggestions where improvement or modernization may be possible. In some cases, the suggested modifications are technologies covered in the unit operations survey (Chapter 7).

The unit operations survey was based on information obtained from the literature and organized in a format that can be supplemented and updated as necessary. More details regarding the scope of the unit operations survey and the approach taken are provided in Chapter 6, with detailed descriptions provided in Chapter 7. Chapter 8 offers a summary of the book and materials presented.