

# CONTENTS

## VOLUME V

### **Industrial Biotechnology** **1**

Jose Manuel Bruno-Barcena, *North Carolina State University, USA*

Faustino A. Sineriz, *University of Tucuman, Argentina*

1. Definition
2. History
3. The Best Biological Agent
  - 3.1 Microorganisms
  - 3.2 Recombinant Microorganisms
  - 3.3 Communities versus Pure Cultures
  - 3.4 Other Biocatalysts
4. The Best Possible Environment
  - 4.1 Selecting the Fermentation System
  - 4.2 Growth Rate and Production Rate
5. Separation and Purification
6. Pilot Plants
7. Good Manufacturing Practice (GMP)
8. Large Scale Fermentation
9. Biopesticide Production
10. Concluding Remarks

### **Enzyme Production** **21**

Rajni Hatti-Kaul, *Lund University, Sweden*

1. Introduction
2. Enzyme source
3. Microbial strain selection
4. Strain development
  - 4.1 Mutation and selection
  - 4.2 Hybridization
  - 4.3 Recombinant DNA technology
  - 4.4 Engineering the enzyme
5. Growth requirements of microorganisms
6. Fermentation
  - 6.1 Submerged fermentation
  - 6.2 Solid state fermentation
7. Isolation and purification of enzymes
  - 7.1 Disruption of cells and tissues
    - 7.1.1 Disruption of microbial cells
    - 7.1.2 Homogenisation of animal/plant tissue
  - 7.2 Clarification of culture broths/homogenates
    - 7.2.1 Filtration
    - 7.2.2 Centrifugation
    - 7.2.3 Pretreatment of broth to facilitate clarification
    - 7.2.4 Flotation
  - 7.3 Concentration and initial fractionation
    - 7.3.1 Evaporation
    - 7.3.2 Ultrafiltration
    - 7.3.3 Precipitation
    - 7.3.4 Adsorption
  - 7.4 Integrated technologies for downstream processing

- 7.4.1 Extraction in aqueous two-phase systems
- 7.4.2 Expanded bed adsorption
- 7.5 High resolution purification by chromatography
  - 7.5.1 Adsorption Chromatography
    - 7.5.1.1 Ion exchange chromatography
    - 7.5.1.2 Hydrophobic interaction chromatography
    - 7.5.1.3 Affinity chromatography
  - 7.5.2 Affinity chromatography
- 7.6 Crystallization for enzyme purification
- 8. Product formulation
- 9. Regulations during enzyme production

## **Production of Alcoholic Beverages**

63

Nduka Okafor, *Nnamdi Azikiwe University, Nigeria*

- 1. Alcoholic Beverages from Cereals
  - 1.1 Introduction
  - 1.2 Barley Beers
    - 1.2.1 Bottom-fermented beers
    - 1.2.2 Top fermented beers
    - 1.2.3 Raw materials for brewing
    - 1.2.4 Brewery processes
      - 1.2.4.1 Malting
      - 1.2.4.2 Milling of malt
      - 1.2.4.3 Mashing
      - 1.2.4.4 Mash operations
        - 1.2.4.4.1 Decoction methods
        - 1.2.4.4.2 Infusion methods
        - 1.2.4.4.3 The double mash method (cooker method)
      - 1.2.4.5 Wort boiling treatment
      - 1.2.4.6 Fermentation
      - 1.2.4.7 Storage or lagerung
      - 1.2.4.8 Packaging
  - 1.3 Sorghum beers
    - 1.3.1 Malting
    - 1.3.2 Mashing
    - 1.3.3 Fermentation
      - 1.3.3.1 Burukutu and pito:
      - 1.3.3.2 Talla
      - 1.3.3.3 Merissa
  - 1.4 Maize and wheat beers
    - 1.4.1 Bouza
    - 1.4.2 Busaa
    - 1.4.3 Tesguino
  - 1.5 Rice wines
- 2. Grape wines
  - 2.1 Processes in wine making
    - 2.1.1 Crushing of grapes
    - 2.1.2 Fermentation
      - 2.1.2.1 Control of fermentation
      - 2.1.2.2 Flavor development
    - 2.1.3 Ageing and storage
    - 2.1.4 Clarification
    - 2.1.5 Packaging
    - 2.1.6 Wine defects
    - 2.1.7 Wine preservation
    - 2.1.8 Classification of wines

3. Palm wines
  - 3.1 Obtaining the sap
  - 3.2 Composition of palm sap
  - 3.3 Microorganisms in palm wine
  - 3.4 Biochemistry of the Conversion of Palm Sap to Palm Wine
4. Alcoholic Beverages from miscellaneous substrates
  - 4.1 Honey Wines
  - 4.2 Wines from Bananas/plantains
  - 4.3 Wines from Plant and Fruit Juices
    - 4.3.1 Cactus fruit, *Opuntia* spp
    - 4.3.2 Sap of Agave
5. Spirit Beverages
  - 5.1 Measurement of the alcoholic strength of spirit beverages
  - 5.2 General principles in the production of spirit beverages
  - 5.3 Various spirit beverages

### Production of Organic Acids

100

Fernando Sanchez-Riera, *Bio-Technical Resources, USA*

1. Introduction
2. Citric Acid
  - 2.1 Historical Development
  - 2.2 Fermentation Basics
  - 2.3 Fermentation Processes
    - 2.3.1 Production with *A. Niger*
    - 2.3.2 Production with Yeasts
  - 2.4 Product Recovery
3. Lactic Acid
  - 3.1 Historical Development
  - 3.2 Fermentation Basics
  - 3.3 Fermentation Processes
    - 3.3.1 Raw Materials
    - 3.3.2 Process Parameters
    - 3.3.3 Process Configurations
  - 3.4 Product Recovery
4. Acetic Acid
  - 4.1 Overview
  - 4.2 Aerobic Production of Vinegar
  - 4.3 Process Configurations
  - 4.4 Anaerobic Production of Acetic Acid
5. Gluconic Acid
  - 5.1 Overview
  - 5.2 Production Process
6. Itaconic Acid
  - 6.1 Overview
  - 6.2 Production Process
7. Other Acids
  - 7.1 Propionic Acid
  - 7.2 Succinic Acid
  - 7.3 Pyruvic Acid
8. Conclusions

### Production of Antibiotics

126

Francisco Javier Casqueiro, *University of Leon, Spain*

Juan Francisco Martin, *University of Leon, Spain*

Santiago Gutierrez, *University of Leon, Spain*

1. Introduction
2.  $\beta$ -lactam Antibiotics as a Model System
3. Penicillin, Cephalosporin and Cephamycin Biosynthesis: An Overview
  - 3.1 Common Reactions to the Penicillins, Cephalosporins and Cephamycins Biosynthesis
    - 3.1.1 Formation of the ACV Tripeptide
    - 3.1.2 Cyclization of the ACV Tripeptide and Formation of the Isopenicillin N
    - 3.1.3 The Last Step in the Penicillin Biosynthesis: Conversion of Isopenicillin N into Penicillin G
  - 3.2 Specific Reactions for the Cephalosporin and Cephamycin Biosynthesis
  - 3.3 The Late Reactions in Cephamycin Biosynthesis
  - 3.4 Alternative Pathway for the Cephamycin C Biosynthesis
4. Regulation of Penicillin Biosynthesis
  - 4.1 Carbon Source Regulation of Penicillin Formation
  - 4.2 Nitrogen Source Regulation
  - 4.3 Regulation by Lysine
  - 4.4 Regulation by Glutamate and Glutamine
  - 4.5 Regulation by Ph
5. Clustering of Genes for the Biosynthesis of  $\beta$ -lactam Antibiotics
6. Strain and Process Improvements
7. Application of the DNA Recombinant Technology to Increase the Antibiotic Production in Filamentous Fungi: Engineering of the  $\beta$ -lactam Antibiotic Pathways
  - 7.1 Increase of the Gene Copy Number
    - 7.1.1 Increase of the Gene Copy Number of the *pcbC* and *penDE* Genes of *Penicillium chrysogenum*
    - 7.1.2 Increase in the Copy Number of the *cefEF* Gene of *Acremonium chrysogenum*
    - 7.1.3 Increase of the *pcbC* Copies on *Penicillium chrysogenum* Industrial Strains
  - 7.2 Increasing the Expression of One or More Genes of the Pathway and Changing their Promoter Regions in Order to Elude the Usual Regulation of These Genes
    - 7.2.1 Over-expression of the *pcbAB* Gene of *Aspergillus nidulans* under the Promoter of the *alcA* Gene
    - 7.2.2 Overexpression of the Genes *IPNS* (*ipnA*) and *AAT* (*acyA*) in *A. nidulans* under the *alcA* Promoter (*alcAp*)
    - 7.2.3 Production of Penicillins in *Acremonium chrysogenum*
  - 7.3 Increasing the Efficiency of the Pathway by Overexpression of Genes that could Improve the Flux of Nutrients or Metabolites to the Pathway
    - 7.3.1 Intracellular Expression of the Vitreoscilla Hemoglobin
    - 7.3.2 Production of Cephalosporin Intermediates by Recombinant *P. chrysogenum* strains
    - 7.3.3 Increasing the Flux of Phenylacetic Acid to the Penicillin Biosynthesis by Disruption of the *phacA* Gene
    - 7.3.4 Increasing the Pool of  $\alpha$ -aminoadipic Acid by Disruption of the *lys2* Gene

## Bioplastic and Biopolymer Production

152

Ian W. Sutherland, *University of Edinburgh, UK*

1. Bioplastics
  - 1.1 Introduction
  - 1.2 Occurrence & Composition
  - 1.3 Biosynthesis
  - 1.4 Products
  - 1.5 Production and recovery
2. Biopolymers (Polysaccharides)
  - 2.1 Introduction
  - 2.2 Occurrence and Composition
  - 2.3 Polysaccharide biosynthesis
    - 2.3.1 Biosynthetic mechanisms
    - 2.3.2 Genetics and regulation of exopolysaccharides synthesis
  - 2.4 Commercial products

- 2.4.1 b -D-Glucans
- 2.4.2 a -D-Glucans
- 2.4.3 Bacterial Alginates
- 2.4.4 Gellan and Related Polymers
- 2.4.5 Hyaluronic Acid and Heparin
- 2.4.6 Succinoglycan and galactoglucans
- 2.4.7 Xanthan
- 2.5 Production of exopolysaccharides
- 3. Future Developments
  - 3.1 Bioplastics
  - 3.2 Biopolymers

**Production of Biosurfactants**

176

Rolf K. Hommel, *CellTechnologie Leipzig, Germany*  
 Hans-Peter Kleber, *University of Leipzig, Germany*  
 Faustino A. Sineriz, *University of Tucuman, Argentina*

- 1. Introduction
- 2. Evaluation
- 3. Structural Types and Producers
- 4. Biosynthesis and Regulation
  - 4.1 Rhamnolipids
  - 4.2 Surfactin
  - 4.3 Other Biosurfactants
- 5. Genetics
- 6. Production
  - 6.1 Screening of Producers
  - 6.2 Factors affecting production
    - 6.2.1 Generic Factors
    - 6.2.2 Precursors
  - 6.3 Batch Cultivation
    - 6.3.1 Glycolipids
    - 6.3.2 Lipopeptides and Lipoproteins
    - 6.3.3 Polymeric Surfactants
  - 6.4 Semicontinuous Cultivation
  - 6.5 Continuous Cultivation
  - 6.6 Processing, Purification, Economy
  - 6.7 Chemical Synthesis and Modifications
- 7. Properties
  - 7.1 Biophysical Properties
  - 7.2 Biological Activities
- 8. Potential Applications
  - 8.1 Environmental Control
  - 8.2 Food
  - 8.3 Cosmetics
  - 8.4 Medicine and Plant Protection
- 9. Concluding Remarks

**Industrial Recombinant Protein Production**

203

Francisco Kuri-Brena, *Probiomed S.A. de C.V., México*  
 Laura A. Palomares, *National Autonomous University of Mexico, México*  
 Octavio T. Ramirez, *National Autonomous University of Mexico, México*

- 1. Introduction
- 2. Markets and Products
- 3. The first step: Selection of an expression system

- 3.1 The importance of the vector and the promoter
- 3.2 The importance of the host
  - 3.2.1 Prokaryotes
  - 3.2.2 Yeast
  - 3.2.3 Filamentous fungi
  - 3.2.4 Animal cells
  - 3.2.5 Transgenic animals or plants
- 4. Bioprocess engineering considerations
  - 4.1 Bioreactor design and operation
  - 4.2 Operational strategies for recombinant protein production
    - 4.2.1 Plasmid instability and copy number
    - 4.2.2 Induction strategies
    - 4.2.3 Production conditions
      - 4.2.3.1 Special considerations for animal cells
  - 4.3 Downstream Processing Considerations
- 5. Biosafety and Regulations
  - 5.1 Containment regulations
- 6. Facility Design
  - 6.1 Validation of facilities
- 7. Product Characterization

**Index** **245**

**About EOLSS** **255**