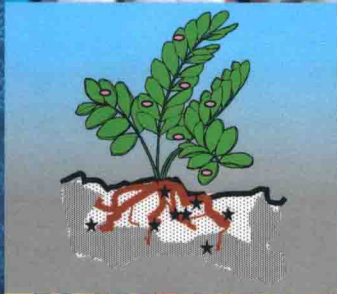
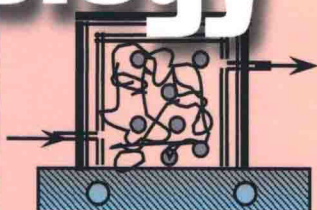
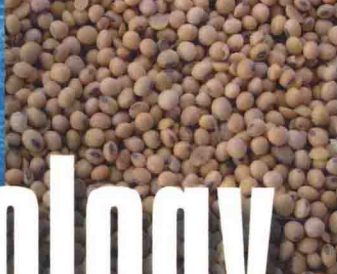


Biotechnology

An Introduction



M. Pele &
C. Cimpeanu



WIT PRESS

Biotechnology

An Introduction

Maria Pele & Carmen Cimpeanu

*University of Agronomic Sciences and Veterinary Medicine,
Bucharest*



WITPRESS Southampton, Boston



Maria Pele & Carmen Cimpeanu
University of Agronomic Sciences and Veterinary Medicine, Bucharest

Published by

WIT Press

Ashurst Lodge, Ashurst, Southampton, SO40 7AA, UK
Tel: 44 (0) 238 029 3223; Fax: 44 (0) 238 029 2853
E-Mail: witpress@witpress.com
<http://www.witpress.com>

For USA, Canada and Mexico

WIT Press

25 Bridge Street, Billerica, MA 01821, USA
Tel: 978 667 5841; Fax: 978 667 7582
E-Mail: infousa@witpress.com
<http://www.witpress.com>

British Library Cataloguing-in-Publication Data
A Catalogue record for this book is available
from the British Library

ISBN: 978-1-84564-666-0
eISBN: 978-1-84564-667-7

Library of Congress Catalog Card Number: 2012935423

No responsibility is assumed by the Publisher, the Editors and Authors for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. The Publisher does not necessarily endorse the ideas held, or views expressed by the Editors or Authors of the material contained in its publications.

© WIT Press 2012

Printed by Lightning Source, UK.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the Publisher.

Biotechnology

An Introduction

WIT*PRESS*

WIT Press publishes leading books in Science and Technology.

Visit our website for the current list of titles.

www.witpress.com

WIT*eLibrary*

Home of the Transactions of the Wessex Institute, the WIT electronic-library provides the international scientific community with immediate and permanent access to individual papers presented at WIT conferences.

Visit the WIT eLibrary at <http://library.witpress.com>

Preface

Strong development of life sciences and biotechnology began in the twentieth century – the century of informatics, biotechnology and telecommunications – with a further exponential growth that opened a vast development potential of the global economy resulting in overall improvement of life in all respects. In this context, the development of training to provide for present and future highly qualified specialists in various branches of biotechnology is not only useful but absolutely necessary.

Biotechnology plays, at the beginning of this millennium, the determinant role for society that was held by physics and chemistry in the late nineteenth century industrial revolution.

To achieve the best results from biotechnology applications it is particularly important to have a thorough understanding of physical mechanisms, biological and obvious chemical processes that occur in the development of various bioprocesses.

This book is written for undergraduate, graduate or postgraduate students studying various aspects of biotechnology, as well as researchers, engineers, teachers and commercial agents such as microbiologists, biochemists, biologists, bioengineers, chemists, veterinarians, physicians and food engineering and pharmaceutical chemists who need at least basic knowledge in the field.

This book is a starting point, a first step for future professionals as it gives readers the basics in various areas in biotechnology applicable.

It is known that biotechnology is a multidisciplinary and interdisciplinary science, using basic sciences such as agriculture, chemistry, biology, microbiology, mathematics, engineering, and others, to develop new fields of life sciences like crop improvement, genetic engineering, in vitro cell cultivation, vitamins, hormones, biofuels, healthy animals, human health, diagnostics, protein engineering, food safety, biomaterials energy and environment management and so on.

Thus the present book covers in a comprehensive form all topics of biotechnology. For each aspect approached it publications of most famous authors in the respective fields of biotechnology from beginning to the latest

publications have been referenced.

The presentation in each chapter is simple and systematic to give a thorough understanding of the core principles of each area, the interrelationships between biotechnology and other disciplines, and how biotechnology affects our everyday lives. It clearly explains the basic concepts of each step to be followed in developing a biotechnology process, such as determining which living agent will be used – microorganism, plant cell, animal cell; parameters that have to be evaluated for each process; product recovery and conditioning operations; bioreactors; and modelling. Their functions are adequately highlighted.

The first chapter considers the history of the exciting developments in biotechnology. Finally, the book presents an annex which contains some of the most important discoveries that led to the development of biotechnology today.

Recent developments in different fields have also been included to provide a contemporary understanding of the subject and the large scope of biotechnology applications.

The last chapter contains some of the most recent examples of trends in this cutting-edge science application, such as the green chemistry and environmental biotechnology.

By presenting the new directions in every area of biotechnology the authors hope to help young researchers and students to choose their best career path in biotechnology.

We are grateful to WIT Press for publishing this book and to the members of the Scientific Advisory Board for careful guidance and diligent support in this book.

Special thanks go to the chief editor Dr Carlos Brebbia and Mrs Elizabeth Cherry, who have supported and encouraged the whole period of elaboration of the publication.

Maria Pele & Carmen Cimpeanu, 2012

Contents

Preface	xi
1 Definitions and historical evolution of biotechnology	1
1 Introduction	1
2 Definitions of biotechnology.....	1
3 Historical evolution of biotechnology.....	4
3.1 Biotechnologies in ancient and Middle Ages history	4
3.2 Classical biotechnology.....	5
3.3 Contemporary biotechnology.....	7
References	9
2 Microorganisms	11
1 General aspects about microorganisms.....	11
2 Characterization of useful microorganisms	14
2.1 Viruses.....	15
2.1.1 Characterization of viruses.....	17
2.1.2 Multiplication of viruses.....	17
2.2 Bacteria.....	21
2.2.1 Bacterial cell structure.....	24
2.2.2 Bacteria multiplication	27
2.2.3 Chemical composition of bacteria.....	29
2.2.4 Classification of bacteria.....	30
2.3 Fungi	32
2.3.1 General morphological characters	33

2.3.2	General physiological characters of fungi	39
2.3.3	Reproduction	40
2.3.4	Classification of fungi	45
3	Metabolism of microorganisms and their metabolites	46
3.1	Energy metabolism	47
3.2	Transport of substances through the membranes	50
3.3	Catabolism	52
3.3.1	Carbohydrate catabolism	52
3.3.2	Lipid catabolism	59
3.3.3	Protein catabolism	59
3.3.4	Hydrocarbon catabolism	61
3.3.5	Compounds with one carbon atom catabolism	62
3.3.6	Alcohol catabolism	63
3.3.7	Fatty acid catabolism	64
3.4	Anabolism	64
3.5	Microbial growth curve	66
3.6	Metabolites	69
3.6.1	Primary metabolites	69
3.6.2	Secondary metabolites	72
	References	74

3 Biotechnological processes 77

1	Biotechnological process assessment criteria	77
1.1	Productivity	78
1.2	Efficiency	79
1.3	Final concentration	81
1.4	Energy consumption	81
1.5	Unproductive consumptions	81
1.6	The quality of biological material	82

2	Biosynthesis processes	82
2.1	Surface cultures	84
2.2	Submerged cultures	85
2.2.1	Anaerobic fermentation	86
2.2.2	Aerobic fermentation	87
2.3	Prefermentation operations	88
2.3.1	Culture media	90
2.3.2	Sterilization	93
2.3.3	Inoculum	96
3	Bioconversion processes	96
3.1	Amino acids through bioconversion	99
3.2	Vitamins through bioconversion	101
3.3	Steroid bioconversion	102
3.4	Bioconversion of antibiotics	104
3.5	Other bioconversions	106
4	Cell culture process	106
4.1	Biotechnology of plant cell and tissue cultures	108
4.1.1	Equipment required for cell cultures	109
4.1.2	Plant cell and plant tissue cultures <i>in vitro</i>	110
4.1.3	Plant cell and plant tissue culture optimization	116
4.2	Biotechnology of animal cell cultures	122
4.2.1	Industrial culture of animal cells	126
4.2.2	Uses of animal cell cultures	132
	References	137

4 Bioreactors 145

1	Introduction	145
2	Types of bioreactors	146
2.1	Operating mode of bioreactors	150

3	Parameters of bioprocesses	152
3.1	Temperature	152
3.2	Agitation	156
3.3	Aeration	159
3.3.1	Solid-state cultivation – stationary cultivation	160
3.3.2	Flasks shaken	162
3.3.3	Stirring bioreactors	164
3.3.4	Bubble-driven bioreactors	164
3.3.5	Packed-bed bioreactors	165
3.3.6	Fluidized bed bioreactors	166
3.4	Carbon dioxide	168
3.5	pH	169
3.6	Redox potential	171
3.7	Foaming	172
3.8	Viscosity	174
3.9	Pressure	175
3.10	Flow	175
	References	175

5 Downstream processing 179

1	Introduction	179
2	Disintegration of cells or tissues	181
3	Extraction	183
4	Filtration methods	186
4.1	Filtration and microfiltration	187
4.2	Membrane filtration	192
4.2.1	Dialysis	193
4.2.2	Ultrafiltration	195
4.2.3	Reverse osmosis	196

5	Centrifugation	197
6	Precipitation	200
6.1	Precipitation with mineral salts	200
6.2	Precipitation with a non-solvent	201
6.3	Crystallization	203
7	Chromatography	205
7.1	Adsorption chromatography	209
7.2	Partition chromatography	210
7.3	Ion exchange chromatography	210
7.4	Permeation chromatography	211
7.5	Affinity chromatography	211
7.6	Covalent chromatography	213
7.7	Electrophoresis	214
8	Drying	215
9	Evaluation of separation process	223
	References	225
6	Bioprocesses modelling	229
1	Introduction	229
2	Kinetic models for cell mass growth rate and formation of products	230
3	The role of oxygen in aerated processes	234
	References	238
7	Some practical examples of biotechnologies	243
1	Introduction	243
2	Food biotechnology	244
2.1	Wine	244
2.2	Food biotechnology and ethics	251

3	Biotechnology of antibiotics	255
4	Enzymes biotechnology	261
5	Environmental biotechnology	267
5.1	Bioremediation	271
5.1.1	Microbial bioremediation	271
5.1.2	Phytoremediation	275
5.2	Prevention	282
5.2.1	Process improvement and product innovation	283
5.2.2	Green energy	286
5.2.3	Organic agriculture	288
	References	291
	Annexe	299
	Index	309

1

Definitions and historical evolution of biotechnology

1 Introduction

It is recognized, worldwide, that the 20th century was the century of developing chemistry, the 21st century is in particular the promoter of biotechnology, information and telecommunications.

In the near and distant future it is expected from biotechnology to solve most problems facing humanity today – pollution, lack of food, diseases, population growth, etc.

It is expected that, by 2025, biotechnology developments will exceed the net economic and social informatics and telecommunications. For example, regarding the financial aspect, it is considered that global bioproducts obtained through biotechnology only will exceed 100 billion dollars annually after 2015.

Today it is considered that a country that does not know or cannot develop an industry based on biotechnology, covering at least partially their country needs, is a country that condemns itself to an economic and intellectual slavery and to a life well below the decent standards.

2 Definitions of biotechnology

Defining biotechnology concerned the scientific community especially in the last two decades, because of the need of a special effort of synthesis and clarity, goals difficult to achieve in the context of an interdisciplinary and multidisciplinary field.

The term *biotechnology* was first used in 1910 by Thomas H. Morgan, with the meaning of “the processes used for obtaining products from raw materials with the help of living organisms”. The word *biotechnology* is a combination of two Greek words – *bio*, from *bios* which means life and *technology* is the word used since the time of Cicero.

2 BIOTECHNOLOGY: AN INTRODUCTION

In the historical evolution of the biotechnology concept we can find several definitions like:

- *Biotechnology is the use of biological systems in industrial processes* – Weide, Paca & Knorr [1].
- *Biotechnology is actually the utilization of bacteria, fungus, animal and plant cell culture whose metabolism and biosynthesis capacity are oriented to manufacture specific substances* – Sasson [2].
- *In relation to its complex and interdisciplinary nature “the concept of biotechnology” could be that of the science of processes with economic and industrial applications based on biological systems work. It involves the use of microorganisms in nature, selected by conventional techniques or rescheduled by genetic engineering techniques. There are active components, of plants, animals and cells derived from plants and animals. In this framework we can include the processes of modern and traditional technologies as well as food, fermentation, of biosynthesis and conventional chemical pharmaceuticals* – Zarnea & Topala [3].
- *Biotechnology is broadly defined as using living organisms, or products of living organisms, for human benefit (or for benefit of human surroundings) to make a product or solve a problem* – Thieman & Palladino [4].

Even today, the definition of biotechnology still attracts many debates in the context of simultaneous development of other related sciences. Ultimately it has outlined two major ways of thinking about biotechnology. Thus the European Federation of Biotechnology, which is the most prestigious professional organization on the continent, bringing together leading experts in the field, grouped in national branches, adopted in 1989 the most comprehensive definition used also by European Commission, in line with majority of opinions and unchanged so far:

- *Biotechnology is the integration of natural sciences and engineering sciences in order to achieve the application of organisms, cells, parts thereof and molecular analogues for products and services* – The European Federation of Biotechnology [5].
- *The application of science and technology to living organisms, as well as parts, products and models thereof, to alter living or non-living materials for the production of knowledge, goods and services* – Biotechnology [6].

In parallel with the European school, which emphasizes the definition adopting the characteristics of biotechnology to study productive systems, the American concept is broader. The American definition of biotechnology includes the study of any techniques that uses living organisms (or parts thereof) in order to obtain or modify products, to improve the characteristics of plants and animals or to develop microorganisms with specific uses. In this characterization we can find again the

genetic techniques for improving the characteristics of plants and animals besides the technological development itself.

- *The application of molecular and cellular processes to solve problems, conducts research, and creates goods and services. Biotechnology includes a wide range of technologies that manipulates cellular, sub-cellular, or molecular components in living things in order to obtain products, discover new information about the molecular and genetic basis of life, or to modify plants, animals and micro-organisms for carrying desired characteristics. Such technologies include, but are not limited to: genetic engineering (e.g. recombinant DNA, gene therapy, cloning, antisense); hybridoma technology (production of monoclonal antibodies); polymerase chain reaction or PCR amplification; gene mapping; DNA sequencing; restriction fragment length polymorphism (RFLP) analysis; and protein engineering – Biotechnology in USA [7].*
- *The use of living organisms or other biological systems in the manufacture of drugs or other products or for environmental management, as in waste recycling; includes the use of bioreactors in manufacturing, microorganisms to degrade oil slicks or organic waste, genetically engineered bacteria to produce human hormones, and monoclonal antibodies to identify antigens – The American Heritage® Science Dictionary [8].*
- *Biotechnology. The scientific manipulation of living organisms, especially at the molecular genetic level, to produce useful products. Gene splicing and use of recombinant DNA (rDNA) are major techniques used – Allender-Hagedorn & Hagedorn [9].*

In general terms, the definitions used currently are those adopted by the European Federation of Biotechnology and European Commission.

However, there is a trend in the expansion of this concept, specialists including in biotechnology the direct creation of one of the main components of the biotechnological system – the biological agent – using recombination and cell engineering techniques and genetic engineering too.

Defining and delimiting the concept of biotechnology became more complicated, this field being integrated with various branches of science and technology such as molecular biology, genetics, genetic engineering, biochemistry, chemistry, microbiology, physical chemistry, electrochemistry, chemical technology, thermodynamics, mathematics, physics, geology and computer science.

Directions of development in the present period of biotechnology are subject to the world needs – food, energy, reuse of raw materials, avoiding/removing pollution, health care, replacing the classical chemical technologies energy consuming and pollutants producing, hydrometallurgical biotechnology (for example extraction of chemical elements – metals – from ore), etc.

3 Historical evolution of biotechnology

In accordance with the relatively recent emergence of the term and the development and definition as a separate branch of science and industry only in the second half of the 20th century, most people believe that biotechnology is very modern, but considering the strict definition of its use of living organisms to produce products and/or services – in fact biotechnology is one of the oldest scientific processes used by humans, with deep historical roots.

Seventy years ago, the name biotechnology was still little used and the scientific and technological activities in this sector are known as the “industrial microbiology” referring to the industrial use of production potential of cultures of microorganisms.

The discovery of DNA structure by Watson and Crick, a scientific revolution in the biotechnology, led to the creation and evolution of unprecedented genetic engineering and has changed the fundamental concepts of biotechnology. As a result, the majority of population, and even a number of specialists from the last decades of 20th century, believed that biotechnology is only one that includes genetic engineering and DNA.

Today it is well established that the development of biotechnology can be divided into three main stages from historical point of view – ancient biotechnology, classic biotechnology and modern biotechnology.

3.1 Biotechnologies in ancient and Middle Ages history

Thousands of years ago, the first settled population, the agricultural one, stored seed plants with properties suitable for use as food, in order to plant them the following year and also kept the biggest, most beautiful animals for breeding, rather than consume them without discrimination. Through these unconscious artificial selection by farmers and livestock breeders in those days gradually, slowly, new varieties of plants and animals were produced, which features wild shape but were also modified for the purposes of presenting benefits to humans such as bigger fruits, plants resistant to drought or/and flood, animals with more meat and higher yields.

The use of microorganisms in the food processing is lost in the darkness of time. Bread, cheese, wine and beer were reached long ago by various processes of fermentation. Written sources – papyruses, clay tablets, the Bible – provided evidence that Sumerians and Babylonians produced beer thousands years before Christ. Egyptians prepared this using leaven bread with 4000 years before Christ while in the Middle East wine was produced.

Thus the process of alcoholic fermentation was observed by all populations as a natural phenomenon that takes place during storage of fruits. Carbohydrates are the energy source and feeding source for a number of microorganisms, in this case

yeast which was found on fruits. We can say that this process was the first known biotechnological process. Evidence of vine cultivation in order to manufacture wine from grapes, dated 2000 years before Christ, and was discovered in Assyria. Existing evidence also shows that on the actual Romanian territory, our ancestors grown vines in order to obtain the wine, at least 2000 years before Christ [10].

Although beer is achieved by a process more complicated than a simple alcoholic fermentation, it was acquired by Sumerians, Babylonians and the Egyptians thousands of years before Christ. The process of beer manufacturing was described on the famous Sumerian slate “Monument Bleu” exposed now at the Louvre Museum [11]. In ancient Babylon the beer was produced according to precise instructions given by the Laws of Hammurabi [12]. Existing evidence shows that Egyptian beer was an amber beer while Babylonian beer was mostly blonde.

In that prehistoric times the preparation of bread using the leaven and obtaining vinegar from fermented juices were processes known by virtually all existing populations.

In Europe, the development of vine cultivation in order to obtain the wine has been promoted by the Greeks and Romans. Thus the Emperor Probus Marcus Aurelius introduced vine cultivation in the valley of the Rhine and Moselle rivers between 276 and 282 after Christ.

Ethanol was recognized for the first time around the year 1150, obtained from distillation of alcoholic beverages produced by fermentation. The term “ethanol” means “spirit/soul of wine” was given by Paracelsus from the Arabic phrase “al ko’hol” means that the finest, most noble.

Ancient biotechnology is not represented only by drinks or bakery products obtained with the aid of microorganisms but also by a number of prepared foods such as fermented milk products [13].

Textiles, footwear and other consumer products to people by natural means using very often part of microorganisms as well were made.

3.2 Classical biotechnology

The term “classical biotechnology” is used for all processes involving fermentation phenomena and the existing ones since antiquity. However, when we speak about “classical biotechnology” we think of biotechnology developed in the mid-19th century up through the 1970s. During this period the fermentation technologies were improved; processes were developed using microorganisms to produce ethanol, acetone, acetic acid, butane and solvents for paints; leguminous plants were inserted into crop rotation in order to enrich the soil with nitrogen; vaccines and Mendel’s laws were discovered; fermentation processes for wastewater treatment and municipal solid waste were developed.