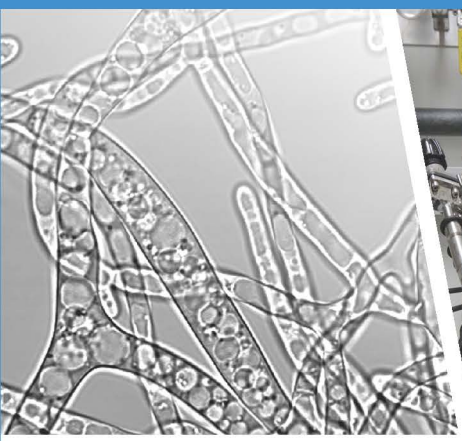
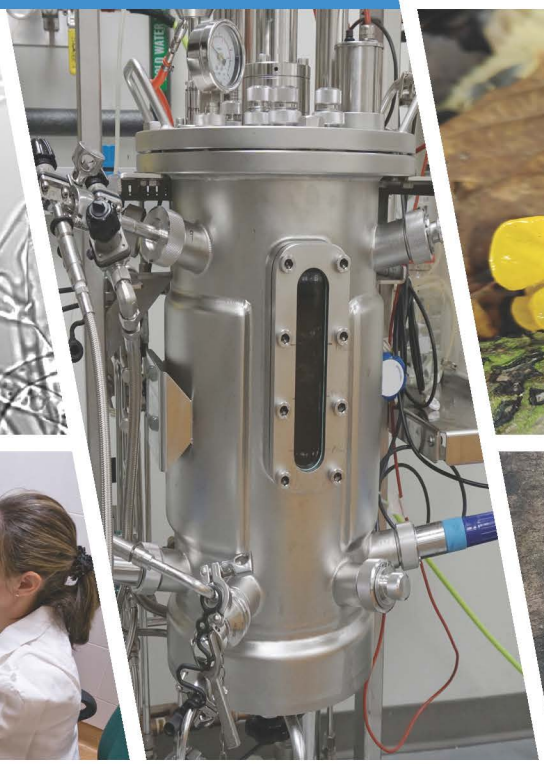


Microbial biotechnology in the laboratory and practice

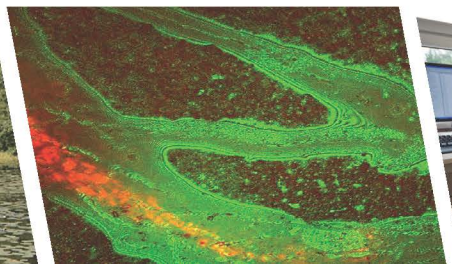
Theory, exercises and specialist laboratories

edited by Jerzy Długoński

WYDAWNICTWO UNIWERSYTETU ŁÓDZKIEGO
JAGIELLONIAN UNIVERSITY PRESS




Microbial biotechnology in the laboratory and practice

WYDAWNICTWO
UNIWERSYTETU
ŁÓDZKIEGO

Microbial biotechnology in the laboratory and practice

Theory, exercises and specialist laboratories

edited by Jerzy Długoński

WYDAWNICTWO UNIWERSYTETU ŁÓDZKIEGO
JAGIELLONIAN UNIVERSITY PRESS
Łódź–Kraków 2021

Jerzy Długoński – University of Łódź, Faculty of Biology and Environmental Protection
Institute of Microbiology, Biotechnology and Immunology
Department of Industrial Microbiology and Biotechnology, 90-237 Łódź, Banacha St. 12/16, Poland

Published by Łódź University Press & Jagiellonian University Press
First edition, Łódź–Kraków 2021

ISBN 978-83-8220-150-5 – paperback Łódź University Press
e-ISBN 978-83-8220-420-9 – electronic version Łódź University Press
ISBN 978-83-233-4984-6 – paper back Jagiellonian University
e-ISBN 978-83-233-7215-8 – electronic version Jagiellonian University

Łódź University Press
Lindleya St. 8, 90-131 Łódź, Poland
www.wydawnictwo.uni.lodz.pl
e-mail: ksiegarnia@uni.lodz.pl
phone +48 (42) 665 58 63

WYDAWNICTWO UNIWERSYTETU ŁÓDZKIEGO

Jagiellonian University Press
Editorial Offices, Michałowskiego 9/2, 31-126 Kraków, Poland
Phone: +48 12 663 23 80, Fax: +48 12 663 23 83
Distribution: Phone: +48 12 631 01 97, Fax: +48 12 631 01 98
Cell Phone: + 48 506 006 674, e-mail: sprzedaz@wuj.pl
Bank: PEKAO SA, IBAN PL 80 1240 4722 1111 0000 4856 3325

JAGIELLONIAN UNIVERSITY PRESS

www.wuj.pl

The book is available in the Columbia University Press catalog:
https://cup.columbia.edu

Table of contents

Foreword

Life sciences, including biotechnology, microbiology, and related disciplines, are among the fastest growing areas of science since the second half of the 20th century. The discovery of the DNA structure in 1953 by Watson and Crick, as well as the development of modern research techniques and methods that allow the analysis and description of the processes taking place in organisms with great accuracy in a short period of time, have contributed to this. This is reflected in the ever-increasing number of scientific publications in the international literature, as well as the emergence of new fields of study at universities, often of an interdisciplinary nature, enabling students to acquire knowledge from different areas of science and facilitating their pursuit of employment in the ever-changing jobs market. Such a role is fulfilled, among others, by biotechnology, which combines the latest achievements of the intensely developing natural sciences with their use in various areas of human activity, as shown in the figure below. The areas of knowledge and practice that are considered both in the upper and lower fields, constitute the main topics that are discussed in this handbook.

The first two chapters discuss the principles of obtaining strains of microorganisms used in practice, the methods of their cultivation, improvement in terms of properties useful from the application point of view, and the most important issues related to storage in conditions conducive to the survival of the microorganisms and maintenance of their desired properties. This part of the handbook omits basic microbiological techniques, assuming that they are known from general microbiology courses. The focus is on familiarising the reader with the latest analytical techniques used in microbiology, biotechnology, and related sciences – confocal and fluorescence microscopy, spectrofluorimetry, isotope techniques,

chromatography, atomic absorption spectrometry. The principles of aerial and satellite remote sensing are also presented here, enabling rapid detection and objective assessment of threats caused by bacterial, viral, and fungal pathogens in agricultural, forest, and urban green areas (Figure 2.6.1.4).

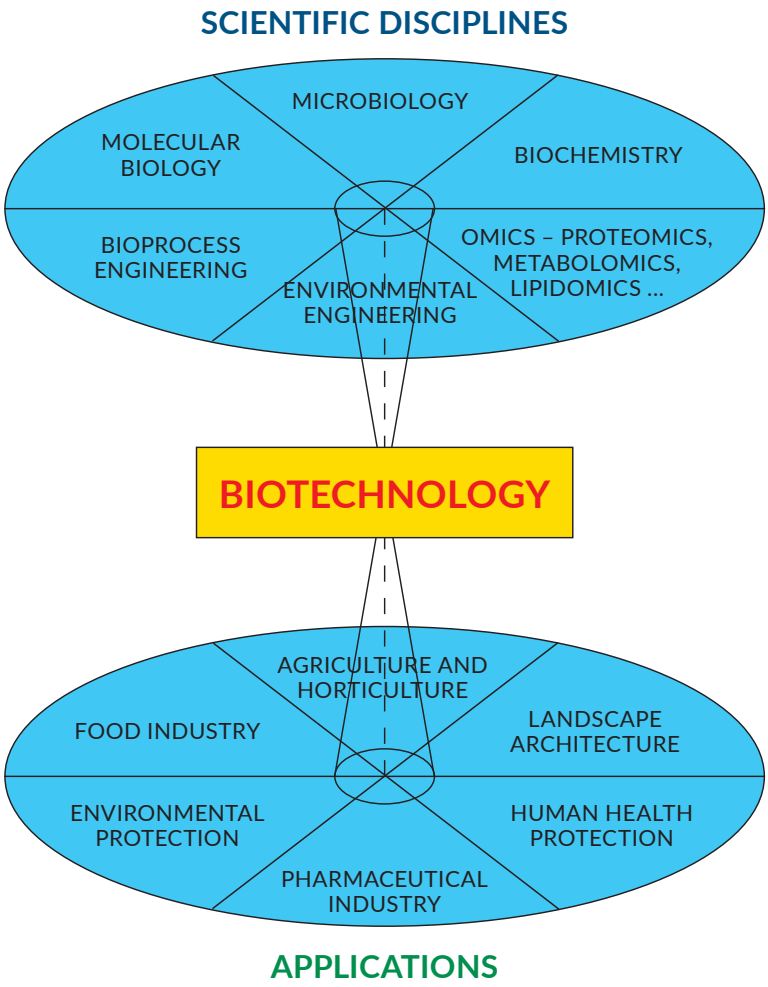


Fig. 1. Relationships between selected natural sciences and areas of human activity.

The third chapter is devoted to the identification of previously isolated bacteria and fungi, considering the use of the latest techniques, including MALDI-TOF/TOF and LC-MS/MS methods already presented in the previous chapter.

The fourth chapter presents the possibilities of using microorganisms on an industrial scale. This part of the publication is largely based on the previously published handbook Microbiological Biotechnology. Exercises and specialist laboratories edited by Jerzy Długoński, which was published in 1997 by the University of Lodz Publishing House. Due to the fact that the layout of that handbook gained the approval of both teachers and students, the division of the discussed issues into biosynthesis, fermentation, and biotransformation processes was maintained, at the same time updating its contents and introducing additional subsections concerning the production of biosurfactants and food produced with the participation of microorganisms.

The rationale for the development of chapter five, the most extensive part and at the same time reflecting the interdisciplinary approach of the authors of individual subchapters to solving problems related to the protection of human health and the environment. These issues were explored in earlier publications, including an extensive monograph Microbial Biodegradation. From Omics to Function and Application (edited by Jerzy Długoński, Caister Academic Press, Norfolk 2016) as well as cooperation with scientists from other fields of science in Poland and abroad.

Chapter six is a collection of examples demonstrating the benefits of using the latest analytical techniques in scientific research and in the protection of health and the environment in a broad sense.

The final part of the handbook (chapters seven and eight) is a list of media and buffers with their composition, used in the practical part of individual subchapters, as well as macroscopic and microscopic images of fungal strains used by the authors of the handbook in research and didactics.

The handbook was prepared on the basis of many years of didactic and scientific experience of the authors, conducting classes in various fields and specialties, which has bestowed a multilateral character on the work. For this reason, the authors of the handbook are convinced that it will be useful for students of biotechnology, microbiology, and ecology, as well as interdisciplinary faculties such as eco-town, environmental

protection, biomonitoring and ecological biotechnology or urban revitalisation.

The authors of the handbook would like to express their heartfelt thanks to the Reviewers, Prof. Grażyna Płaza, Prof. Jerzy Falandysz, Prof. Dominik Kopeć and Dr. Alwyn Fernandes whose valuable comments and remarks contributed fruitfully to the preparation of its final version. Special thanks are also addressed to Aleksandra Góralczyk-Bińkowska, MSc, Dr. Anna Jasińska, Dr. Katarzyna Zawadzka, Dr. Andrzej Długoński and Małgorzata Krokocka, MSc for taking care of the careful editorial and graphic design of the handbook.

With a view to a possible next edition of the handbook, the authors will be grateful if any critical comments were sent to the scientific editor (jerzy.dlugonski@biol.uni.lodz.pl).

Łódź, 12 December 2019

Jerzy Długoński

Foreword to the handbook *Microbiological Biotechnology*. Exercises and specialist laboratories from 1997

According to the definition adopted by the European Federation of Biotechnology, "biotechnology is an integrated application of natural and engineering sciences with the aim of using living organisms, cells and their component parts for products and services". One of the sciences that is part of biotechnology is industrial microbiology, which deals with the use of microorganisms in practice. The most important factors that favour the widespread use of microorganisms in biotechnological processes include relatively easy, large-scale culturing techniques, the ability to use various raw materials as a source of carbon and energy, high metabolic rates, the well-known genetic structure of some species and the ease of genetic manipulation. It should be noted, however, that to take full advantage of the properties of microorganisms, it is necessary to know the physiology of industrial strains and the mechanisms regulating the changes taking place inside the cell. These issues are the main subject of specialist laboratories and exercises in industrial microbiology for students of the 4th year of biotechnology and microbiology at the Faculty of Biology and Environmental Protection, University of Łódź. Students, when they join classes conducted in semesters 8 and 9, and already have a considerable amount of knowledge in the fields of chemistry, general microbiology, biochemistry, mycology and microbial genetics. Therefore, the handbook does not discuss the basic techniques used in laboratory work, but focuses mainly on issues concerning the physiology of industrial microorganisms and their use in biotechnological processes.

The handbook has been developed on the basis of many years of didactic and scientific experience of the employees of the Department of Industrial Microbiology of the University

of Łódź. The authors would like to express their deep gratitude to the organiser and long-term manager of the Department of Industrial Microbiology, Prof. Leon Sedlaczek, without whose contribution to the didactic and scientific achievements of the Department, it would not be possible to develop this handbook.

Equally sincere thanks are due to the Reviewer of this study, Prof. Aleksander Chmiel, whose valuable remarks and suggestions have helped to create the final version of the handbook.

The handbook was written primarily for students specialising in biotechnology and microbiology. Nevertheless, it will be useful for students of other specialisations interested in microbiology and for various people involved in the use of microorganisms in practice.

Łódź, 27 June 1996

Jerzy Długoński

1.

Methods of screening, culturing, improvement and storage of microorganisms of industrial importance

1.1. General characteristics of microorganisms used in biotechnological processes

Although more than a century and a half has passed since Louis Pasteur created microbiology and biotechnology, it is estimated that only 5% of microfungi (filamentous fungi and yeast) and 12% of eubacteria and archaeon species, capable of growing in the laboratory, are now known. This creates a large field of action for biologists, especially microbiologists, especially since microorganisms are characterised by a number of valuable properties favourable for their use in technological processes:

- rapid, in relation to other organisms, course of metabolic processes;
- well-known genomes of numerous species of microorganisms and ease of genetic manipulation;
- well-developed methods of culturing on a laboratory and industrial scale;
- cheap and widely available ingredients for culture media;
- diversity of metabolism (different products can be obtained from the same raw materials, using different strains of microorganisms);
- adaptability of microorganisms (using the same microorganism, the same product can be obtained from different raw materials, e.g., in alcoholic fermentation – ethanol production).

The features limiting the use of microorganisms include:

- production of large biomass and thus the consumption of medium components and the costs of disposing of biomass and/or post-culture liquid;
- unprofitable production of most of the low molecular weight substances (except for the food industry and compounds used in medical treatment).

When isolating microorganisms from different environments and then assessing their suitability for use in different technological processes, not only the ability to synthesise, transform or degrade various compounds is considered, but also:

- nutritional properties (low nutritional requirements);
- temperature properties (thermophilic strains are preferred, due to the lack of need to cool the culture in large-volume bioreactors);
- process type (continuous cultures);
- no harmful effects of the microorganism on the equipment components (biological corrosion);
- stability of morphological and biochemical characteristics;
- resistance to bacteriophages and bacterial infections;
- susceptibility to genetic manipulation (acquisition of mutants and GMMs based on the starting strain);
- high performance (based on substrate conversion);
- high productivity (product performance per unit of time);
- product recovery (cost of extraction and disposal of by-products present in the extract).

Table 1.1. Examples of negative and positive manifestations of microbial activity (from the point of view of human health and activity)

Microorganisms (dominant)	Practical application	Adverse activity
Claviceps purpurea	Biosynthesis of psychotropic drugs	Ergot poisoning ("Fire of St. Anthony")
Numerous species of *Bacillus*, *Pseudomonas*, *Micrococcus*, *Flavobacterium*, *Streptomyces*, *Aspergillus*	Production of numerous complexes, agri-food industry waste treatment, composting, sewage treatment	Decomposition of raw materials and food products
Bacillus, *Pseudomonas*, *Acinetobacter Micrococcus*, *Sphingomonas*, *Mucor*, *Phanerochaete*	Bioremediation of areas contaminated by petroleum substances, production of biosurfactants	Mucosalisation and degradation of petroleum products (oils, lubricants, gasoline)
Bacillus, *Pseudomonas*, *Trichoderma harzianum*, *Paeciliomyces marquandii* *Cunninghamella echinulata* *Umbelopsis isabellina* *Nectriella pironii* *Myrothecium roridum*	Biodegradation of toxic xenobiotics, including EDCs (Endocrine Disrupting Compounds), industrial sewage decolorisation, biosynthesis of hydrolases, laccases and other enzymes	Biological corrosion of construction materials, library resources, interior design elements, plant diseases

When analysing the properties of isolated microorganisms and their suitability for use in specific technological processes, it should also be remembered that the same microorganism (or groups of interacting microorganisms) can be useful in several

different branches of industry or environmental protection, but they can also cause serious damage. Examples are the so-called acetic acid bacteria of the genera *Acetobacter* and *Gluconobacter*, used for the production of acetic acid or sorbose, and concurrently posing a serious threat in the production of wine or beer. Other similar examples are presented in Table 1.1.

1.2. Screening of microorganisms useful in microbiological processes

1.2.1. Suitability of various environments for isolation of microorganisms used in industrial processes

Microorganisms with specific metabolic characteristics are widely used in modern biotechnological processes. The main source of microorganisms is the natural environment, especially the soil. The soil contains bacteria, actinomycetes and micromycetes that are capable of biosynthesis, decomposition and biotransformation of many compounds important for humans (antibiotics, vitamins, organic acids, steroid compounds), as well as elimination of pollutants, including toxic xenobiotics. Genetic diversity of soil microorganisms can be an excellent source of strains with recognised biotechnological properties, used in modern technologies.

The exploration of strains useful in industrial processes, from less typical (extreme) microbial habitats, e.g. saline water reservoirs, waters of polar and high mountain lakes, hot springs, geysers, etc. should also be considered.

By isolating microorganisms from sites polluted with xenobiotics, such as industrial landfills, strains capable of detoxifying and degrading them can be obtained. Most ecological niches are useful reservoirs of microorganisms with unique properties, often very valuable from the biotechnological point of view. In particular, microorganisms capable of surviving in unusual, often extreme conditions (extremophiles), can be a source of enzymes used for industrial, therapeutic, scientific purposes (Table 1.2.1).

Thermophiles (organisms adapted to live above 80°C) are successfully used in various industries. They are usually isolated

from volcanic areas (soils). A classic example is *Thermus aquaticus* – a species that produces thermostable DNA polymerase, used for the PCR reaction. A lot of enzymes obtained with the use of this group of microorganisms (e.g. protease, lipase) are used in industry, e.g. food, pharmaceuticals and dyes. Furthermore, microorganisms inhabiting cold environments, such as the polar and tundra biomes, cold ocean waters, glaciers or underground caves (psychrophilic microorganisms) are a source of enzymes, commonly used as an additive to washing powders that are active at low temperatures. In turn, proteases and lipases, obtained from psychrophiles, are also used in the food industry, e.g. for cheese production.

Another group of microorganisms, inhabiting unusual environments, are acidophilic microorganisms living in acidic environments. They were identified for the first time in the waters created in the process of mine drainage. This group of bacteria oxidises inorganic sulphur compounds, playing an important role in the geochemical cycle of this element. They have also found practical use in the mining industry for desulphurisation of hard coal or for the recovery of rare metals, including those of strategic importance, such as uranium. The discussed group also includes lactic acid bacteria that have found use in the food and pharmaceutical industries, e.g. probiotics (Section 4.2.2). Alkaliphiles – microorganisms living in alkaline environments – are a very valuable source of proteases used in the food industry, as well as in the production of cleaning products and amylases used in the brewing, food and baking industries. Moreover, the treatment of highly saline wastewater can be considerably assisted by the presence of NO_2 and NO_3 reductases.

1.2.2. Soil as a source of potential producers of biologically active compounds

The soil is a rich and inexhaustible source of microorganisms. There are about 10^6–10^8 bacterial cells, 10^4–10^6 actinomycete conidia, 10^2–10^4 fungal spores in 1 g of arable soil. They are very important in the course of biogeochemical cycles and the mineralisation of organic remains of plant and animal origin, improving at the same time the fertility (humus formation) and productivity of soils. Soil microorganisms also affect the functioning of ecosystems and the condition of plants. Moreover, microorganisms with properties

that make them useful in biotechnology have been isolated for years, from the soil environment.

Soil is a natural habitat for many species of microorganisms. It consists of mineral compounds and organic substances (50% of the soil composition), soil gas (about 35%) and soil solution (15%). The solid part of the soil consists mainly of soil colloids. The most important function is performed by organic colloids (humic compounds), which are formed as a result of the decomposition of organic matter by microorganisms. Mineral colloids, on the other hand, determine water-air relations. Soil water, containing dissolved organic and mineral substances, is called soil solution. This soil fraction has buffer properties and determines the pH of the soil. The space between soil particles (unoccupied by soil solution) is filled by the soil gas. The soil gas consists mainly of NH_3, CO_2, N_2 and O_2, also of smaller quantities of H_2S and CH_4. Soil structure is composed of mineral grains, which are glued together by humic compounds and mucous, forming 0.5–5 mm size particles. The microorganisms are located on the surface of particles in humic substances, as well as in organo-mineral complexes.

Soil is rich in microbiota in partially decomposed organic matter (humus). Larger clusters, the so-called soil aggregates, are built from soil particles. The exterior of these clusters is inhabited mainly by spore-forming bacteria, actinomycetes and filamentous fungi. The inner part is colonised by bacteria assimilating mineral nitrogen compounds, other (mainly Gram-negative) bacteria, and *Fusarium* moulds.

Soil microbiota are abundant and depend on the type of soil, including chemical composition (inorganic and organic compounds), pH, oxygen availability, humidity, temperature, depth, geographical zone, presence of plants. In general, soil microbiota are divided into: autochthonous microbiota (constantly present in the soil) and zymogenous microbiota (introduced periodically). The source of carbon and energy for the autochthonous microbiota are humus substances (compounds formed from the decomposition of organic matter of plant and animal origin). The development of the zymogenous microbiota depends on the inflow of easily assimilable organic matter.

Filamentous fungi and a significant proportion of bacteria prefer moist environments. Microorganisms with low water requirements

develop in dry soils – they are xerophilic microorganisms. Actinomycetes and nitrifying bacteria of the *Arthrobacter* genus also inhabit dry soils. Wet soils, which have a limited amount of nutrients and oxygen, are abundant in anaerobic and facultative microorganism. Biodiversity of soil microbiota also depends on temperature. The best time of year for the microorganism's isolation is spring. In winter, the soil microbiota is significantly reduced. An important parameter that determines the development of microorganisms in the soil is pH. Soils with alkaline or neutral pH contain several times more microorganisms than acidic soils (e.g. peatlands). Fertile soil, rich in humus substances, is also rich in microbiota compared to compacted meadow soil.

Microorganisms are very sensitive to sunlight (UV solar radiation) and wind (drying), so very few dwell on the soil surface.

Most microorganisms are present in the root zone (rhizosphere) and on the root surface (rhizoplane). On the other hand, the number of microorganisms is much smaller in deeper soil layers (at the depth of 1–2 m).

1.2.3. Microbial screening

Special screening methods have been developed in order to obtain new strains from the environment. The screening can be defined as a set of selective procedures aimed at isolating from a large number of microorganisms, only those that meet specific technological requirements. Screening also includes a preliminary assessment of their suitability for a given process, e.g. assessment of their biodegradation potential. In the initial stage of screening, we are dealing with microorganisms with undefined systematic affiliation, which may also include pathogenic strains. Usually strains isolated directly from the environment produce small amounts of metabolites of interest. Only after they have been enhanced, using genetic and microbiological techniques (see Section 1.6), can strains be used on an industrial scale. An effective and simple technique for the isolation of microorganisms is the selective multiplication of cultures. In this case, strictly defined media and specific culture conditions are used, i.e., medium composition, temperature, pH, osmotic pressure, light availability, oxygen availability, which allow the growth of a specific group of microorganisms (Table 1.2.1).

Culture conditions	Type of isolated microorganisms
Extremely acidic pH (pH 2–4)	Acidophiles
Low temperature (4–15°C)	Psychrophiles
High temperature (42–100°C)	Thermophiles
NaCl at great concentration	*Nocardia*, halophiles
Presence of atmospheric nitrogen (N_2)	Anaerobics
Chitin as growth substrate	*Lysobacter*
Tree bark, roots	Myxobacteria
Grain pollen	*Actinoplanes*
Media with heavy metals addition	Microrganisms accumulating heavy metals

Table 1.2.1. Methods of stimulating the growth of selected groups of microorganisms by application of appropriate culture conditions

The search for microorganisms with desired characteristics can also be accelerated by inoculation of the samples taken from prepared extracts, directly onto differential media plates, allowing the isolation of microorganisms with specific metabolic activity (Table 1.2.2).

Table 1.2.2. Examples of methods that favour the acquisition of microorganisms with specific metabolic activities

Producers	Pre-selection rules
Antibiotics	Inoculation on agar plates with strains of test microorganisms, e.g. *Staphylococcus aureus*, *Proteus vulgaris*, *Candida albicans*, *Penicillium avellaneum*. Growth inhibition zones are an indicator of activity.
β-lactamase-resistant antibiotics	Inoculation on agar plates with β-lactamase addition.
Proteases	Inoculation on agar plates with casein. Colonies that produce lightening zones in the medium are selected.
Amylases	Inoculation on agar plates with starch. Selection of colonies after staining with Lugol's iodine (J/KJ).
Lipases	Inoculation on agar plates with emulsified oil. Selection of colonies after precipitation of free fatty acids with calcium (Ca) ions.
Phosphatases	Inoculation on agar plates with phenolphthalein diphosphate. Selection based on the colour change of the substrate around the colony.
Cellulases	Inoculation on agar plates with phosphocellulases. Selection of colonies that produce transparent zones of enzyme activity.
Pectinases	Inoculation on agar plates with pectins. Selection of colonies producing bright zones on an opalescent background – reaction with a solution of CTAB (cetyltrimethylammonium bromide).
Xenobiotic decomposing microorganisms	Inoculation on agar plates with selected xenobiotic, being the only or main source of carbon and energy. Analysis of the growing colonies in terms of their degradation activity.

The screening stage includes the selection of the collection site and the method of isolating microorganisms using appropriate media. If we are looking for protease-producing bacteria, a convenient place to collect samples may be, among others, the areas around industrial plants producing significant amounts of waste containing proteins, e.g. dairies, meat plants. At a further stage, pure microbial cultures are isolated, on the basis of which liquid cultures are prepared. A preliminary assessment of the suitability of the isolated strains for use in a specific technological process is made. If a specific product is desired, process optimisation is carried out, often combined with improving the isolated strain and increasing the scale of culture. The final stage of screening includes purification of the product (if necessary), patenting of the relevant (key) production steps and commercialisation of the obtained research results. The key stages of screening are shown in Figure 1.2.1.

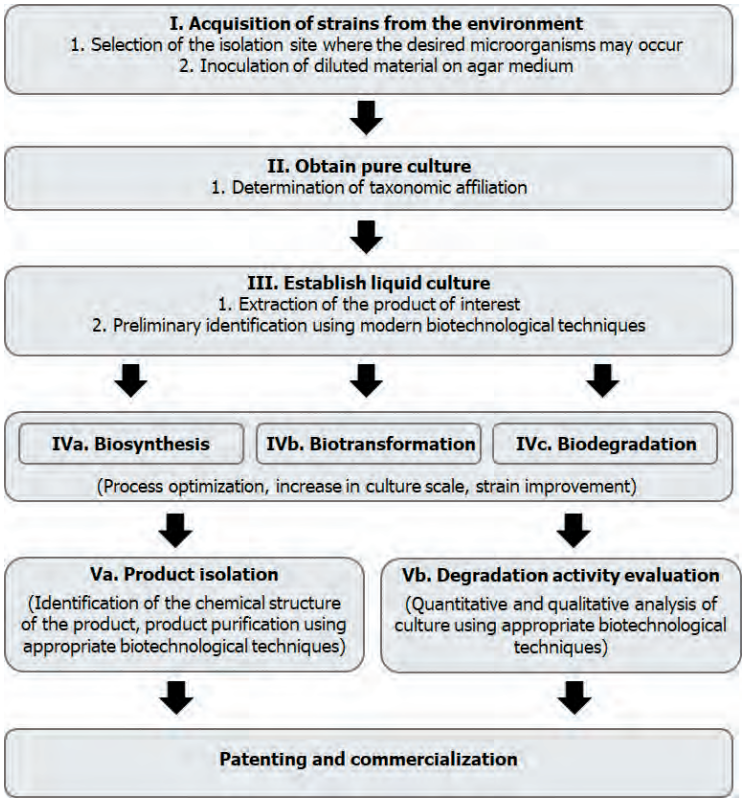


Fig. 1.2.1. Stages of screening microorganisms that are useful in biotechnological processes.

1.2.4. Soil of polluted environments as a source of microorganisms used in environmental protection processes

A significant increase in environmental pollution has been observed as a result of intensive industrial development, which has a very high impact on soil composition and biodiversity. A large part of the species of microorganisms inhabiting the soil have the ability to biodegrade xenobiotics. The degradation potential of microorganisms depends primarily on their environmental biodiversity but also on physical and chemical parameters (temperature, oxygenation, pH, availability of nutrients) of soil. Of all the pollutants, aromatic hydrocarbons are particularly dangerous. They exhibit strong toxic and carcinogenic properties. Contamination with petroleum products is greatest in the vicinity of refineries, petrol stations, airports, freeways, railroads and diagnostic stations, repair shops and car washes. Nevertheless, the contamination of green areas of cities by aromatic hydrocarbons and other harmful substances, and the related revitalisation of degraded areas, are important problems. These issues are discussed in more detail in Section 5.1. Petroleum products, especially polycyclic compounds, are resistant to degradation and some may remain in the environment for many years. Elimination of these compounds is mainly caused by microorganisms. Therefore, the search for strains capable of using oil derivatives as carbon and energy sources or of converting them into environmentally non-toxic products is a very important task of modern environmental biotechnology.

Chemical structures of selected aromatic hydrocarbons are presented in Figure 1.2.2.

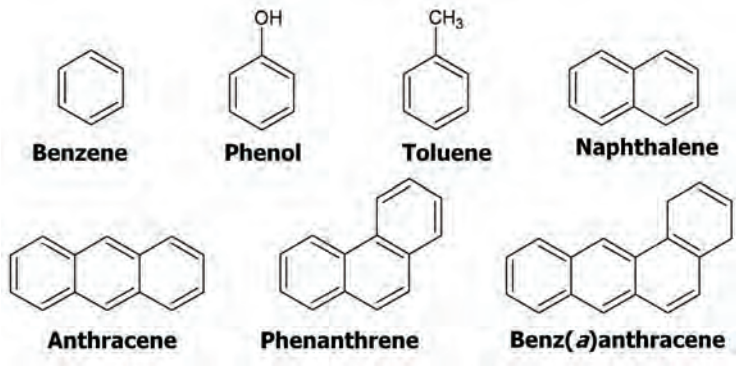


Fig. 1.2.2. Structure of selected aromatic hydrocarbons.

The rate of degradation of these compounds depends on the number of aromatic rings in the molecule. Today, numerous microorganisms capable of degrading low molecular weight hydrocarbons are known, but not many that can degrade high molecular weight polycyclic aromatic hydrocarbons (PAHs). Aromatic hydrocarbons can be partially or completely degraded (mineralised) by consortia of microorganisms and less frequently by individual species or strains.

Prokaryotic microorganisms used in the degradation of aromatic hydrocarbons mainly use dioxygenases, which are capable of introducing two oxygen atoms into the aromatic ring. Then, in the dehydrogenation stage, dihydroxy derivatives (catechols) are formed. The catechol ring, a key intermediate metabolite formed, is further degraded via enzymes of *ortho* and *meta* cleavage pathways. Subsequent reactions produce metabolites that are included in the Krebs cycle (Figure 1.2.3).

Some microorganisms, such as filamentous fungi, are capable of biotransforming aromatic hydrocarbons into intermediate products. These organisms use monooxygenases containing cytochrome P-450 that introduce one oxygen atom into the aromatic ring. The resulting epoxide is then converted to phenol or dihydrodiol and these can be conjugated with polar compounds such as glucose, glucuronic acid and xylose (Figure 1.2.4).

In addition, PAH biotransformation processes in both fungi and bacteria can be catalysed by oxidising and reducing enzymes such as laccases and peroxidases. The mechanism of these reactions is described in Section 5.11.

The susceptibility of hydrocarbons to biochemical degradation is determined not only by the chemical structure of petroleum products. Other factors influencing this process include substrate solubility and production of surfactants by microorganisms, hydrocarbon adsorption processes, number of microorganisms, concentration of nitrogen and phosphorus biogenic compounds, concentration of hydrocarbons, pH, humidity, temperature, oxygen content, presence of carbon sources for microorganisms other than hydrocarbons and the presence of toxic compounds.

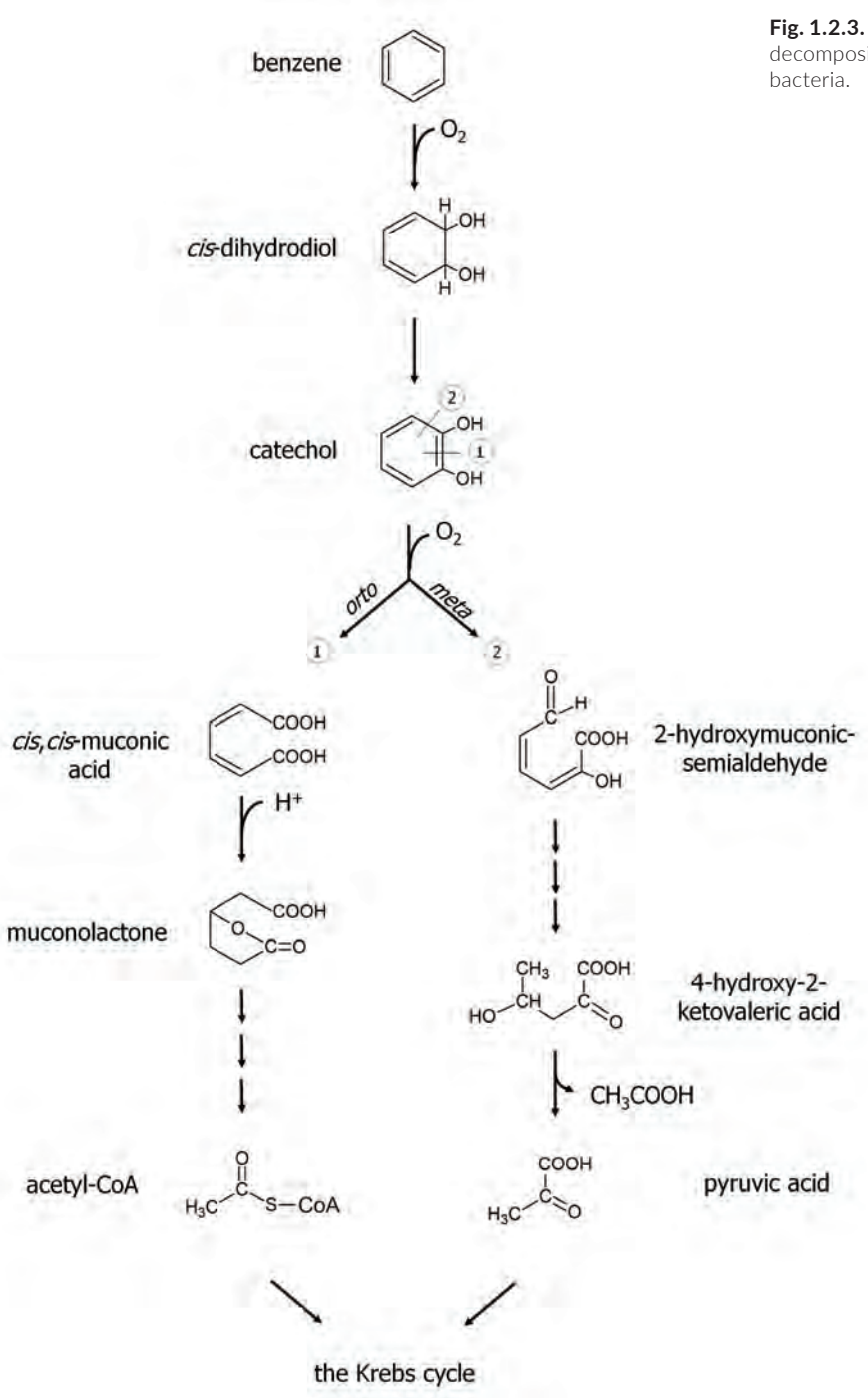


Fig. 1.2.3. Benzene decomposition by bacteria.

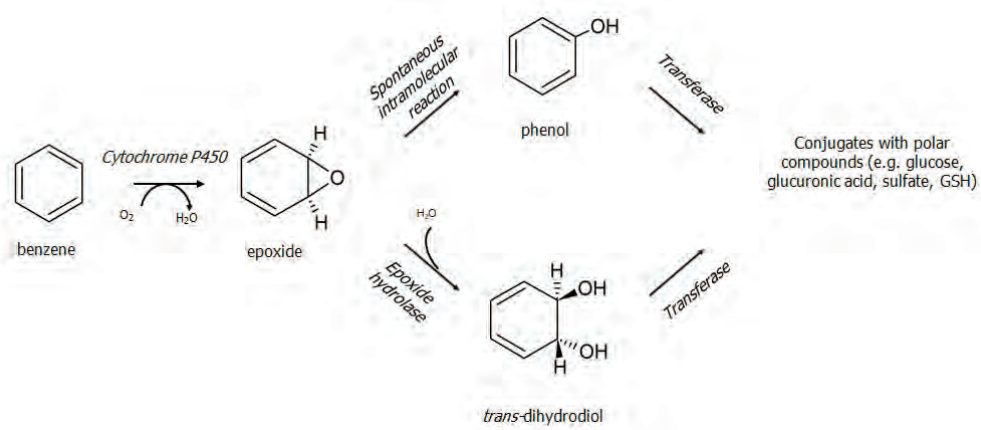


Fig. 1.2.4. Biotransformation of benzene by filamentous fungi.

Only a small number of soil bacteria (0.01–0.3%) are capable of using hydrocarbons as sole source of carbon and energy. Screening methods are very useful in isolating microorganisms capable of degrading xenobiotics. The easiest way to do this is to take a soil sample and perform an inoculation from appropriate dilutions on selective media with the addition of xenobiotics as a source of carbon and energy. Growing colonies are then isolated and checked for their degradation activity.

PRACTICAL PART

Materials and media

1. Source of isolated bacteria: garden and meadow soil, as well as soil samples taken from the area of a dairy, a gas station or freeway.
2. Microorganisms:
   a) Strains isolated from soil samples;
   b) Reference bacterial strains: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 15442.
3. Solid media: agar, ZT medium, universal, maltose, universal with anthracene and phenanthrene (0.1 g/l), medium with milk, with starch, Tween 80.
4. Reagents: anthracene, phenanthrene.