

Chapter 2

Basics of Biotechnology for Civil and Environmental Engineers

2.1 References for the Chapter

This chapter is given without references considering its use as an introduction for the engineers starting work in the interdisciplinary area of Construction Biotechnology. The major references that can be used additionally to this text are (Ivanov 2015; Madigan et al. 2014; Wang et al. 2010; Bhattacharyya and Banerjee 2008; Fulekar 2010).

2.1.1 *Biotechnology*

Biotechnology is a scientific and engineering knowledge on the use of microorganisms or their products in large-scale industrial processes. Depending on the area of application, the microbial biotechnologies are differentiated as

- Food Biotechnology, which was started in ancient times from the production of bread, wine, beer, cheese, yogurt, pickled vegetables, fermented souces, etc.;
- Medical and Veterinary Biotechnologies, deals with industrial production of antibiotics, vaccines, and other pharmaceuticals, as well as with microbial probiotics—live microorganisms for enhancement of physiological activities of human or animals;
- Environmental Biotechnology, maintains clean and sustainable environment using the microbial processes in water, wastewater, polluted soil, and polluted air;
- There are also developing areas of Agricultural (biofertilizers), Mining (bioleaching of metals), and Energy (production of biofuels) Biotechnologies;
- Construction Biotechnology, described in this book, is a new discipline developing in two directions: (1) biotechnological production of construction materials, and (2) biotechnological construction processes in situ.

2.2 Applicability of Construction Biotechnology

Biotechnology can be applied for the production of construction materials due to four reasons:

- low cost due to use of mining or organic wastes as raw materials;
- lower cost in comparison with the products of chemical industry due to simpler and less energy consuming technology;
- lower toxicity of biomaterials than chemical materials;
- sustainability of the biotechnological production.

Construction Biotechnology is usually applied in geotechnical engineering for bioaggregation, bioclogging, and biocementation of porous soil or fractured rocks in situ by the same reasons but there are important additionally features such as:

- low viscosity of biogrouting and biocementing solutions and deep penetration of this solution into porous soil or fractured rocks;
- ability to control rate of biochemical reactions in situ by the concentration or activity of biomass or enzyme;
- ability for self-multiplication (proliferation) of microbial cells in situ;
- better public acceptance of biotreatment of environment rather than chemical treatment.

However, a combination of biotechnological, chemical, and mechanical treatments may be more efficient than a single type of treatment. So, it is a common rule that a combination of biological and chemical materials, and technologies and proper mechanical optimization ensures the development of the most effective technology. There is almost no example of application-only bioprocess without chemical or mechanical processes in Construction Biotechnology.

2.3 Bioprocesses Used in Construction Biotechnology

The following bioprocesses are mainly used in Construction Biotechnology:

- 1) Exponential growth of biomass X

$$dX/dt = \mu X = Y(dS/dt)$$

where μ is specific growth rate and Y is growth yield; $\mu = \Delta \ln X / \Delta t$ for the time interval time Δt .

- 2) Linear growth or decay of biomass

$$dX/dt = k$$

where k is the rate of growth or decay.

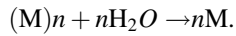
- 3) Primary biosynthesis of metabolite P (production of the substance P depends on biomass)

$$dP/dt = f(X).$$

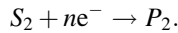
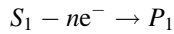
- 4) Secondary biosynthesis of metabolite P (production of substance P is independent of biomass)

$$dP/dt = f(t).$$

- 5) Enzymatic hydrolysis, which is decay of oligomer or polymer by the addition of molecule of water between monomer units (M):



- 6) Coupled oxidation/reduction of two substances, S_1 and S_2 , with the formation of products, P_1 and P_2 :



This oxidation–reduction can be coupled with the microbial growth:

$$dS_1/dt = YdX/dt$$

but can be also independent of the growth rate:

$$dS_1/dt = f(t).$$

2.4 The Stages of Biotechnological Process

Any biotechnology includes:

- A preliminary step (upstream processes)
- A cultivation or biogeochemical activity step (core process)
- A posttreatment step (downstream processes)
- Process monitoring and control.

2.5 Upstream Processes in Construction Biotechnology

Upstream processes include:

- Pretreatment of raw materials
- Preparation of a medium for cultivation
- Selection, isolation, and collection of microbial strains
- Preparation of inoculum.

2.6 Upstream: Pretreatment of Raw Materials

Pretreatment of raw materials in Construction Biotechnology includes:

- Crushing, grinding, sieving, and homogenization of the particles;
- Homogenization (mechanical or by ultrasound) of suspended hydrophobic substances;
- Chemical oxidation of hydrophobic substances by hydrogen peroxide or ozone for better dissolution and oxidation;
- Chemical treatment with alkali or acids to hydrolyze and dissolve nutrients for faster assimilation;
- Chemical treatment with alkali or acids to disinfect raw materials;
- Preliminary washing by surfactants to clean up surface from hydrophobic substances;
- Thermal pretreatment of raw materials for disinfection or faster assimilation;
- Freezing pretreatment of raw biomass for disinfection or faster assimilation after killing of cells by ice crystals.

2.7 Upstream: Preparation of a Medium for Cultivation

A medium (pl. *media*) is an artificial environment for the cultivation of microorganisms in the form of solution, suspension, or solid matter. A medium in Construction Biotechnology can be a waste that is undergoing biological treatment. Preparation of the medium includes:

- Mixing
- Addition of concentrated nutrients
- Addition of microelements
- pH and oxidation–reduction potential (ORP) adjustment.

There may be also:

- Addition of indicators of medium quality (pH, ORP, or sterilization quality indicators)
- Sonication of suspension
- Thermal pretreatment of the medium
- Sterilization of the medium
- Conservation of the medium.

To store the medium without deterioration, it can be cooled, frozen, dried, pasteurized, or sterilized.

2.8 Upstream: Components of Medium

The major elements of microbial biomass are C, H, O, and N. Carbon content in biomass is usually 50 % (w/w). The average content of dry matter of bacterial cells is as follows: protein, 55 %; RNA, 15 %; polysaccharides, 10 %; lipids, 5 %; DNA, 5 %; and monomers and inorganic ions, 10 %. The water content in the cells is 70–80 %.

The average content of major elements in biomass can be shown by the empirical formula $\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$. The sources of C, H, and O are usually carbohydrates (empirical formula CH_2O) such as disaccharide saccharose and lactose (empirical formula $\text{C}_{12}\text{H}_{22}\text{O}_{11}$), polysaccharides starch or cellulose (empirical formula of monomer unit is $\text{C}_6\text{H}_{10}\text{O}_5$), alcohols (mainly ethanol $\text{C}_2\text{H}_5\text{OH}$), and organic acids (mainly acetic acid CH_3COOH). The sources of N are usually inorganic compounds: salts of ammonium, nitrate, or urea. There is also need for growth of other macronutrients of biomass (P, S, K, Na, Mg, Ca, Fe) and micronutrients (Cr, Co, Cu, Mn, Mo, Ni, Se, W, V, Zn), which are supplied as the salts or as extracts from meat, microbial biomass, and plants. Iron salts, which are very important for life activity of microorganisms, are unstable at neutral pH and form iron hydroxides so ferric or ferrous ions in the medium must be chelated, usually by organic acids, to be protected from oxidation of ferrous ions or hydrolysis of ferrous and ferric ions. Some microorganisms, adapted to live in rich environment and called auxotrophic strains, require an addition to the medium the growth factors, which are organic compounds such as vitamins, amino acids, and nucleosides. The simplest and most economical way to supply all the necessary nutrients for growth is to use digests or extracts of natural ingredients such as meat broth, hydrolyzed soya beans, casein, or a cheapest organic material—biomass of activated sludge of municipal wastewater treatment plants. This rich medium must be supplemented with the source of energy, usually carbohydrate, because the components of the medium extracted from animal or plant biomass are used for the production of a new microbial biomass but not for the production of biological forms of energy used by microbes for the life activities.

In the majority cases, medium is used as a solution or suspension. pH of the medium is adjusted to optimum for growth and biochemical activity value. If the

pH is changed during cultivation due to accumulation of acid or alkaline, pH buffering substances are introduced into medium or titrants such as NaOH or HCl are added automatically to maintain optimum pH during cultivation. Common pH buffers are a mixture of $\text{H}_2\text{PO}_4^-/\text{HPO}_4^{2-}$ salts, $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ salts, and CaCO_3 .

If the aeration rate and the content of surfactants (proteins, polysaccharides, and salts of long-chain fatty acids) are high, the formation of foam in bioreactor can destroy cultivation of microorganisms, so the antifoaming substances have to be added to the medium at the beginning or during cultivation.

In the cases of solid-phase fermentation, a medium as a mixture of solid substances with optimum content of water for microbial growth and activity, usually it is within the range from 50 to 80 % (w/w).

The calculation of the medium components can be made using balance of elements. Roughly, the growth yields of aerobic and anaerobic growth using carbohydrates as the sources of carbon and energy are 0.5 and 0.1 g of dry microbial biomass/g of consumed carbohydrate, respectively.

2.9 Upstream: Isolation and Selection of Microbial Strain (Pure Culture) for Bioprocess

Unicellular microorganisms can be isolated from nature as a strain (the cells of one colony) or a clone (the cells originating from one cell). Isolation of a pure culture (microbial strain) is usually performed by spreading diluted microbial suspension on a Petri dish with a semisolid medium to produce from 10 to 50 colonies on the dish after several days of cultivation. The cells of one colony are picked up for the next round of cultivation on a semisolid or liquid medium. Some advanced methods can also be used for the isolation of a pure microbial culture:

- Mechanical separation of cells by micromanipulator;
- Sorting of cells or microbeads with immobilized cell using a flow cytometer;
- Magnetic or immunomagnetic separation of cells;
- Cell chromatography.

2.10 Upstream: Acquiring of Microbial Strain from Collection

Known and useful microbial strains are stored in the culture collections, which are the centers for deposition and storage of strains for further study and use. Deposition of the commercially important strain in the national collection is a mandatory condition for the strain patenting and protection of the patent rights. The universities and national collections contain also type strains to serve as the

standards of the characteristics attributed to a particular species. The best-known national culture collections are, for example, American Type Culture Collection (ATCC) and Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). These collections are a nonprofit bioresource centers that provides biological products, technical services, and educational programs to industry, government, and academic organizations around the world. Their functions are to acquire, authenticate, preserve, develop, and distribute biological materials, information, technology, intellectual property, and standards for the advancement, validation, and application of scientific knowledge. A strain is identified by its assigned number in a microbial collection and the name of the species. For example, name “*Bacillus subtilis* ATCC 6633” refers to a strain of the species *Bacillus subtilis* that is stored under number 6633 in the ATCC.

However, the microbial strain acquired from the culture collection cannot be adapted to the conditions of the real bioprocess. Therefore, an isolation of a strain from the site of application or from the site with the conditions similar to the conditions in the site of application could be often a better option than acquiring the strain from the culture collection.

2.11 Upstream: Selection of an Enrichment Culture

The autoselection of an enrichment culture refers to the autoselection of a microbial community with one or several dominant strains that are preferably growing in the bioreactor as a result of the selection pressure, i.e., of selective conditions of medium and cultivation for these strains. Enrichment culture can be used in Construction Biotechnology for the performance of large-scale construction bioprocesses in situ. Selection pressure for the production of an enrichment culture is as follows: (1) source(s) of energy; (2) source of carbon; (3) source of nitrogen; (4) source of phosphorus; (5) temperature; (6) pH; (7) presence of a specific antibiotic in the medium; (8) concentration of dissolved oxygen or oxidation–reduction potential (ORP); (9) osmotic pressure of the medium; (10) spectrum and intensity of light; (11) settling rate, etc.

The mechanisms for autoselection of an enrichment culture are as follows:

1. Faster or more efficient growth of one or several strains (positive growth-related autoselection).
2. Faster or more efficient biochemical functions of one or several strains (positive metabolic autoselection).
3. Slower or less efficient growth of one or several strains (negative growth-related autoselection).
4. Slower or less efficient biochemical functions (negative metabolic autoselection).
5. Better survival under harmful conditions (positive survival-related autoselection).

6. Weaker resistance to some environmental factors (negative survival-related autoselection).
7. Stronger or more specific adherence of cells to surface (positive or negative cell adherence-related autoselection).

The selected properties of an enrichment culture can be genetically unstable and may disappear after several generations of cells in case when the selection pressure is absent. This instability is known for cell surface hydrophobicity, which can be significantly increased using the retention of cells on a hydrophobic carrier over several cell generations. Conversely, cell surface hydrophobicity can decrease for several cell generations if there is no selection pressure (retention on hydrophobic carrier) in the medium (Stabnikova 2000). This feature is important in case of microbial construction processes on hydrophobic surface.

2.12 Upstream: Selection of an Ecosystem

Main property of ecosystem is either physical boundary such as air–water or water–soil interphases or chemical boundary such as steep gradient of concentrations separating this ecosystem from other parts of environment. The selection of an artificial microbial ecosystem is similar to the selection of an enrichment culture, but (1) it must be within system separated by the boundary; and (2) there may be several changed selective factors (selection pressures) ensuring dominance of several microbial communities with different, even alternative, physiological functions. For example, the aerobic and anaerobic microbial communities can simultaneously exist in a selected artificial microbial ecosystem due to the presence of both aerobic and anaerobic conditions in the ecosystem. The external and self-developed internal selective pressure is a major factor of self-formation of an artificial microbial ecosystem. The selection mechanisms for an artificial ecosystem can be positive or negative interactions such as commensalistic, mutualistic, amensalistic, antagonistic, or parasitic relationships between the microbial communities of ecosystem.

2.13 Upstream: Construction of Genetically Engineered Microorganisms

Microorganisms that are suitable for the production of construction materials or construction processes in situ can be isolated from the natural environment. However, their ability can be modified and amplified by artificial alteration of the genetic (inherited) properties of these microorganisms. Natural recombination of the genes occurs during DNA replication and cell reproduction. It includes the breakage and rejoining of chromosomal DNA molecules and plasmids

(self-replicating mini-chromosomes containing several genes). Recombinant DNA techniques and genetic engineering can create new, artificial combinations of genes and increase the number of desired genes in the cell. Genetic engineering of recombinant microbial strains for Construction Biotechnology involves typically the following steps:

- DNA is extracted from a cell and is cut into small sequences by specific enzymes.
- Small sequences of DNA are introduced into DNA vector (either a virus or a plasmid).
- A vector is transferred into the cell and self-replicated to produce multiple copies of the introduced genes.
- Cells with newly acquired genes are selected based on their specified activity, e.g., the production of defined enzymes, and the stability of the acquired genes.

The main problem in an application of recombinant strains is maintaining stability of the plasmids in these strains. Other technological and public concerns include the risk of the application and release of genetically modified microorganisms into the environment.

2.14 Upstream: Preparation of Inoculum

The organisms used to start the core process are called the *inoculum* by microbiologists, or sometime as the “seeds” by engineers. The inoculum could be a suspended, frozen, dried, or cooled biomass of microorganisms. Cultivation of the inoculum is performed usually in batch process mode. The volume of inoculum for microbial batch cultivation in the bioreactor must be about 5–20 % of the bioreactor volume. However, for application of microorganisms in the construction process the dosage of inoculum depends on the process conditions and properties of microorganisms and could be as small as few grams of biomass per 1 m³ of soil/particles. Inoculum for biotreatment of soil can be selected using following microbiological and molecular biological methods:

- Obtaining and testing of the microbial strains from national collections of microorganisms, for example American Type Culture Collection (ATCC, USA) or German Collection of Microorganisms and Cell Cultures (DSMZ, Germany).
- Isolation, identification, and testing of wild strains from natural sites with environmental conditions close to the conditions that are needed for the biotreatment, for example, with high salinity, high or low temperature, aerobic or anaerobic conditions, alkaline or acid pH. However, many bacteria are pathogenic (causing diseases) for human, animal, and plants. Therefore, bio-safety of biotechnological process is always an important issue and only non-pathogenic isolated strains of bacteria can be used for Construction Biotechnology applications.

- The biomass of the isolated and identified strain can be produced in bioreactor and used as a starter culture for nonaseptic environmental processes for faster start-up and increased biosafety (Ivanov et al. 2006b; Ivanov and Tay 2006a, b).
- Autoselection in continuous culture, selection of the mutants, and construction of the recombinant microbial strains from wild strains for the biotreatment. However, there are many restrictions on the applications of recombinant microbial strains so they can be used mainly for industrial production of such construction materials as polysaccharides or bioplastic.
- Selection and testing of suspended enrichment cultures using such selective conditions (selection pressure) as source of energy, carbon, nitrogen and phosphorus, temperature, pH, salinity (osmotic pressure), concentration of heavy metals, concentration of dissolved oxygen, and spectrum and intensity of light (for photosynthetic microorganisms). Some autoselected features of the enrichment culture can be genetically unstable and could disappear after several generations when the selection pressure will be absent.
- Selection and testing of aggregated enrichment cultures, such as flocs, biofilms, and granules using such selective pressure as settling rate of microbial aggregates and adhesion of cells to solid surface. An example is the formation of bacterial cells aggregates that cannot penetrate inside sand, settled onto the surface of sand and formed calcite crust (Stabnikov et al. 2011; Chu et al. 2012a).

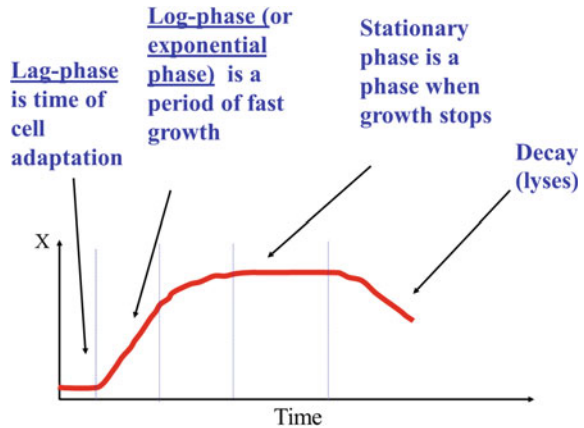
2.15 Core Biotechnological Process: Batch Cultivation of Microorganisms in Bioreactor

The volume of bioreactor varies from 100 mL in laboratory to several thousand cubic meters in industry. The most useful aseptic bioreactors in industrial biotechnology are 50–100 m³ aerobic and anaerobic tanks with complete mixing and the devices for the bioprocess monitoring and control. Cultivation in the bioreactors of bigger volume is performed in nonaseptic conditions, i.e., without sterilization of medium and equipment.

For cultivation of microorganisms in industrial biotechnological plant, bioreactor is cleaned, washed, filled with medium, sterilized in the case of aseptic cultivation, the temperature, aeration rate, stirring rate, and monitoring system are adjustment to the required parameters. Then, suspension of inoculum for microbial batch cultivation is introduced into the bioreactor in a dosage of 5–20 % of the bioreactor volume.

The system of microbial cultivation with gas exchange only is called as batch culture and the system with gas and liquid exchange is called as continuous culture. The following phases can be separated in batch culture (Fig. 2.1): lag phase (adaptation of the cells), log phase (exponential growth), stationary phase (absence of the growth), and death phase.

Fig. 2.1 Phases of growth in batch culture



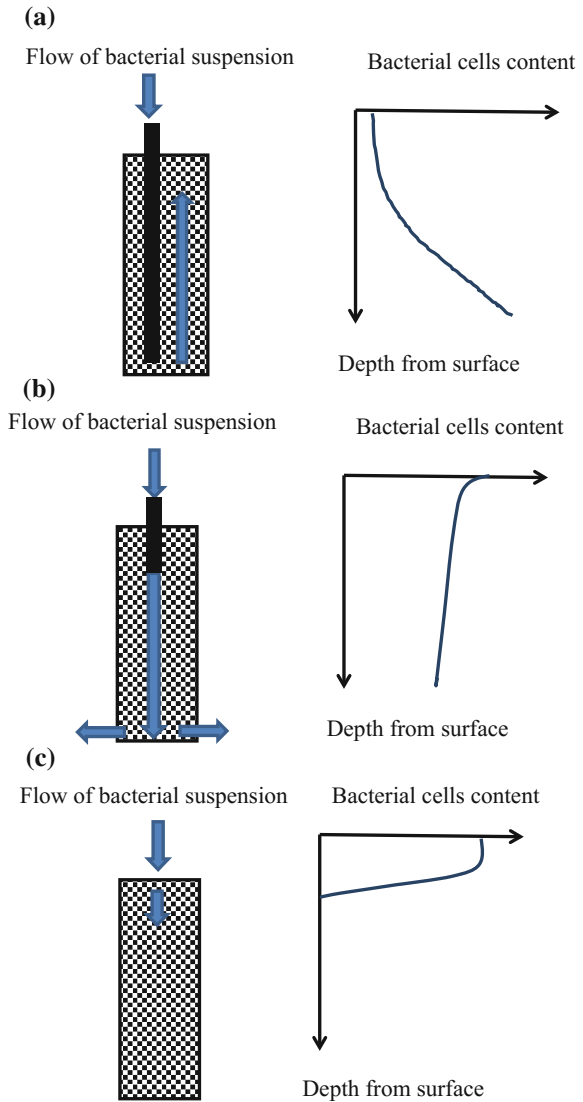
To minimize duration of lag phase, the conditions in bioreactor must be close to optimal values and the inoculum must be in the state of the fast growth. The rate of exponential growth of microbial biomass diminished after short period of maximum growth rate due to exhaustion of nutrients or accumulation of products inhibiting growth. Stationary phase can be short or long, depending on the properties of microbial strain and conditions of cultivation. Gram-negative bacteria usually dying after stationary phase, but gram-positive bacteria and fungi usually produce anabiotic forms of cells like endo- and exospores and cysts that can survive long-term starvation, presence of toxic compounds, low activity of water/high osmotic pressure, drying, and high temperature.

2.16 Core Biotechnological Process: Batch Cultivation of Introduced Microorganisms in Soil

Growth of microorganisms introduced as suspension into porous soil also can be considered as a batch cultivation process with the similar specific phases, but there are following features of this cultivation:

- Inoculum cannot be bigger than 0.1–1.0 % w/v ($\approx 1\text{--}10$ kg of dry biomass/ m^3 of soil) because of economic reasons. Therefore, lag phase can be relatively long.
- Conditions in soil—temperature, pH, and content of heavy metals—are not optimal for microbial growth, so biomass growth rate and biogeochemical activity of microorganisms in soil will be significantly lower than in industrial or laboratory bioreactor.
- Introduction of microorganisms in soil will be accompanied with adsorption of cells on the soil particles. Slow injection of bacterial suspension into the bottom part of the soil column will give the highest concentration of bacterial cells in the bottom part (Fig. 2.2a) because of the cells settling.

Fig. 2.2 Effect of injection on the distribution of adhered bacterial cells in soil column



An injection of suspension into the upper part of the soil column will give almost even distribution of bacterial cells in the soil column (Fig. 2.2b) because of fast flow under gravity and fast distribution of bacterial suspension in the volume of the pores. A spraying of small volume of bacterial suspension on the soil surface will produce a surface layer with high concentration of adhered bacterial cells (Fig. 2.2c) because liquid will be delayed in a surface layer of porous soil by the capillary forces.

- Usually, laboratory or industrially grown microorganisms released into soil die; so the cell death rate, cell survivability, or decrease of the biogeochemical activity of cells must be evaluated and accounted in the design of the bioprocess in soil. A rule of the thumb is that applied microorganisms must have some reasonable limits of lifetime or limit their biogeochemical activity in the treated and surrounding areas. This short lifetime can prevent accumulation or spread of unwanted microorganisms in the environment.

2.17 Core Biotechnological Process: Batch Cultivation of Indigenous Microorganisms in Soil

In some cases, when soil is rich with indigenous microorganisms having needed biogeochemical function, for example urease activity, soil biotreatment can be performed only by indigenous microorganisms, without preparation and supply of microbial inoculum (Burbank et al. 2011, 2012a, b; Weaver et al. 2011). To enhance the needed biogeochemical function of indigenous microorganisms, soil can be amended with the related reagent. For example, to enhance urease activity of indigenous microorganisms before the biotreatment, urea can be added to soil (Burbank et al. 2011).

However, if microorganisms, used in construction bioprocess, are indigenous it does not mean that they are safe for human, animals, and plants because nonselective conditions of the soil bioprocess, especially in the case of application of nutrients-rich medium, can enhance the proliferation of pathogens or opportunistic pathogens in soil. Additionally, it may take several weeks for ureolytic indigenous groundwater microorganisms to grow and become ureolytically active (Tobler et al. 2011). So, application of known safe strain grown in industrial bioreactors is more preferable than use of unknown indigenous microorganisms.

2.18 Core Biotechnological Process: Continuous Cultivation of Microorganisms in Bioreactor

Microbial continuous culture is open for exchange by gasses and liquids. The bioreactors, which are used in continuous cultivation are as follows:

- Complete mixing bioreactor. The most common type of this reactor is a chemostat, where the dilution rate (D) is maintained constant. $D = F/V$, where F is a flow rate and V is a working volume of the bioreactor. If $D < \mu_{\max}$ the stability of the system is maintained due to feedback interactions between the specific growth rate (μ), the concentration of

the substrate (S), and the biomass concentration (X): increase of X decreases S , and then μ , which decreases X at a constant D .

- Plug-flow system, whose parameters are constant in time but changed along the length of the reactor; one long plug-flow bioreactor can be replaced by a series of complete mixing reactors connected consecutively.
- Bioreactors with the retention of biomass. In Fixed Biofilm Reactor (FBR), microbial biomass is attached to the carrier/support material with a big specific surface inside FBR. In Membrane Bioreactor (MBR) biomass is retained using membrane filtration inside or outside of MBR and removal from MBR only solution but not biomass. Semi-continuous and Sequencing Batch Reactor (SBR), which is continuous cultivation with the periodical addition of nutrients and removal of suspension. Very often SBR is used for selection of fast settling cell aggregates and retention of biomass aggregates inside SBR due to sedimentation. A sequencing batch reactor retains biomass due to the periodic settling of the biomass followed by removal of the liquid. It sustains a semi-continuous cultivation with the periods of the biomass settling, removal of the liquid (effluent), and addition of fresh medium (influent).

The retention or recycling of biomass in all types of the bioreactors during continuous cultivation ensures the following properties:

- The flow rate through bioreactor can be higher than the specific growth rate of microorganisms so that slow-growing microorganisms can be maintained continuously in the bioreactor.
- The concentration of biomass and the rate of biogeochemical reactions in the bioreactor can be higher than in the chemostat.

2.19 Core Biotechnological Process: Continuous Cultivation of Microorganisms in Soil

Growth of microorganisms, during continuous or semi-continuous introduction of inoculum and medium into porous soil and removal or dispersion of effluent in surrounding environment. The porous soil or fractured rocks cannot be considered as complete mixing bioreactor, but conventionally can be considered as a plug-flow bioreactor or fixed biofilm bioreactor. The boundaries of this natural “bioreactor” are determined by the steep gradient of physical properties of soil such as hydraulic conductivity, compressive strength, porosity or chemical concentrations of oxygen, ferrous ions, hydrogen sulfide, values of pH, and oxidation–reduction potential separating for example of aerobic and anaerobic zones in soil and the layers with different properties.

Adsorption of bacterial cells and formation of biofilm on the surface of soil particles or rock fractures are most important stages of this continuous cultivation. Adsorption of bacterial cells depends on the method of injection and velocity of the

medium, which affects the cells distribution pattern in soil. Same as in batch cultivation, an injection of bacterial suspension into the bottom part of the soil column will give the highest concentration of bacterial cells in the bottom part (Fig. 2.2a) in case if the flow velocity is lower than settling velocity of cells. There can be sorption/desorption equilibrium described by Freundlich equation:

$$Q = KC^n$$

where: K and n are the coefficients characterizing adsorption, Q is a mass of adsorbed cells per unit mass of adsorbent (adsorption capacity); X is the concentration of bacterial cells in fluid. Adsorption capacity varies from one to several thousand bacterial cells/mg of soil and depends on physicochemical properties of soil particles and cell surface. Parameters of adsorption K and n can be determined from the slope and intercept on the linear graph derived from the Freundlich equation:

$$\log Q = \log K + n \log X.$$

An injection of suspension into the upper part of the soil column will give the almost even distribution of bacterial cells in the soil column (Fig. 2.2b) in case when downward flow is high and there is no equilibrium between cell sorption and desorption. A spraying of bacterial suspension on the soil surface will produce a surface layer with high concentration of adhered bacterial cells (Fig. 2.2c) because the liquid medium and the bacterial suspension will be delayed in surface layer of the porous soil by capillary forces. The patterns of the biogeochemical activities of cells are complicated by the medium flow, which can be from the bottom or from the top of soil, i.e., as a parallel flow or a counterflow with the flow of bacterial suspension. Continuous flow of the medium through soil increases risk of accumulation or spread of unwanted microorganisms in the environment.

Porous space of soil or fractured rocks contains diverse microenvironments during microbial cultivation. These microenvironments are characterized by the microgradients of chemical concentrations in the micrometer scale, and that are formed due to microbial activity in biofilm. The typical scale of such microbial microenvironments as cell aggregates, biofilms, or microbial mats is between 0.1 and 100 mm. Microbial cells are concentrated on a liquid–solid interphase or a liquid–gas interphase because the concentrations of the nutrients are higher there than in the bulk of liquid. Due to the presence of different microenvironments, the physiological groups of microorganism living there could be also very different, for example anaerobic, facultative anaerobic, microaerophilic, and aerobic microorganisms growing together in one treated volume of soil or fractured rocks. Presence of microenvironments in soil enhances the opportunities for the engineering bioprocesses in soil.

2.20 Downstream Processes

Typical downstream processes include

- Separation and concentration of biomass and products from the culture liquid
- Drying/dewatering of the biomass
- Packing/disposal of secondary waste.

2.21 Downstream: Separation and Concentration of Biomass

Simplest way for biomass separation and concentration is sedimentation. The sedimentation velocity (V) of cells and cell aggregates with diameter D and density ρ_p can be described by Stokes' law:

$$V = D^2(\rho_p - \rho_l) / 18 \mu g$$

where ρ_l is the liquid density, μ is the viscosity of the liquid, and g is the gravitational acceleration.

Because of the small size of bacterial cells and their density of 1.04–1.10, which is close to the density of water, their sedimentation rate by gravity settling is too low to be used in practice. Therefore, bacterial cells are separated from culture liquid, and are concentrated by three ways:

- centrifugation with acceleration (centrifugal force) $> 5000 \times g$;
- membrane filtration with diameter of pores below $0.2 \mu\text{m}$;
- bacterial cells aggregation and settling of the aggregates by gravity.

2.22 Downstream: Aggregates of Cells

A multicellular aggregate is formed and separated from its surrounding environment due to:

- Aggregation resulting from hydrophobic forces, electrostatic interactions, or salt bridges.
- A loose polysaccharide or inorganic matrix (iron hydroxide, for example) combining the cells by mechanical embedding, chemical bonds, hydrogen bonds, electrostatic forces, or hydrophobic interactions.
- Formation of mycelia, which is a net of branched cell filaments.
- A polysaccharide matrix with a filamentous frame.

- Coverage by a common sheath of organic (polysaccharides, proteins) or inorganic origin (iron hydroxide, silica, calcium carbonate); a common sheath can also be made from the dead cells of an aggregate (the “skin” of a microbial aggregate).

Bacterial cells can be aggregated by adjustment of pH to 3–5 to minimize zeta potential of cell surface but cells can be inactivated at low pH. Another way for production of bacterial cells aggregates is selection of fast settling flocs or granules with the sizes ranging from 50 μm to several mm using retention of self-formed cell aggregates in enrichment culture. Settling an aggregate of cells for 20–30 min and retaining or recycling of the settling aggregate in a bioreactor is a method for the selection of flocs. Intensive aeration for mechanical compaction of the aggregate by air bubbles, settling of the aggregate for 2 min, and retention of this settling aggregate in the bioreactor is a method for selection of granules. A simple method for selection of cellular aggregates is using the largest colonies grown in semisolid medium in a Petri dish. The largest colonies originated from not one bacterial cell but from an aggregate of several cells.

Cell aggregates are most suitable for the biotreatment of soil surface for soil bioaggregation and formation of soil crust but not for biotreatment of the bulk of soil for its bioclogging and biocementation.

2.23 Downstream: Separation and Concentration of Products

Different methods are used for separation and concentration of microbial products from the culture liquid. Depending on the product and its application there may be used:

- precipitation of exopolysaccharides (cement admixtures) and enzymes by ethanol or salts;
- Reverse Osmosis (RO) filtration for concentration of biopolymers;
- Adsorption on Granulated Activated Carbon, aluminum oxide, and hydrophobic sorbents;
- Flotation—concentration in foam formed by the gas microbubbles;
- Evaporation of volatile substances.

Separation and concentration of intracellular enzymes and intracellular accumulated bioplastic polyhydroxyalkanoates (PHAs) requires preliminary disruption of cells using chemical or mechanical treatments of microbial biomass.

2.24 Downstream: Drying, Mixing and Packing of Biotechnological Products

Drying of cells requires a soft regime to avoid inactivation of cells or enzymes. Best but the most expensive way is freeze-drying, but soft regime of spray drying at temperature of air below 50–60 °C could be also suitable for some microbial biomass and products. The very important point for practical applications is that dry biomass can be easily suspended as the separated cells or it forms in suspension the cell aggregates.

Dry biomass or microbial product has to be mixed with chemicals to produce ready to use construction material. Calcium chloride must be used for the mixing in hydrated form to avoid overheating of biomass or enzyme. In the mixtures with cement, the particles of biomass or microbial product must be encapsulated, coated with insoluble polymer to protect biosubstances from inactivation under high pH of cement.

All dry biomaterials are highly hygroscopic substances, attracting and holding water, so they must be sealed from air. Shelf-life of biomaterials is usually few months, but the spores of gram-positive bacteria can be stored for years.

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