Chapter 25

SCREENING AND SELECTION OF MICROORGANISMS FOR THE ENVIRONMENTAL BIOTECHNOLOGY PROCESS

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1. Major Physiological Groups of Microorganisms

Due to their long-term evolution, microorganisms have two major mechanisms for biological energy generation: (1) chemotrophy is the generation of biologically available energy due to the oxidation and reduction of chemical substances and (2) phototrophy is the generation of biologically available energy due to the capture

and transformation of light energy. The related physiological groups are chemotrophs and phototrophs, respectively.

There are three different ways to generate biologically available energy by chemotrophs: (1) fermenting microorganisms produce biologically available energy under anaerobic conditions using intramolecular oxidation/reduction; (2) anaerobically respiring (or anoxic) microorganisms produce biologically available energy under anoxic ("no oxygen") conditions using oxidation of organic matter by acceptor of electrons other than oxygen, for example, Fe^{3+}, SO_4^{2-}, and CO_2; and (3) aerobically respiring microorganisms produce biologically available energy under aerobic conditions by aerobic respiration, using reduction of oxygen.

There are also three different ways to generate biologically available energy by phototrophs: (1) use of the products of fermentation (organic acids, alcohols, and hydrogen) as donors of electrons and light as a source of energy to reduce CO_2; (2) use of the products of anoxic respiration (H_2S; Fe^{2+}) as donors of electrons and light as a source of energy to reduce CO_2; and (3) use of the product of aerobic respiration (water) as donor of electrons and light as a source of energy to reduce CO_2.

2. Periodic Table of Prokaryotes

Three major types of biological energy generation are the results of evolution of Earth's atmosphere from anaerobic to aerobic one. Therefore, microbial physiological diversity can be shown as created in three evolutionary periods related to fermenting, anaerobically respiring and aerobically respiring microorganisms. There are also intermediate groups, for example, microaerophilic or facultative anaerobic microorganisms between these groups.

These groups exist in three parallel semi-independent but coordinated phylogenetic lines: (1) line of aquatic organisms; (2) line of terrestrial organisms; and (3) line of organisms in extreme environment. These lines are semi-separated because of low frequency of genetic exchanges between organisms in aquatic, terrestrial, and extreme environments.

Prokaryotes of aquatic, terrestrial, and extreme environments are in the following lines: (1) Gram-negative bacteria (Gracilicutes), cells with thin wall, originated from environment with stable osmotic pressure (water, tissues of macroorganisms); (2) Gram-positive bacteria (Firmicutes), cells with rigid cell wall, originated from environment with changeable osmotic pressure (soil); (3) line of Archaea (Mendosicutes), cell without conventional peptidoglycan, originated from environment with some extreme conditions, usually temperature- or oxidation-reduction potential.

Table 1. Evolutionary Lines and Periods of Periodic Table of Chemotrophic Prokaryotes. (Selected Examples of Conventional Genera are Shown in the Groups.)

	Evolutionary Period		
Evolutionary Line	Fermenting Prokaryotes	Anoxic Prokaryotes	Aerobic Prokaryotes
Prokaryotes of aquatic origin (Gram-negative type of cell wall, Gracilicutes)	*Bacteroides* *Prevotella* *Ruminobacter*	*Desulfobacter* *Geobacter* *Wolinella*	*Pseudomonas* *Acinetobacter* *Nitrosomonas*
Prokaryotes of terrestrial origin (Gram-positive type of cell wall, Firmicutes)	*Clostridium* *Peptococcus* *Eubacterium*	*Desulfotomaculum* *Desulfitobacterium*	*Bacillus* *Arthrobacter* *Streptomyces*
Prokaryotes of extreme environment origin (Archaea)	*Desulfurococcus* *Thermosphaera* *Pyrodictium*	*Methanobacterium* *Thermococcus* *Haloarcula*	*Picrophilus* *Ferroplasma*

Therefore, the physiological diversity of chemotrophic prokaryotes can be shown as three periods of three parallel lines of the periodic table of prokaryotes (Table 1).

This periodic table is a logical basement to understand microbial physiological diversity. It gives the predictive power of not discovered yet groups of prokaryotes and clarifies the evolutionary connection between microbial groups.

Phototrophic organisms are also inserted into this periodic table as three sublines of aquatic, terrestrial, and extreme environment origin. Three periods of phototrophic organisms are integrated with three period of chemotrophs: (1) first period related to phototrophs using products of fermentation (organic acids, alcohols, and hydrogen) as donors of electrons and light as a source of energy to reduce CO_2; (2) second period related to phototrophs using products of anoxic respiration (H_2S; Fe^{2+}) as donors of electrons and light as a source of energy to reduce CO_2; and (3) third period related to phototrophs using product of aerobic respiration (water) as donor of electrons and light as a source of energy to reduce CO_2. Integrated periodic table of chemotrophic and phototrophic prokaryotes are shown in Table 2.

Some groups of phototrophic prokaryotes have not been discovered yet, such as phototrophic Gram-positive bacteria and Archaea, using the products of fermentation or anaerobic respiration as electron donors for CO_2 reduction. The existence of these groups in nature can be predicted following the logical basis of periodic table of prokaryotes.

Eukaryotic microorganisms, such as microscopic fungi, algae, and protozoa have no relation to the phylogenetic lines of aquatic, terrestrial, and extreme origin.

Table 2. Evolutionary Lines and Periods of Periodic Table of Chemotrophic and Phototrophic Prokaryotes. (Selected Examples of Conventional Genera are Shown in the Groups.)

		Periods of Evolution		
Evolutionary Line	Sub-Line	Fermenting Prokaryotes or Prokaryotes Using the Products of Fermentation as Electron Donors	Anoxic Prokaryotes or Prokaryotes Using the Products of Anoxic Respiration as Electron Donors	Aerobic Prokaryotes or Prokaryotes Using the Products of Aerobic Respiration as Electron Donors
Prokaryotes of aquatic origin (Gram-negative type of cell wall, Gracilicutes)	Chemotrophs	*Bacteroides* *Prevotella* *Ruminobacter*	*Desulfobacter* *Geobacter* *Wolinella*	*Pseudomonas* *Acinetobacter* *Nitrosomonas*
	Phototrophs	*Rhodopseudomonas*	*Chlorobium* *Rhodocyclus* *Chromatium*	Cyanobacteria *Prochloron*
Prokaryotes of terrestrial origin (Gram-positive type of cell wall, Firmicutes)	Chemotrophs	*Clostridium* *Peptococcus* *Eubacterium*	*Desulfotomaculum* *Desulfitobac-terium*	*Bacillus* *Arthrobacter* *Streptomyces*
	Phototrophs	*Heliobacterium* *Heliobacillus*	Not known yet	Not known yet
Prokaryotes of extreme environment origin (Archaea)	Chemotrophs	*Desulfurococcus* *Thermosphaera* *Pyrodictium*	*Methanobacterium* *Thermococcus* *Haloarcula*	*Picrophilus* *Ferroplasma*
	Phototrophs	Not known yet	Not known yet	*Halobacteria*

3. Use of Periodic Table for Theoretical Selection of Prokaryotes in Environmental Engineering

Almost all microbial groups are used in environmental engineering[1] and the selection of correct group is important at the primary stages of the development of any specific environmental biotechnology.[2] The periodic table of prokaryotes provides a theoretical understanding of microbial diversity. At the same time, it can be used for the selection of microbial group suitable for different environmental engineering processes. Depending on the available conditions in the designed system, one or several group from periodic table can be selected for the process performance.

For example, if there is variable osmotic pressure and low or absent concentration of dissolved oxygen, the group of Gram-positive bacteria, which are intermediate between the groups of Gram-positive anoxic and anaerobic bacteria, i.e., the group of Gram-positive facultative anaerobic bacteria, are most suitable for the process performance. For the physical isolation of these bacteria from nature, the samples of

soil must be inoculated in the medium, cultivated under changed osmotic pressure due to the cycles of drying and suspending and low or absent concentration of dissolved oxygen due to weak aeration.

Another example is the selection of microbial groups for sequential treatment of organic and inorganic substances in wastewater at nonextreme conditions. It is clear from the periodic table of prokaryotes that Gram-positive and Gram-negative bacteria can exist altogether in the medium with stable osmotic pressure but not in the medium with unstable osmotic pressure. Therefore, for the treatment of wastewater bacteria with stable osmotic pressure, bacteria from both lines can be used but Gram-negative bacteria will have evolutionary preference because of their aquatic origin. Physiologically, this preference can be explained by lower cost of materials and energy for the synthesis of thin cell wall of Gram-negative bacteria. There may be coexistence of Gram-negative fermenting and anoxic bacteria in one biotope because anoxic bacteria are using the products of fermentation as electron donors. There may be also coexistence of Gram-negative anoxic and aerobic bacteria in one biotope because aerobic bacteria are using the products of anaerobic respiration as electron donors and anoxic bacteria are using the products of aerobic respiration as electron acceptors. Therefore, the best groups for the treatment of wastewater could be (1) association of the groups of fermenting and anoxic Gram-negative bacteria under anaerobic conditions; (2) association of the groups of anoxic and aerobic Gram-negative bacteria under variable anoxic or aerobic conditions; and (3) some group of aerobic Gram-negative bacteria under stable aerobic conditions.

4. Connection between Cell Shape and Physical Properties of Medium

There may be more detailed classification parallel sub-groups inside the groups of the periodic table of prokaryotes. For example, it is known that prokaryotic cell shape is an important feature of classification. That is why it was and until now is used for the identification and classification of prokaryotes. The shape can be considered as an evolutionary adaptation to environment as described below.

- Spherical cell (coccus) is an adaptation to homogenous environment, without gradients of nutrients. Example of this environment is fresh- or seawater.
- Elongated rod-shaped cell (bacillus) is an adaptation to heterogenous nonviscous environment with gradients of nutrients. It is most abundant shape of prokaryotes. Elongated shape increases vector of directional movement of cell toward the source of nutrients, i.e., increases efficiency of chemotaxis or phototaxis. Example of this environment with gradients of nutrients is every aquatic microenvironment,

which is close to the surface of solid matter of soil particles, suspended particles, bottom sediments, as well as to the surface of animal or plant tissue.

- Curved (vibrio) or spiral shape (spirillum) of prokaryotic cell is adaptation to viscous environment. This shape ensures spiral rotating movement of cell through viscous environment thus decreasing resitance of directional movement of cell toward the source of nutrients. Examples of this environment are viscous bottom sediments of aquatic ecosystems and mucous surfaces of animal and plant tissues.
- Filamentous cell is an adaptation to heterogenous environment with the particles, where the nutrients are concentrated on the surface of these particles. Therefore, the spread of filamentous cells onto the surface of the particles is optimal way to obtain these nutrients. Examples of this environment are soil particles, dead organic matters, and suspended particles in water.

This explains the domination of cocci in oligotrophic (not polluted) fresh- and sea-water, the domination of bacilli in eutrophic (polluted) water, vibrio-like forms (curved cells) and spirilla (spiral cells) in bottom sediments, and mucous surface of tissues, as well as the domination of filamentous cells on surface of the soil and suspended in water particles.

All these cell shapes exist in three lines of Gram-negative bacteria, Gram-positive bacteria, and Archaea as parallel sublines. Therefore, additional theoretical selection of microorganism for environmental engineering process can be made on cell shape depending on viscosity and physical structure of the medium. For example, filamentous microorganisms will be most suitable for the bioremediation of soil polluted with substances adhered to the surface of soil particles. Cocci could be most active in bioremoval of soluble pollutants from wastewater. Vibrios or spirilla are most suitable for the biotreatment of sediments.

5. rRNA-Based Phylogenetic Classification Cannot Be Used for Theoretical Selection in Environmental Engineering

The existing classification of prokaryotes is based mainly on phylogeny of 16S rRNA gene. There are practically useful classification and related fast methods for the experimental identification of microbial species and their phylogenetic interrelations. Identification performed by PCR of 16S rRNA gene and gene sequencing requires few hours and can be performed by nonexperienced researcher but identification of microbial strain using physiological, cytological, and biochemical methods requires at least several days and must be performed by significantly more experienced researcher. Therefore, rRNA-based classification is dominating in experimental research. However, there are no physiological connections between phylogenetic groups and there is no predictive power in the current rRNA-based phylogenetic classification. As a result of the widespread use of 16S rRNA-based

phylogenetic classification in experimental research, microbial diversity is often perceived by the young researchers as a random mixture of microbial species with different physiological properties.

The physiological groups often do not correspond to rRNA-based phylogenetic groups. Some examples are the grouping of microaerophilic and aerobic prokaryotes in one β-subdivision of Proteobacteria and grouping of facultative-anaerobic and aerobic prokaryotes in the γ-subdivision of Proteobacteria. Almost all divisions and subdivisions consist of species with a mixture of physiological and cytological features. Small evolutionary distance between two species of different physiological groups reflects short evolutionary time after speciation. An example is the small evolutionary distance between *Nitrobacter winogradski* and *Rhodopseudomonas palustris*, which are an aerobic chemolithotroph and an anaerobic phototroph, respectively. The small evolutionary distance between their 16S rRNAs can be explained as thus: the branch of *N. winogradski* originated from the line of *R. palustris* a short evolutionary time ago. Another contradiction is that the physiological properties of some of these representatives may be very similar but the dissimilarity between their rRNAs would be very large because of the accumulation of a large number of mutations in 16S rRNA over a long period of evolution.

That is why current 16S rRNA gene-based prokaryotic classification cannot be used for the selection of specific physiological groups needed for the different processes of environmental engineering. Probably, future classification, which will be based on the complete genome of prokaryotes and will be created in 2020s, will be close to physiological classification of prokaryotes and periodic table reflecting this classification.

6. Methods of Selection and Isolation of Microorganisms

Theoretical selection of microbial group that is most suitable for the specific environmental engineering process is the first step of selection. This selection is used for the specification of the conditions, which are most suitable for the experimental selection of microorganisms for the defined environmental engineering process. However, there are often possible multiple choice of suitable microbial groups and, respectively, multiple choices of conditions for the experimental selection of microorganisms.

Microbiological methods that are used for selection of microorganisms needed for the performance of environmental engineering process are as follows:

- selection and isolation of pure culture,
- selection of enrichment cultures, and
- selection of artificial ecosystem.

Isolation of pure culture (of microbial strain) is usually performed by spreading a diluted microbial suspension on a Petri dish with a semisolid medium to produce 10–50 colonies on the dish after several days of inoculation. Cells of one colony are picked up for the next round of inoculation on a semisolid or liquid medium. Usually, there is no problem of cultivability for environmental engineering strains because the major function of selected microorganisms is biodegradation of different natural or similar to natural organic compounds.

Instead of isolation of strain on semisolid medium other methods can be used, for example, (1) mechanical separation of big microbial cells by micromanipulator; (2) sorting of cells or microbeads with immobilized cell using sorter of flow cytometer; (3) magnetic or immunomagnetic separation; (4) cell dielectrophoresis; (5) cell chromatography.

Pure culture could be selected not for the performance of the technology but as the object of the monitoring of environmental engineering process.[3]

Selection of enrichment culture or autoselection refers to the selection of the microbial community with one or several dominated strains, which are accumulated in the system of cultivation due to the preferred conditions (selection pressure) for these strains. Enrichment cultivation is often used in environmental engineering to select microorganism(s) capable of particular metabolic transformations. Selective conditions (selection pressure) for the production of enrichment culture are as follows:

- source of energy,
- source of carbon,
- sources of nitrogen and phosphorus,
- temperature,
- pH,
- concentration of heavy metals,
- presence of specific antibiotic in a medium,
- concentration of dissolved oxygen,
- osmotic pressure of a medium, and
- spectrum and intensity of light.

The mechanisms of selection of enrichment culture are as follows:

- faster or more efficient growth of one or several strains (positive growth-related autoselection),
- faster or more efficient biochemical functions of one or several strains (positive metabolic autoselection),
- slower or less efficient growth of one or several strains (negative growth-related autoselection),

- slower or less efficient biochemical function (negative metabolic autoselection),
- better survival under harmful conditions (positive survival-related autoselection),
- weaker resistance to some factors of environment (negative survival-related autoselection), and
- stronger or more specific adherence of cells to surface (positive or negative cell adherence-related autoselection).

Autoselected features of the enrichment culture can be genetically unstable and could disappear after several generations of cells when the selection pressure will be absent.[4] This is known, for example, for cell surface hydrophobicity, which can be enhanced several times during retention of cells on hydrophobic carrier during several cell generations or can disappear during several cell generations if there will be selection pressure in medium. This feature is important in the cases of microbial remediation of oil spills, where cell must float at water surface, or in case of formation of microbial granules, which can replace conventional flocks of activated sludge in municipal wastewater treatment.

Selection of artificial microbial ecosystem is similar to the selection enrichment culture but there may be several alternative or changed selective factors (selection pressures) ensuring dominance of several microbial communities with different, even alternative, physiological functions. For example, in selected artificial microbial ecosystem may be simultaneously aerobic and anaerobic microbial communities were selected due to the presence of both aerobic and anaerobic conditions in the environmental engineering system.[5] The set of the mechanisms of selection and the selective pressures is specific factor for the selection of artificial microbial ecosystem. Additional mechanism of selection of artificial ecosystem can be positive or negative interactions between the microbial communities of the ecosystem: commensalistic, mutualistic, amensalistic, antagonistic, or parasitic relationship.

Important element of selected ecosystem is the boundary between an ecosystem and its surrounding environment, which could be a steep gradient of physical or chemical properties. The physical boundary is formed by an interphase among solid and liquid phases, solid and gas phases, and liquid and gas phases. For example, the microbial ecosystem of an aerobic tank for wastewater treatment is separated from the environment by the reactor walls and air–water interphases. The boundaries of this ecosystem are as follows: (1) side walls of the equipment with a fixed microbial biofilm; (2) bottom of the equipment with the sediment of microbial aggregates; and (3) gas–liquid interphase with accumulated hydrophobic substances (lipids, hydrocarbons, and aromatic amino acids) and cells or aggregates with high hydrophobicity of their surface or cells and aggregates containing gas vesicles. Due to these interphases, there are at least 10 different microbial communities in aerobic tank of wastewater treatment (Fig. 1).

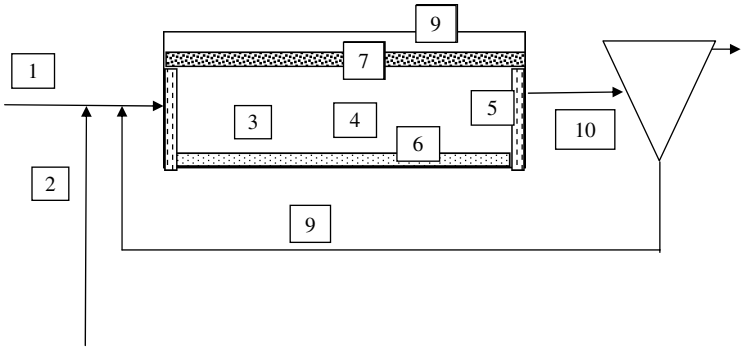

Figure 1. Different microbial groups in aerobic tank of municipal wastewater treatment plant. The components are as follows: (1) influent of raw sewage into aeration tank (contains dead cells of obligate anaerobes, spores, and live cell of facultative anaerobes and aerobes); (2) influent of return liquor (rejects water after anaerobic digester) into aeration tank (contains dead cells of obligatory anaerobes, spores, and live cells of facultative anaerobes); (3) suspended cells (contain aerobes); (4) activated sludge flocs (contain aerobes and facultative anaerobes); (5) attached biofilm on the walls of aerobic tank (contains anaerobes and aerobes); (6) bottom sediment of aerobic tank (contains aerobes and facultative anaerobes); (7) foam layer of aerobic tank (contains hydrophobic cells of aerobes and spores); (8) bioaerosol released from aerobic tanks (contains hydrophobic cells of aerobes and spores); (9) influent of settled activated sludge and ecosystem of the settling tank (contains aggregated cells of anaerobes, facultative anaerobes, and aerobes); and (10) effluent from aerobic tank (contains dead and live hydrophobic cells and floating aggregates).

The steep gradient of chemical substances, for example, oxygen, ferrous, hydrogen sulfide, etc., forms a chemical barrier. Such barriers separate, for example, aerobic and anaerobic ecosystems in a lake. The steep gradient of conditions can be also created by cell aggregation in flocs, granules, or biofilms. The main function of the boundary is to maintain integrity of an ecosystem by controlled isolation from the environment and to protect an ecosystem from the destructive effects of the environment. Defined ecosystem cannot exist, cannot be selected, and maintained without defined boundary.

7. Selection of Microbial Aggregates

A multicellular aggregate is formed and separated from its surrounding environment due to following processes[6–9]:

- aggregation by hydrophobic force, electrostatic interactions, or salt bridges,
- loose polysaccharide or inorganic matrix (iron hydroxide as example) combining the cells altogether by mechanical embedding, chemical bonds, hydrogen bonds, electrostatic forces, or hydrophobic interactions,
- formation of mycelia, which are net of branched cell filaments,

- polysaccharide matrix with a filamentous frame, and
- coverage by a common sheath of organic origin (polysaccharides and proteins) or inorganic origin (iron hydroxide, silica, and calcium carbonate); common sheath can be made also from dead cells of aggregate ("skin" of microbial aggregate).

Usually, matrix of aggregate is structured with the layers or sub-aggregates. Therefore, a microbial aggregate can be considered as a multicellular organism because its parts have some extent of coordination and synchronization of physiological functions, i.e., synchronous growth, motility, sexual interactions, assimilation of atmospheric nitrogen, production of extracellular polysaccharides, transport and distribution of nutrients, oxidation of electron donors, and reduction of electron acceptors.

Three major types of microbial aggregates used in environmental engineering are floc (loosy suspended aggregate of irregular shape), biofilm (attached to surface microbial aggregate), and granule (dense suspended aggregate of regular shape). The selection pressures for these aggregates are (1) settling of aggregate for 20–30 min and retain or recycle of this settling aggregate in bioreactor is used for the selection of flocs; (2) intensive aeration for the mechanical compaction of aggregate by air bubbles, settling of aggregate for 2 min, and retain of this settling aggregate in bioreactor are used for the selection of granules; (3) attachment and retain of microbial aggregate at surface are used for the selection of biofilm.

Therefore, the selection of microorganisms able to form aggregates can be performed by settling rate or filtration. Simple selection of microbial strain able to form cellular aggregates can be performed on Petri dish by the size of the colonies of pure culture. The colonies of the biggest size were most probably originated not from one cell but from the aggregate of several cells.

The interactions of microorganisms in aggregates are usually positive because of close physical proximity of cells. Physiological cooperation in aggregates is supplemented and supported by its spatial structure, i.e., formation of microhabitats for individual populations. Some examples of mutualism in microbial aggregates are (1) syntrophy ("co-eating"), both microbial groups supply nutrients or growth factors; (2) sequential biodegradation of xenobiotics, when the product of biodegradation inhibits biodegradation; and (3) biochemical oxidation and reduction of element by two microbial groups.

8. Growth-Related and Survival-Related Selection of Microorganisms

Growth-related selection of microorganisms can be performed in batch or continuous cultures. Such parameters of microbial growth as (1) current specific growth rate;

(2) maximum specific growth rate; (3) affinity of specific growth rate to different nutrients, first of all to the sources of carbon, energy, oxygen, nitrogen, phosphorus, and iron; (4) growth yield from different nutrients; and (5) optimum of temperature, pH,[5] oxidation–reduction potential (ORP) for growth rate and for growth yield, specificity of electron acceptor and surface for cell adhesion[10] can be used for growth-related autoselection of enrichment culture.

Continuous culture is most effective way for the selection of microorganisms with the highest growth rate and growth yield. Following continuous cultivation, reactors can be used for this type of selection: (1) bioreactors of complete mixing, for example a chemostat, where the dilution rate (D), which is a ratio between flow rate (F) and working volume of the reactor (V), is maintained constant; (2) plug-flow bioreactor or consecutively connected bioreactors of complete mixing that form a plug-flow system; (3) fixed biofilm reactor or retained biomass reactor with the flow of medium through it; biomass is retained in the reactor due to adhesion, sedimentation, cell aggregation,[9] or membrane filtration; (4) complete mixing or plug-flow reactor with the recycling of microbial biomass; and (5) semi-continuous and sequencing batch reactor, where the periodical addition of nutrients and removal of suspension are used.

Survival-related selection of microorganisms is usually performed in batch culture. There are always, even under optimal conditions for growth, high percentage of dead cells in microbial population. Negative factors of environment, such as starvation as well as nonoptimal pH, ORP, concentration of dissolved oxygen, can increase significantly this percentage of dead cells in microbial culture.

Effect of starvation is the most often used in practice. There are three typical responses of microorganisms to starvation, i.e., to shortage of some nutrients in a medium. The microorganisms known as R-tactics are fast growing in a rich medium but can quickly die under a shortage of nutrients. Typical representatives of this group are *Pseudomonas* spp. The L-tactics microorganisms are fast growing in a rich medium but under starvation, they form dormant spores and cysts. Typical representatives of this group are *Bacillus* spp. This feature is used in environmental engineering for the selection of microorganisms, which can survive for a long time as dry cells. K-tactics microorganisms are adapted to grow slowly in the medium with a low concentration of the nutrients. Typical representatives are the oligotrophs *Hyphomicrobium* spp.

Significant role in survivability has an ability of microorganisms to accumulate storage compounds as carbon and energy sources and nitrogen or phosphorus source. Therefore, survivability during starvation is used in environmental engineering for the selection of microorganisms that are able to accumulate glycogen, polyhydroxibutirate, lipids, or polyphosphate. Some storage compounds (polyols, disacharides,

and aminoacids) are serving also as osmoprotectors of cells under high osmotic pressure.

Survival of anaerobic microorganisms under presence of oxygen is most important way of selection of oxygen-tolerant anaerobes or facultative anaerobic bacteria. Resistance to heavy metals, antibiotics, oxidants, and the organic solvents such as ethanol or butanol[11] also can be used for the selection of environmental engineering microorganisms with specific properties.

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