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Bioelectrochemical Hydrogen and Electricity Production

Theoretical bases, description and modeling of the process

Lublin 2013

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Preface

Prominent scientists of today declared that the twenty-first century will be the era of biological sciences and other science would recede into the background. Therefore, for example, predictions by the famous physicist of modern age N. Bohr, are already coming true. In 1950s he repeatedly emphasized that in the near future intensive penetration into the mysteries of nature would be the preferences not of physics, but namely of biology. For the most part of it, the current scientific literature is to any extent devoted to the study of wildlife. Today, problems of biological systems are concerned by tens of sciences both in fundamental and application aspects.

Sciences related to implementing of modern biological advances are very productive. Thus, solving of many crucial problems of the present, such as energy, food, medicines and other useful substances production, ensuring of sustainability and environmental quality improving, environmental monitoring, is connected with active implementation of biotechnology.

Such a rapid progress of biology and biotechnology would not be possible without their active interaction with other sciences such as chemistry, physics, physical chemistry, electrochemistry and others. The decisive difference of modern state of science is in that a big amount of research is going on at the intersection of science, and the involvement of scientists of various specialties is essential for the practical solving of the problem. Nowadays ideas and methods of biology, physics, chemistry, mathematics, electrochemistry, electronics, nanotechnology and other fields of knowledge are closely intertwined in solving biotech problems. The authors of this monograph are the representatives of different but closely related sciences: electrochemistry, environmental biotechnology and environmental engineering – technology of water and wastewater treatment. Therefore successes and challenges of bioelectrochemistry are close for them and they are very concerned about them.

This monograph is devoted to a very timely topic. Bioenergy production via bioelectrochemical systems is of the great interest because of its potential applications in environmental biotechnology and renewable energy. Energy and water supply are two of the biggest challenges facing humanity in the coming decades. The efforts of modern scientists to develop strategies for the recovery or efficient usage of resources are, therefore, highly justified. Groundbreaking technology is needed to implement novel means of converting and conserving resources. BESs fit this global goal. Issues of mathematical modeling presented in the monograph deserve a particular attention. The basis of BES is a biofilm with exoelectrogens. Biofilm formation is determined by a variety of biological, chemical, and physical processes. By generating quantitative predictions from descriptions of biofilm characteris-

tics that have been turned into the rational form of a complete set of equations, mathematical models can lead to deeper and broader understanding.

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List of acronyms

AC – anodophilic consortium
ADP – adenosine diphosphate
AP – action potential
ATP – adenosine triphosphate
BEP – bioelectric potentials
BES – bioelectrochemical system
BFC – biofuel cell
BLM – bilayer of lipid membrane
CCS – chemical current source
CEM – cation exchange membranes
CNT – carbon nanotubes
COD – chemical oxygen demand
DMRB – dissimilatory metal-reducing bacteria
DNA – deoxyribonucleic acid
EMF – electromotive force
FC – fuel cell
MFC – microbial fuel cell
MMFC – modified microbial fuel cell
NHE – normal hydrogen electrode
OCV – open-circuit voltage
SHE – standard hydrogen electrode

Introduction

Nature has taken care of everything
so that all you find is what you can learn from.
Leonardo da Vinci

Bioenergy, biology and electrochemistry are inextricably linked. In XVIII century it was known that electrochemical processes play an important role in nature. The intensive studies of recent decades have convincingly demonstrated that biological processes, especially associated with energy transformation, have electrochemical nature. A growing interaction of biology and electrochemistry is mutually very beneficial. Then electrochemistry has found a new and extremely productive area in biology for applying its capabilities (*Kuzminskiy and Shchurska, 2010*).

The subject of electrochemistry is the study of the laws related to mutual transformation of chemical and electrical energy. Chemical reactions are usually accompanied by absorption of heat or heat release (reaction thermal effect), but not electricity – electrical energy. But the electrochemistry studies reaction either occurring being externally fed by electricity or being the source of electricity. In electrochemical reactions electrons and charged particles (cations and anions respectively) are necessarily involved. Naturally, the energy of these particles is a function of the electric field generated at the boundary layer "electrode – electrolyte". Thus, the rate of electrochemical reaction depends not only on the temperature, the activity of the catalyst and reaction participants, that is the same factors that determine the rate of a chemical reaction, but also on the potential on the boundary layer of phases with different conductivity. Boundary layer potential variation at the constant concentrations of electrochemical reaction participants and temperature allows changing the rate of reaction in tens, hundreds and thousands times and in some cases even products composition. This makes the electrochemical reactions more manageable and easily controlled compared with the chemical ones. Electrochemical reactions can be defined as chemical reactions, the rate of which is a function of potential. Therefore, the electrochemical reactions differ from chemical not only in energy generation process effect and also in the amount of activation energy (*Antropov, 1984*).

At the present stage of science and technology development nanotechnology could significantly expand the scope of electrochemical and bioelectrochemical systems and devices and raise them to a new level. Nanotechnology is a basic priority for all existing industries being not simply specific technologies or new techno-

logical directions, but a modernization of existing technologies on a fundamentally new atomic level. Nanotechnology is suprasectoral foundation of all without exception areas of the new knowledge-based economy of postindustrial society. Today we can formulate the main features of the present stage of development of scientific and technical areas.

First, it is a transition to nanoscale due to the possibility of manipulating of atoms and molecules as components of any substance. Combining individual atoms and molecules in certain way it is possible to construct new substances. The paradigm of science has changed from the understanding of how the world functions, to purposeful creating of some of its items. However, the main problem is that the resources available to the mankind are limited. Hence, there is a new formulation of the problem: a new system of priorities needs to be developed and bioelectrochemical energy is one of these priorities.

The second characteristic of scientific development at this stage is the convergence of the organic world (the world of wildlife) with inorganic. A significant progress has been made in the last decade in this issue. As a result, the approach to the R&D organization has been fundamentally changed: it is necessary to move from the highly specialized research method to the interdisciplinary one. The bioelectrochemical energy and, in particular, bioelectrochemical systems (BESs), are examples of such interdisciplinary research.

The third direction of nanotechnology is the combination of today's technological capabilities with constructions patterned after wildlife. For example, the creation of semiconductor nanostructures with quantum dots is an important achievement in inorganic material science. The basis of the formation of these structures is the principle of self-organization, which is a basic principle of nature. Another example is BESs as technical devices that copy the energy supply system of wildlife.

One of the illustrative examples that demonstrate the efficiency of interdisciplinary approach is bioenergy. Bioenergy is a term of a broad sense. On the one hand, it is a science of general laws of energy transformation in living systems (cells, organisms, ecosystems, etc.). On the other hand, the bioenergy can be interpreted as the direction of technical (alternative) energy related to using of alternative energy sources of biological origin. Technical bioenergy is mainly engaged in the conversion of various biological materials and organic waste into biofuels (solid, liquid, gaseous) or directly into electrical energy (*Kuzminskiy et al., 2009*). Both mentioned areas of bioenergy are interrelated, while developing they harmoniously complement each other (*Kukhar et al., 2005*).

The first fundamental characteristic of bioenergetics is in the fact that organisms are open systems, which function only under conditions of constant exchange of materials and energy with the environment. The thermodynamics of such a system is essentially different from classical thermodynamics. The main concept of

equilibrium states, which is a basic principle of classical thermodynamics, is replaced by the concept of stationary states. It is the reason for the entropy changes in similar systems which take place not according to the second principle of thermodynamics (the principle of the increase of entropy) but to Prigozhin's theorem.

A second very important characteristic of bioenergetics is associated with the fact that the processes in the cells occur in the absence of jumps in temperature, pressure, and volume. Nature, in contrast to the technology, could not afford the high temperature, pressure and other conditions characterizing modern internal combustion engines and other similar heat machines. The transition energy of chemical bonds into biological useful work in a particular cell or the whole organism occurs without converting chemical energy into thermal energy used by the mechanisms that enable the direct conversion of one form of free energy to another, bypassing its conversion into heat.

One of the main results of bioenergy development for recent decades is in the establishment of similarity of energy processes throughout the living world, from bacteria to humans. It was found that all substances as well as the processes where energy is collected and used are identical for all the flora and fauna. Technical and biological systems of chemical energy conversion into electrical energy are also fundamentally similar; the differences are in detail only. Any problems with insulation during the creation of technical electrochemical systems are not usually arisen because they are surrounded by an air, which is a dielectric medium. In addition, metals with high electrical conductivity are used in the technical devices such as electrodes and conductors. Instead, wildlife established its electrochemical devices within the electrolyte solution that is the non-dielectric environment. Besides, there are no any metal conductors.

Another illustrative example of the use of developed natural approaches to solving the energy problems of mankind is the analogy between man-made electrochemical generators, fuel cells, and energy supply of living organisms. The researchers have found as if hydrogen-oxygen fuel cell (FC) has been mounted in the living cell (*Kuzminskiy et al., 2003*).

The intensive search of renewable and environmentally friendly fuel is conducted because of the progressive shortage of fossil fuels. Hydrogen is the most promising second generation fuel. Widespread use of hydrogen will reduce air pollution, eliminate greenhouse effect and, consequently, lead to slowing global warming (*Kozin and Volkov, 2002*). FC, i.e. systems that carry out the energy of direct hydrogen oxidation into electrical energy are traditionally used for hydrogen energy generation. These systems have an efficiency of over 80%, while the best efficiency of internal combustion engines is only 20%, and, in contrast to combustion systems the hydrogen combustion does not form oxides of carbon, nitrogen and sulfur, i.e., have the high ecological purity. The implementation of FC in cars was seen

only as science fiction 15 years ago. Now almost all automobile Companies have demonstrated prototypes of cars that are driven with FC, and the United States have adopted a program of transferring of road transport on FC since 2010 (*Dudnik et al., 2000; Brodach and Shilkin, 2004; Kovtun and Polunkin, 2006*).

In this regard, the special attention should be drawn to electrochemical systems of power where the microorganisms (or enzymes) directly convert the energy of chemical bonds of organic molecules into electricity in the most effective way in biofuel cells (BFC) (*Kuzminskiy et al., 2003; Kuzminskiy et al., 2008; Kuzminskiy et al., 2009*). Using biofuel cells technology it is challengeable to solve not only the energy problems, but also environmental problems of waste utilization, because microorganisms have the ability of adaption to the environment. As the aquatic environment is a necessary condition of life of all organisms, thus, the wastewater containing organic contaminants are most attractive substrate for the implementation in the declared approach. The urgent need for waste of organic origin disposal and management, as well as an intensive search for alternative energy sources is an important incentive for large-scale research of BFC. It is necessary to emphasize that their payback is determined not only with accumulated electricity, but also with decreased in value of wastewater treatment. For example, Professor Bruce E. Logan of the University of Pennsylvania is confident that large-scale use of BFC in wastewater treatment will annually save about U.S. $ 25 billion (*Min et al., 2005*).

Thus, wastewater with high concentration of organic compounds is a renewable source for electricity or hydrogen gas production. Highly effective receiving of these energy sources is possible in devices bioelectrochemical microbial fuel cell (MFC). Bioelectrochemical method of energy generation implemented in the biofuel cells is a totally new way to produce renewable energy with simultaneous wastewater treatment. In MFC microorganisms oxidize the organic matter and transfer electrons directly to the anode. There are the bacteria able to transfer electrons to the electrode directly or through endogenous mediators: *Geobacter*, *Shewanella*, *Pseudomonas* and others. Electrons perform useful work and are transferred to the cathode where they combine with oxygen and protons with subsequent formation of water molecules. This process occurs in conventional MFC.

Also emitted protons and electrons during the oxidation of organic compounds can be recombined to molecular hydrogen. This requires strict anaerobic conditions in MFC and additional potential for the cathode, because higher cathodic potential in the MFC electrical circuit enables direct production of hydrogen from protons and electrons generated by bacteria. Such modification of traditional MFC reduces energy requirements for the hydrogen production from organic matter in comparison with the production of hydrogen from water by the electrolysis.

The MFC have gained new impetus during recent years due to the growing interest in the production of so-called “green” (or environmental) electricity because

microorganisms use as fuel a huge spectrum of organic compounds including a variety of wastes. It was already noted that this enables simultaneous solving of both environmental and energy problems. Although developed laboratory MFCs are far inferior in terms of power densities in comparison with conventional wastewater treatment technology with simultaneous electricity generation (UASB-biogas reactors) (*Kuzminskiy et al., 2007; Kuzminskiy et al., 2008*), but there are several ideas of effectiveness improvement (*Kuzminskiy and Shchurska, 2010b; Kuzminskiy et al., 2011; Shchurska et al., 2011;*) that will be discussed in this monograph.

Given all the above this monograph that is devoted to one of the least studied areas of renewable energy, namely the study of the possibility of bioelectrochemical production of electricity and hydrogen in the biofuel cells will be useful for researchers working in the field of energy conversion systems and in particular, in bioenergy, and for teachers and students who are specialized in environmental biotechnology, bioelectrochemistry and bioenergy.

1 Bioelectrochemistry as a theoretical basis of biofuel energy

Every science, as every truth
is a child of its time.
Michele Giua

Electrochemistry and biology are related genetically as Professor A. Ksenzhek pointed out in his paper (*Ksenzhek, 2000; Kuzminskiy and Shchurska, 2010*). The ability to use electrochemistry for solving "biological" problems is based on similarity between biochemical and electrochemical redox reactions: (I) both types of reactions are heterogeneous (the first ones take place on the electrode surface, the second ones occur at the interface between the phases "enzyme-solution"); (II) proceed in the same range of pH and in solutions of the same ionic strength; (III) occur in aqueous environments and in the same temperature range; (IV) include the stage of the substrate orientation.

Electrochemical processes play an important role in wildlife because the electrochemical stages occur in all the processes of energy transformation in the cells of bacteria, fungi, plants, animals and of course in human body. Thus, in humans the total surface of cell membranes reaches a few hectares. All cells are filled with biological fluids containing ions, i.e. electrolytes. Although biological systems do not have metal electrodes, but there are special systems, electron transport chains that carry out the function of electron transfer. These are the classic objects of electrochemistry so far. Besides, the processes of photosynthesis and respiration include electrochemical stages as an integral part.

It should also be noted that the overall capacity of the electrochemical processes that occurs in the biosphere is extremely large. It exceeds the scope of the technical world energy use enormously. The urgent need to present bioelectrochemistry as a separate discipline is contained in particular in M. Faraday's, one of the founders of electrochemical laws, statement: "Ironically, the electrical phenomena within the inorganic matter pale in comparison with those in nervous system and life processes". And as a result of establishing the electrochemical nature of a number of processes in living organisms bioelectrochemistry appears to be a new trend of science and technology.

Analyzing in terms of methodology of the experiment the popularity of electrochemical methods is stipulated because of the following:

- theory of electrode reactions and methods of electroanalysis facilitating the consideration of analytical signal formation using enzyme electrodes are worked out in details;
- methods of electroanalysis are universal, some of them (pH, dissolved oxygen and hydrogen peroxide concentration measurements) can be used for determination the activity of not one but a number of enzymes under the same conditions of measurement;
- electrochemical methods, in general, can improve analytical characteristics of enzymatic methods by specific way and the design of biosensors based on immobilized enzymes (biological component sorption at controlled potential, using electrocatalysis and homogeneous electron transfer approaches, etc.);
- electrochemical methods allow to use a standard element base and measurements (glass electrodes for pH measurement, ionomer resin, Clark oxygen electrodes, volt-ampere graphs et al, that are accessible and easy to operate);
- theoretically and experimentally processed a traditional fuel cell is a good basis for development of the theory and practice of biofuel components.

All this allows reducing the costs of initial studies of new “biological” devices for energy conversion, standardizing the measurement and integrating the biochemical methods of analysis into the existing systems of automatic control in the nearest future. The processes of charge transfer on the phases’ interface are the basis of electrochemical processes of energy conversion. Even if the enzyme is in solution or is used the soluble electron transfer mediator, the measurement signals (electrical conductivity, potential, current, Faraday’s impedance) are always associated with the change of the surface of the transducer. These electrochemical methods are fundamentally different from optical, photometric, fluorometric in which the origin of the signal is due to changes in solution. Thus, both electrochemical and optical methods can solve the same problem.

Heterogeneous character of the signal has both advantages and disadvantages. For example, it includes the possibility of creation of electrochemical systems for energy conversion, or sensors, when the phase interface is both a place of localization of the enzyme or a microorganism in the electrode biofilm. There are a number of cases when the mass transfer process on the phases interface can significantly improve the analytical ability to detect components of enzymatic reactions. The definable component sorption as well as its selective transfer to the enzyme through an auxiliary membrane can serve as an example of the above-mentioned.

Potential limitations of electrochemical techniques include the limitation on the stage of mass transfer on the "transducer-solution" interface. It can lead to the general increasing in its measurement durability or decreasing in its sensibility.

The interest in biology among electrochemists has dramatically increased after it occupied a frontline position in science and technology. This interdisciplinarity gives birth to bioelectrochemistry – the new scientific direction for studying electrochemical principles of functioning of biological or "living" systems functioning (*Antonov, 1996; Opritov, 1996; Chizmadzhev, 2000; Ksenzhek, 2000; Kuzminskiy et al., 2007*) on the one hand and such bioelectrochemical devices as biofuel cells (*Kuzminskiy et al., 2003; Kuzminskiy et al., 2008; Kuzminskiy et al., 2008a; Samarukha et al., 2008; Kuzminskiy et al., 2009; Kuzminskiy et al., 2009a; Kuzminskiy et al., 2011*) on the other hand.

Electrochemistry is the fundamental science for biology, medicine and bioenergy. However, besides being able to give a lot to medicine and biology, bioenergy and biosensors technology but the electrochemistry itself can obtain a big amount of knowledge and approaches through detailed studying of bioelectrochemical processes. Modern biology and bioenergetics are developing very rapidly and this is a stimulus for almost all who are involved in related disciplines including electrochemists. But to have the opportunity to widely practically apply the bioelectrochemical concepts and methods, the electrochemists are bound to meet the needs of today as well as study modeling systems as close to real biological objects as possible. They are: the investigations of model systems as close as possible to real biological objects. Therefore, the authors of this monograph consider it appropriate to introduce students, teachers and researchers of various scientific fields with some classical concepts and present achievements in bioelectrochemistry.

1.1 Specificity of biological objects

The development of biology has shown that the study of elementary biological phenomena requires a use of concepts and methods of science. This approach is justified by the fact that all biological objects, ultimately, are the complex of atoms and molecules and are a subject to physical and chemical laws. However, the biological systems are the systems capable to self-organization and self-healing and those which were created during evolution and have properties that have no place in inanimate nature.

Biological objects (cells and organisms) are different from inanimate in an ability to perform metabolism and reproduction (with genetic information transfer).

Biological objects are open thermodynamic and relatively isolated cybernetic systems. This means that beings are in exchange of energy, substances and, particu-

larly, information with the environment. Exchange with the environment is realized by using of substrates that are the sources of free energy and order (of information) that leads to a decreasing of entropy and increasing of the level of living systems organization. The Information about all the features of beings identifying their individual and species specificity is embedded. Phenotype is a set of characteristic and properties of being that have been formed during the interaction of its genetic structure (genotype) and external environment. Genotype is the genetic (hereditary) information that is almost invariably reproduced and transmitted from generation to generation, but may not appear in every generation. Phenotype is formed during the beings life under the influence of genotype which is expressed as the external traits and individual experience of interaction with the environment and other organisms.

Exchange with the environment is mainly subject to the Le Chatelier's principle and leads to a steady state system. (Le Chatelier's principle: “If a chemical system at equilibrium experiences a change in concentration, temperature, volume, or partial pressure, then the equilibrium shifts to counteract the imposed change and a new equilibrium is established”). Stationary state is a dynamic condition in which at any given period of time (long enough, such as night for a man) the system receives as much substance and energy from the environment as it returns to the environment. As a result, the amount of substance and energy in the system remain unchanged. This distinguishes living objects from inanimate (isolated) systems that are in time-independent equilibrium. In abiotic or isolated systems the amount of substance and energy is constant, metabolism and energy interchange with the environment do not take place and therefore all processes are terminated.

The living organism processes run in time and space. Biological systems of various complexities differ in spatial structure and life cycle. Thus, the life cycle for microorganisms with fission reproduction and somatic (unsexual reproduction) cells of pluricellular organisms is the period between two divisions; for organisms with sexual reproduction is the period from zygote formation from gametes till death and so on.

The structures of living organisms consist of organic and inorganic substances that have a certain spatial configuration. The reaction ability and biological activity of many substances in biological systems depend on the configuration of molecules.

Specificity of living organisms is that the information is involved into the process of their constructing, because it is arisen in the course of evolution and founded in the genome. This information appears in the structure of beings that are ordered, thermodynamically non-equilibrium and adapted to perform a specific function.

The structure of living organisms is quite similar to an artificial construction which is also based on information accumulated by mankind. All levels of biological and artificial structures are similar: the molecular (proteins, nucleic acids, etc.), cellular (organelles and membranes) and organismal. There are two ways of consid-

eration of biological information contained in the object: direct and indirect. Direct route involves the construction of a living object (in all its levels, from molecular to organism) based on information that is embedded in the genome. In nature this way is realized in ontogenesis i.e. in the development of the organism from a fertilized egg. The methods of synergetics – the self-organization theory and mathematical modeling are used in the theoretical studies in biophysics. Indirect way is the experimental study of biological structure of the object by using all known physical and physicochemical methods. X-ray structural analysis gives a lot of information at the macromolecular level; the electron microscopy is used in investigations of membranes and cell organelles; at higher levels microscopy and anatomy are used. The obtained information is equivalent to biological information inherent in the object. So if the construction is known it is not necessary to know the information on which it was constructed. Further study of the behavior of the object is based on the laws of physics and chemistry including the investigating object design. Both directions are being developed in biophysics but the second one dominates in specific problems solving.

1.2 General provisions of bioelectrochemistry

Within the bioelectrochemistry the mechanisms of processes that occur in living cells are studied by using of various models and methods of electrochemistry. Now it is obvious that electrochemical patterns are the basis of not only information systems but also systems of energy conversion within organisms (*Chizmadzhev, 2000; Kuzminskiy et al., 2007*). In other words, the "membrane potential" is not only the way of "cells dialogue" but also the connecting factor that converts chemical energy, light energy and even energy of mechanical motion. It was found that the internal membrane electric field controls the interaction and fusion of cell membranes. Therefore, it is clear that electrochemistry is fundamental to biology and biotechnology and, especially, for bioenergy technologies and biosensors. (*Kuzminskiy et al., 2003; Kuzminskiy et al., 2007*).

It has been studied that biological membrane is a polyfunctional system, but the main task of the cell membrane and organelles is in being a barrier to prevent penetration of substances that are on both sides of the membrane, rather more precisely, should have selective membrane permeability.

Membranes may be permeable not only for ions and neutral compounds, but also for electrons because they are insulators with a low value of the coefficient of dielectric constant ($\varepsilon = 2$) and have very high resistance. It can be confidently asserted that the electrochemical properties of biological membranes are not something exotic in wildlife, but they underlie in such important life processes as animals breathing and plants light energy conversion. From the physicochemical point

of view the membrane with electron transfer may be represented as a dielectric layer that separates two phases with different redox systems that are in contact with opposite sides of the membrane. Thus, for the understanding of the processes of electron transport through biological membranes it is important to explore and understand the features of the processes at the interface “insulator-electrolyte”.

The charges formation after chloroplasts lighting and their absorption by the reaction centers on the surface of lipid-protein complex is one of the issues in photosynthesis investigation. To understand the process, the knowledge of electrochemical photoinjection at the interface of aqueous phase and dielectric medium is required. In such cases the phenomenon that occurs at the interface of a heterogeneous “membrane-water” environment is the most important. Analysis of charges transport processes in the cells and model membranes has led to the need of development of new theories of facilitated diffusion mechanisms and migration of ions in the narrow channels as well as dependency of the energy profiles of ion diffusion path on the measure of filling of the vacant focuses and so on. All these problems are largely due to the necessity of a detailed study of the properties of the interface “membrane-electrolyte”.

Electrochemical methods allow obtaining information about the redox potentials, the number of electrons, the reaction mechanisms and so on. Capacitive measurements provide important knowledge about the adsorption properties of low molecular weight and macromolecular biologically active compounds (nucleic acids, proteins, etc.). Study of mechanoelectrical phenomena such as movement and orientation of cells in the external electric field, membrane structural rearrangements during electrical breakdown and electrostimulated cells merge are the fundaments for different areas of medical engineering technology and biotechnology (medicine artificial carriers, diagnostic membrane, obtaining hybrid cells using electrical maintenance). The substantiation of chemiosmotic Mitchell hypothesis is the significant achievement that was made during experiments with modeling and reconstruction of membrane systems as well as liposomes. The development of ion selective microelectrodes for intracellular use, microelectrodes for intracellular injections of electrochemically active materials, electrochemical biosensors (bacterial and tissue electrodes) and ion selective electrodes using the ionophores are among the achievements of applied bioelectrochemistry. Medical and engineering applications include the study of extracellular electric fields and mechanisms of influence of external fields and currents on physiological processes including tissue regeneration.

All examples described above suggest that the processes of energy and substance transformation in living organisms have the electrochemical stage. One of the significant results of bioenergy development during last decade is in establishment of energy processes similarity in all living organisms – from bacteria to hu-

mans. The substances in which energy is accumulated and the processes by which such storage is carried out are the same for all plant and animal organisms. This similarity describes the processes and the use of accumulated energy in these substances.

Technical and biological systems for chemical energy conversion into electricity are similar in general, there are only differences in detail. No isolation problems usually occur in R&D of technical electrochemical systems, because they are surrounded by air as dielectric medium. In addition, the metals with high electrical conductivity are used to be electrodes and conductors. Instead, the wildlife establishes its electrochemical devices in nondielectric environment that is in the electrolyte solution. Besides, there are no metal conductors. For these reasons, "Biological Electrochemistry" seems to be the inverse to the familiar "Technical Electrochemistry" (Fig. 1.1).

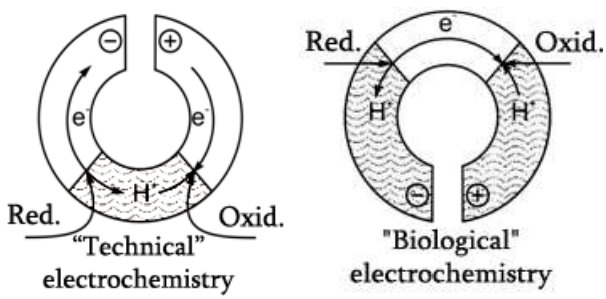


Fig. 1.1 The scheme of processes in the "technical" and "biological" systems (*Kuzminskiy and Shchurska, 2010*)

In this case not the electronic conductor, but the electrolyte phase is divided into two volumes. The thin film of cell membrane serves as the insulation layer between them. Potential difference in such a system is generated between the volume of electrolyte that is divided by a membrane. Similarly, mitochondria and chloroplasts are organized similarly. These subcellular components are the biological electrochemical generators, i.e. cells power plants. Although the potential difference generated on the membrane is low (150–200 mV), but the power that referred to unit of mitochondria volume reaches 10–50 kW/m^3 that even more than for industrial electrolyzers. In turn, the total current which passes through the membrane of mitochondria in the cells of the human body can reach values of 400–800 A. For example, electric ray (*Torpediniformes*) generates pulses of 50 A at a voltage of 60 V and an electric eel (*Electrophorus electricus*) "shoots" of voltage pulses over 500 V! Their electric organ consists of a stack of items (the electric ray has 1000 ones) that are connected in series. These batteries (the electric ray has 2000 ones)

are in parallel. Hence, however, it generates high values of amperage and voltage (*Kuzminskiy and Shchurska, 2010*). In animals, the heart is probably the most powerful generator (the amplitude of signals at cardiogram varies in a range of 0.002–0.003 V); the brain generates a weak pulses that reach about 0.0001 V.

For the time science has been studying bioelectric potentials (BEP) a big amount of material was accumulated that shows their close relationship with almost all life functions (*Kuzminskiy and Holub, 2007*). This was the basis for widely used BEP registration for diagnosing the physiological state. This area is particularly well-developed in medicine. BEP registration also is used in agriculture for the prediction of practically important qualities of animals and plants. It was strongly substantiated that BEP is an inevitable "product" of physiological, biochemical, biophysical and bioelectrochemical processes. This has been caused by the overriding of independent role of BEP in the body. The exception, perhaps, is in establishment of the role of the BEP in the generation and distribution of reaction in nerve. However, the analysis of available data shows that participation BEP in the living organisms processes is very versatile and diverse, so a variety of ways of such participation can be identified – they are listed below.

Firstly, it is the energy role. Surface plasma membrane of cells is always polarized: its outer side is charged positively and internal one has negative charge. This potential difference on the membrane varies in different cells in the range from –100 mV to –300 mV. This resting membrane potential generated by the operation of passive and active mechanisms, like ATP, may be considered as a peculiar form of the energy storing (saving) in the cell. Today, the opinion that the criterion of the cell energy capacity is not only a number of substances with high-energy chemical bonds, but the value of the membrane potential – is spread widely. It is especially significant that electric energy accumulated on the membrane could be transferred very easily into other forms of energy during the course of several processes. It especially refers to the membrane transport which provides an exchange of substances between cells and medium. Under the influence of electric fields on the membrane the movement of ions through channels is accelerated or slowed down (depending on the charge). The electrical polarity of the membrane has the significant importance for the active transport of substances through the membrane by means of special compounds-carriers. The role of BEP in electroosmotic phenomena within the membrane is also investigated (*Chizmadzhev, 2000*). Thus, the utilization of BEP energy for different modes of charged substances transport through the membrane is the phenomenon that is widely represented in living cells.

Secondly, it is the regulatory role. Along with energy exchange the BEP act as an important regulatory unit. This becomes especially clear if we take into account the voltage of the electric field on the membranes. If the value of transmembrane potential difference is 0.1 V (although, as it has been noted above, the one can be

2–3 times greater) and the average thickness of membrane is 10 nm then the electric field strength across the membrane will be at least 105 V/cm. Such enormous electric field strength has a significant effect on structural organization of the membrane because the molecules or parts of molecules of proteins and lipids have the dipole moments. Changes in membrane electric field lead to the change of orientation of the membrane protein molecules or changes in conformation of functionally important centers. Also, there are the change of lipid matrix viscosity and the phenomenon of electrostriction (electromechanical compression). Structural changes in lipids, in turn, can affect the protein conformation. All this leads to the changes in membrane potential difference under the influence of various factors that affect membrane proteins such as enzymes, receptors, carrier protein etc. Thus, the BEP has the regulatory role in the functional activity of cells.

Thirdly, it is the information role. The greatest perfection of information role of BEP is reached in nerve fibers of animals. The action potential (AP) arising under the influence of various external stimulus spread with high speed (up to 100 m/s) and provide information transfer from one part of the body to another. The process of distribution of AP is the temporary depolarization of the membrane and results from the electric mechanism (*Kuzminskiy and Holub, 2007*). So-called "local" currents flow from excited to unexcited nerve areas that cause depolarization within the unexcited areas and the AP shift. The described method can provide distribution AP without attenuation. It is observed in most animals having some peculiarities However, it would be wrong to assume that the generation and distribution of AP in response to external stimulation is unique for animals only. At the beginning of the century the Indian scientist Dzhahdish Chandra showed that the motion of the mimosa petal is caused by the BEP fluctuations similar to the BEP oscillation in animal nerves. It was found by the Russian scientist I. I. Gunnar that not only plants with rapid motion activity (mimosa, sundew, Venus' flytrap, etc.), but the ones without fast movement (pumpkin, beans, sunflower, etc.) generate the AP in response to external irritation (burning, mechanical stimulus).

Finally, the BEP plays a role in self-organization of living systems. The cells or tissue with electric potentials create an electric field around themselves. These fields are very weak, but they can be measured using special techniques. It is believed that these electric fields form the kind of biopower matrix that determines the growth and development of organs and tissues. Besides, the electric field of one object (e.g. seed) may affect the electric field of the other one (if they are nearby) and thereby ensure a collaboration of their physiological processes. However, at present the role of BEP in self-organization of living systems is not enough investigated.

1.3 Nanobioelectrochemistry

The most typical feature of scientific development at this stage is the approximation of the organic world (the world of wildlife) with inorganic. And, therefore, the approach to the research has been changed fundamentally in order to access the interdisciplinary of research. The scientists who manipulate with atoms creating new substances are the physicist, chemist and biologist at the same time. This scientist is the naturalist, as Newton was more than 300 years ago, but on-at a qualitatively new level. Therefore, the nanotechnology is, in fact, the name of scientific and technological revolution of the beginning of the XXI century. Formerly, the scientists moved their investigation toward reducing the size of objects that had been created. It is "top-down" paradigm in R&D. Thus, we can imagine the line: we hew the tree – then saw it on board – produce some goods, i.e. cut through unnecessary. As a result, we get useful goods, but most of the efforts (financial and technological) result in the creation of waste and pollution. However, a new "from below" paradigm makes the scientists start from the level of atoms to create materials and systems with desired properties. In fact, it is necessary to create technologies and equipment for atomic and molecular design of any material that is obviously possible only via creation of adequate diagnostic methods with atomic resolution. In such way the change-over to nanotechnology and atomic design gives the most important result, namely, the industry materialization and dramatic qualitative reduction of energy and resource costs, maintaining environmental sustainability. However, using this approach it is necessary to conduct a scrupulous assessment of possible consequences, because the whole is somewhat greater than the sum of its parts.

Unlike information technologies that are mainly virtual, nanotechnology, in fact, is material.

Nanotechnology is:

- base priority for all existing industries and, as a result, will change information technology;
- not only the individual technologies or new technological trends, but also modernization of existing technologies for brand new atomic level;
- foundation of all the sectors of the new knowledge-based economy of postindustrial society.

What happens in the "nanobio" world? What are the trends that certainly deserve the attention? There are many instruments designed to respond to emerging issues. However, from the analytic materials that are created by different groups of experts, the "Roadmap of Nanotechnology", composed by Corporation RAND (Research And Development, USA), and "Roadmap of the European Commission" (Nanoroadmap Medical and Health) (*Androshchuk et al., 2009*) that was established in preparation and implementation of the Seventh Framework Programme of the

European Union Research and Technological Development are the most noteworthy. Both documents do not only analyze the situation in nanobiotechnology, but also offer a forecast of certain areas of nanobiotechnology in the near future (2015–2020 years). In addition, experts analyze the situation in those areas that may give an access to production and become commercially available. American experts pick out the representative number of nanotechnologies, above all, in biomedicine that include eleven directions: bioengineering of living tissue and regenerative medicine, biological nanostructures, encapsulation and targeted drug delivery, molecular imaging, biophotonics, biocompatible implants, bioanalytical membrane, molecular biosensors; biochip and lab on a chip (Lab-On-A-CHIP); functional molecules: switches, pumps, carriers. Experts of the European Commission have made a list of the most important nanobiotechnology sections: drug delivery, molecular imaging, cosmetics, new drugs R&D, methods of diagnosis, surgery (including transplantation of tissues and organs), tissue engineering, food technology, genomics and proteomics, molecular biosensors and other technologies. It is obvious that both lists are fairly close. However, the experts distinguish four main trends of nanobiotechnology (see. Table 1.1.).

Table 1.1. The main trends in nanobiotechnology development (*Androshchuk et al., 2009*)

Used properties	Metal nanoparticles	Carbon nanoparticle	Nanoparticle of silica gel	Nanomicelles and others
Chemical	+	+ +	+ +	+ +
Electric	+ +	+ +	–	–
Optical	+ +	+ +	–	–
Thermal	+	+	–	–
Magnetic	+ +	–	–	–

Within certain areas nanotechnology is a combination of existing technological possibilities of microelectronics and structures that are modeled as in wildlife. For example, the creation of the semiconductor nanostructures with the quantum dots is an important achievement of the inorganic materials science, but the formation of these structures is based on the principle of self-organization which is a basic principle of wildlife. Another example is the fuel and biofuel cells. Table 1.2 represents a small part of what the market of nanotechnology products offers today.

Table 1.2 Market of nanobiotechnology products (*Kuzminskiy and Shchurska, 2010*)

Finished Products are on the market	Products that are ready to enter the market in 3–5 years	Future products and developments
Nanodispersed materials Polymeric materials	Carbon materials	Production of nanobiotechnology
	Nanoelectronic devices	
Coating		
Composites	Means of delivery of drugs	
Ceramics	(nanocapsules)	Hybrid devices and bionic apparatus
Catalysts		
Membranes	Microsystem Technology	
LEDs		
Sensors	Medical diagnostics	Nano biosystem and devices
Biochips		

Modern nanocomposites materials that are synthesized with various combinations of nanoparticles and matrix materials differ markedly by strength increasing, thermal and electrical conductivity. Thanks to the unique properties and versatility of carbon the carbon nanomaterials are more and more used in various engineering and medical fields of application, particularly in bioelectrochemistry (*Giersig and Khomutov, 2008; Shang et al., 2008; Viertelov et al., 2007*). For example, carbon nanoparticles are distributed in the matrix materials such as epoxy, polyurethane, rubber, cement give unique electrical and magnetic properties to the matrix material and discover several new and interesting applications.

Carbon nanotubes (CNT) that are produced by chemical vacuum deposition can be connected in blocks to create structures with nanometers diameter and with the length of several microns to 2 cm. CNT grown as an array or “forest” are called “black cotton”. This material is promising for the development of unique mechanical stress sensors, executive devices, biosensors, electrodes for the various electrochemical devices, nanocomposites for multifunctional applications. For example,

the structural system of sensors can be made by application of spray to make a grid of nanoparticles solution. Electrochemical impedance (resistance and capacitance) of the system may change due to destruction of this structure. These kinds of materials with carbon nanotubes can also be used for manufacturing smart fabrics having many unique properties, e.g. fabric composites, sensors, etc. Thanks strength and inertia typical of CNT based nanoelectrodes that can penetrate the living cell and be used as miniature biosensors are being developed. The nanotubes structures for fixation neurons to further recover damages of the central nervous system are also being developed.

Graphene is the latest discovery of carbon nanoform (graphene substances is not found in nature). Graphene is an allotrope of carbon whose structure is a single planar sheet of sp^2 -bonded carbon atoms, which are densely packed in a honeycomb crystal lattice. The term graphene was coined as a combination of graphite and the suffix -ene by Hanns-Peter Boehm, who described single-layer carbon foils in 1962. Graphene is most easily visualized as an atomic-scale chicken wire made of carbon atoms and their bonds. The carbon-carbon bond length in graphene is about 0.142 nm. Graphene sheets stack to form graphite with an interplanar spacing of 0.335 nm. Graphene is the basic structural element of some carbon allotropes including graphite, charcoal, carbon nanotubes and fullerenes. It can also be considered as an indefinitely large aromatic molecule, the limiting case of the family of flat polycyclic aromatic hydrocarbons. Today, graphene is the most topical issue of research on condensed matter physics and material science owing to its newly discovered, completely exceptional electronic properties, particularly, electrocatalytic capabilities which is an important issue for fuel and biofuel cells. Researchers at the University of Ulster (UK) Dr. P. Papakonstantinu and Dr. N. Shang proposed a new efficient technology of growing graphene films of large area with strong electrocatalytic and biosensor properties (high sensitivity to the biological components) (*Shang et al., 2008*). For example, changes in the concentration of a known neurotransmitter dopamine that plays a decisive role in the kidney activity, central nervous, hormonal and cardiovascular systems are closely related to human health, and therefore it is important to accurately measure its concentration. Using of traditional dopamine sensitive electrode gives a significant distortion of information because of the presence of ascorbic or uric acid in the solution resulting in low sensitivity and thus in significant distortion of the true concentration value. In contrast, the graphene electrodes provide accurate results in dopamine, ascorbic and uric acid.

Graphene electrodes have a wide field of possible applications in nanobioelectrochemical electroanalytical devices, biosensors, storage devices and energy conversion systems (fuel cell, biofuel cell) and can successfully compete with similar products that are made of carbon nanotubes. Nanobioelectrochemistry is a section of nanotechnology in which various biochemical and bioaffinity processes are studied using electrochemical methods both as nanoscale biological objects and using nanotechniques (nanoelectrodes, nanostructured electrodes, highly sensitive sensors, nanocomposite materials).

Surface electrodes design is an important factor in the study of direct electron transfer in heterophase reactions between solid electrode and colloidal solution of protein on the electrode surface. Metal nanoparticles (gold, silver, palladium, and others) are used in bioelectrochemistry for effective electron transport between the electrode and biopolymers (proteins, nucleic acids). This inorganic nanostructure consists of nanoparticles of metals and nanoscale biomolecules which include proteins with characteristic 1–100 nm dimensions. Combination of membrane similar LCD films and nanoparticles of metals allows obtaining the stable films with high ability to transfer electrons between the electrode and hemoprotein. When the gold nanoparticles are included into the membrane similar substances films the increasing of sensitivity and stability of electrochemical parameters of electrodes is observed. This is due to the fact that the behavior of metal nanoparticles on the electrode is alike that of nanoelectrode systems.

Biosensor based on Prussian blue (active and selective electrocatalyst of H_2O_2 reduction) is a good example of nanoparticles application in electrochemical methods of analysis (in Fig. 1.2 and Fig. 1.3 and tabl.1.3).

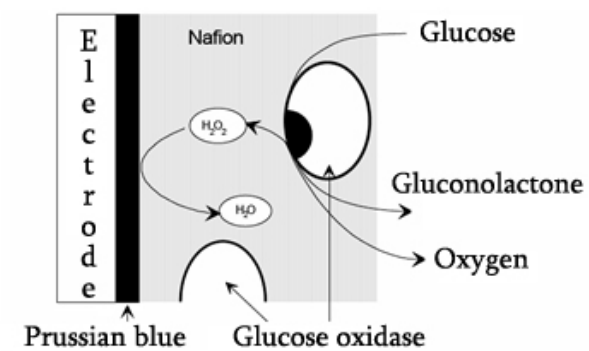


Fig. 1.2 Biosensor is based on Prussian blue (*Karyakin et al., 1994*)

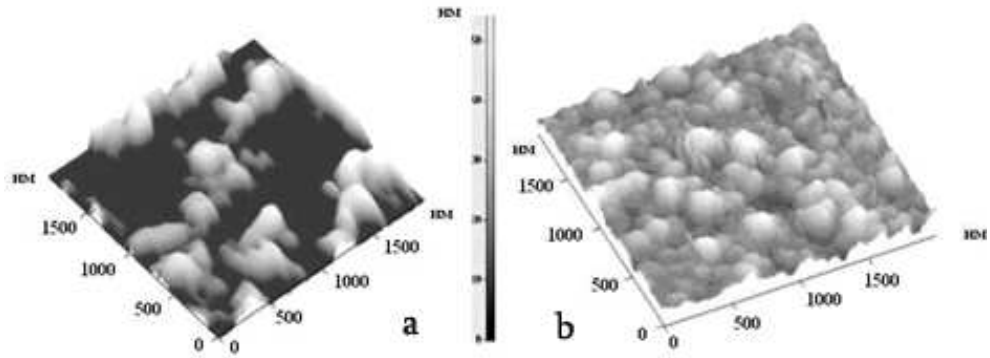


Fig. 1.3 Nanoparticles in electrochemical methods of analysis: the image of nanostructured (a) and normal (b) Prussian blue (using atomic force microscopy) (*Karyakin et al., 1994*)

Table 1.3 Prussian blue $FeIII_4[FeII(CN)_6]_3$ – active and selective electrocatalyst of H_2O_2 reduction (*Kuzminskiy and Shchurska, 2010*)

Electrocatalyst	Selectivity, $j(H_2O_2)/j(O_2)$	Electrochemical constant, $cm \cdot s^{-1}$
Platinum	0.1	$4 \cdot 10^{-6}$
Peroxidase	30–40	$1 \cdot 10^{-3}$
Prussian blue	400–600	$1 \cdot 10^{-2}$

The research of the laboratory of bioelectrochemistry at the Institute of Electrochemistry of A.N. Frumkina RAS in the field of membrane nanostructure that are involved in merger, division and transport (*Sokolov and Kuzmin, 1980; Chizmadzhev et al., 1998; Frolov et al., 2000; Kuzmin et al., 2001; Maksaev et al., 2001; Frolov et al., 2003; Akimov et al., 2004*) is a more striking example. The problem of distribution of nerve impulses on smooth homogeneous fiber and then in the myelin was solved in analytical form. The methods for describing the spreading of excitation in neural networks were developed. The theory of moving carriers (valinomycin + K^+) and single-row (relay) transport (gramicidin + K^+) was developed which then was spread to Na^+ and K^+-channels of electrically excitable cell membranes. These results formed the basis for the development of the membrane ion transport in biological systems of information and energy conversion. There are two original methods allowing studying the charged particles adsorption process, to determine the charge

for any besieged monolayer, to find the boundary potential that is generated by the surface dipole. The elaborated theory explains the dependence of membrane functioning duration on voltage. Thus, the experiments with a bilayer structure led to the discovery of the so-called reverse breakdown. It is the phenomenon that is caused in response to the step by step imposition of external electric field to the cell membrane and leads to formation of small radius pores, but the membrane maintains the stability and pores disappear after removal of voltage. The theory of electroporation (or electropermeabilization) has been recognized worldwide. The study of the breakdown of cell membranes showed that this process is reversible. It had been the opportunities for a variety of medical and biotechnological applications had been discovered. Bilayer of lipid membrane (BLM) was an ideal model for studying the mechanism of membrane fusion. Thus, the theory that substantiated that there is a lintel between the nearest monolayers the expansion of which leads to merger of the last ones if it is energetically favorable has been developed. These ideas about the mechanism of fusion were widely recognized by the international scientific community. It was shown that the liposomes capture DNA using the mechanism of endocytosis, i. e., after the DNA adsorption the invagination is formed on the membrane and the septum decayed and packed in a piece of lipid membranes DNA gets into liposomes. It was shown that DNA enters the cells through pores during membranes electrical processing. The electric field has two functions: (I) to initiate pore formation, and (II) to electrophoretically involve charged macromolecules into cytoplasm. Direct experiments showed that the DNA greatly enlarges the pores in the cell membrane during the electrophoresis. These results are used in cancer electrotherapy.

1.4 Electrochemical basis of biofuel cells

The evident example of the use of approaches of nature for the solving of the worldwide energy problems is an analogy between man-made electrochemical generators (fuel cells) and the energy supply of living organisms. Thus, it was founded that it is “mounted” hydrogen-oxygen fuel cell in living cell (Fig. 1.4) (*Kuzminskiy et al., 2003; Kuzminskiy et al., 2007*).

In such a fuel cell (FC) coming from the environment to human lungs (animals) oxygen is the oxidant. It absorbs into the blood, combines with hemoglobin and then is distributed to all tissues of the body. The food serves as the source of the hydrogen (fats, carbohydrates, proteins) which is converted into water, carbon dioxide and protons in the Krebs cycle using biocatalyst (enzymes). Electrons move through mitochondria chain enzymes (“respiratory chain”) and then combine with protons and oxygen atoms to form water as the final product. The main elements of the “respiratory chain” are a number of oxidative enzymes because of

concerted efforts of which and by oxidation of organic compounds on the mitochondrial membrane as well electrochemical potential is created. This potential is used for the synthesis of compounds with particularly high chemical bonds energy (ATP) using other enzymes.

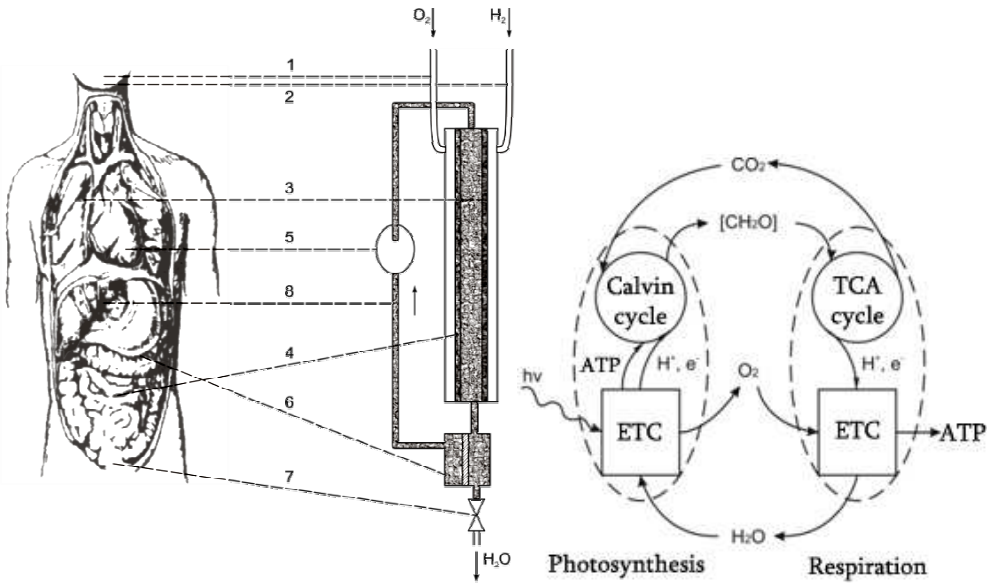
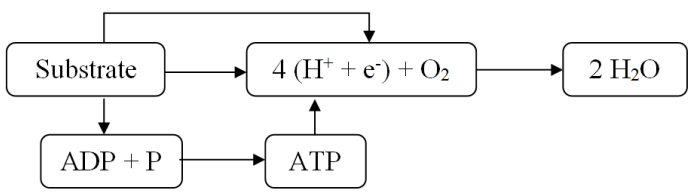


Fig. 1.4 I – Analogy of various human organs and hydrogen-oxygen fuel cell, II – The scheme of the main flows of energy and matter in the biosphere

At the above figure numbers and abbreviations represents following elements of described systems and processes: 1 – supply of oxygen, 2 – fuel supply, 3 – lungs and oxygen electrode, 4 – alimentary system and hydrogen electrode, 5 – heart and pump that drives the electrolyte, 6 – kidney and cell for the regeneration of the electrolyte, 7 – urinary bladder and valve for the emission of water, 8 – blood vessels and piping for the electrolyte), ETC – electron transport chain, ATP – adenosine triphosphate.

Bioenergetic processes in mitochondria can be represented as:



In fact the top of the diagram is the same as in FC. But there is no high temperature; the process goes smoothly and with high efficiency. And what happens to electrons? In FC the flow of electrons in the outer circle can feed, for example, lights, and what happens to it in the living cell? Thus, electrons start the chemical reaction interaction of adenosine diphosphate (ADP) with phosphate (P) by moving through “respiratory chain” from hydrogen to oxygen and using its energy (because the electric potential decreases continuously). Adenosine-5'-triphosphate (ATP) is formed that is the energy rich chemical compound and universal source of energy for various processes of wildlife. Thus, in the FC the chemical energy of fuel is converted into electrical energy; the chemical energy of food (fuel) also first transforms into electrical form in the nature, but then immediately conserves into the energy of chemical bonds. Accumulated chemical energy of ATP can be converted into electricity (nerve cells, electric organs of certain fish species), light (fireflies), mechanical energy (muscle, heart), osmotic work (secretion), heat (thermal regime support for animals and human), etc.

In 1911, the British scientist J. Potter observed that phenomena of the emergence of a potential difference between two electrodes: one of which was immersed in the bacteria suspension and the other one was in sterile environment. This, perhaps, was the first biofuel cell (BFC) where the electricity is generated by microorganisms. The development of these devices is the highly developing and perspective trend because it could convert any biological and other waste (wastewater, compost, garbage, etc.) into electricity using bacteria and their enzymes. The need for recycling and waste management of organic wastes is a crucial incentive for R&D in the field of biofuel cells. As it has been already mentioned the biochemical redox reaction does not significantly differ from the inorganic redox processes. The only difference is in biocatalyst because their activity is better than the best one of metal catalysts. However, the dynamic nature of biological processes in organisms determines the features and specifics of the biocatalyst. In chemistry the catalyst is operated under the cyclic pattern and brings out of the reaction in the same way as it enters it. This ideal system corresponds to the results of experiments with the simplest chemical catalysts. However, the complication of the chemical structure of the catalyst decreases the possibility of its return to the original state after the reaction. It is known that the chemical activity of catalysts is significantly reduced during the process. But in the organisms that function normally the biocatalysts concentration maintains at a level that meets the conditions of the environment of an organism. That is why dynamic equilibrium is supported when biocatalysts decay is coincided to their controlled synthesis. Biocatalysts are the compounds with complex structure and high degree of organization while the most of the molecules of substances that are converted with biocatalysts have a less complex structure. The energy flow is accumulated in high-energy bonds of molecules which are formed.

Pure enzymes and bacterial enzymatic complexes are able to accelerate chemical and biochemical reactions in the BFC and biosensors. There are three ways of biocatalysts preparation depending on the degree of isolation of active components: the living organisms, unrefined extracts or purified enzymes. As a part of a living organism the enzyme acts within cells that is why the reaction rate depends on the characteristics of the cell structure. The rate of the biochemical process can be determined by the rate of substance supply to the enzyme and depends on the activity of the enzyme. Unrefined extracts are obtained from the organisms (bacteria, fungi) and used in the form of powders or raw enzymes. Unrefined extracts are active within a relatively short period of time and cannot be recovered; however in isolated state they can be stored for a long time. Extracts provide higher rate of electrochemical incineration than living organisms from which they are extracted because of excalation of removable diffusion control (cells do not prevent the delivery of substance to the enzyme). Purified enzymes are the most effective biochemical catalysts if they are considered in electrochemical terms. Purified enzymes are attractive because of the rapidity they bring to the reactions: the number of elementary actions for many of purified enzymes varies in 10^6–10^8 per second. Currently none of the industrial catalysts allow reaching such a speed of chemical processes.

Enzymes are usually composed of two parts: a huge protein molecules and tiny active center with only tens of atoms. Oxidoreductases are one of the six classes of enzymes (Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases, Ligases) that is of the greatest interest for electrochemistry. It is a group of enzymes that catalyze oxidation/reduction reactions. There are active reductants of oxygen in the group (Cytochrome oxidase, Laccase and other enzymes) and natural oxidant of hydrogen (Hydrogenase). A variety of metals (iron, copper, molybdenum) that are in nonprotein active centers play an important role in the catalytic activity of these enzymes. In addition to extremely high efficiency for electrocatalysis enzymes are attractive because of the high selectivity that is essential for biosensors. They usually catalyze the conversion of the only one compound (or a limited group of similar substances) and are inert to other processes. Unlike biocatalysts “normal” electrocatalysts do not have this property.

The investigation of the mechanism of biocatalysts allowed recording the act of electrons transfer from organic molecules (substrate) which are oxidized to the enzyme molecule and observe the dynamics of this process in the experiment. These developments have allowed testing of biosensors that are the stabilized membrane with a specific enzyme and the conductive substrate. When the fluid (e.g. blood plasma) contacts with biosensors the enzyme reacts quantitatively with the certain substances that are registered. Although the recorded current is negligible the fact of creating an artificial system that directly converts chemical energy to electricity is important. The prospect of practical use of such systems as an energy

source seems to be unlikely. However, it should be noted that the biosensors are dealing with scarce space, but electrochemical processes in living organisms occur over large areas. And the nature using electric eels and rays as an example shows that the energy produced in membrane processes in cells can be converted to electrical energy one of high power.

Thus it is summarized that for a bioenergy installations a series of interrelated technological tasks should be solved. Firstly, the task is to develop the technology of similar to biosensor stabilized membranes with large areas and work out the conditions for the formation of compact three-dimensional structures. Second, it should be investigated how to include biocatalysts into the membrane complex and ensure full oxidation of certain organic substances. In addition, the mechanisms and arrangements for regulation the intensity of oxidation process need to be developed, then it is necessary to ensure its cycle to regulate the flow of energy from bioenergy sources in general.

By a similar principle, solar panels can also be built on a new basis. If scientists include chlorophyll from plants and several auxiliary enzymes in these stabilized membranes then the excitation energy of chlorophyll by photons of light will be taken directly to the conductive substrate.

In fact, all these processes are complex processes of extraction and transformation of energy in animal and plant organisms. As it was schematically considered above, the processes are made of a number of simple reactions in which substances (reductants) are oxidized to carbon dioxide and water. If one or more electrons are transferred in a separate stage of the overall process, such a reaction can be used in BFC. Biochemical reactions are easily carried out in relatively soft natural conditions, so it is promising to apply them to biofuel cell, the electrochemical energy converters. And there is a need to hold the extensive R&D in this field to bring this promising approach to industrial biotechnology.

An evident example of the use of produced natural approaches to solve the energy problems of mankind is biofuel cells that are considered in the V Chapter and are the main subject of this monograph. It should be noted that the aquatic environment is a necessary condition of life of all organisms; hence, the wastewater containing organic contaminants is one of the most attractive substrate for the implementation of the declared approach. Also the development of the application of BFC in electronics and medicine is considered, because these industries require miniature power sources that can withstand prolonged operation without human intervention. Hence, the urgent need for recycling and waste management of organic origin as well as an intensive search of alternative energy sources is an important incentive for large-scale BFC research. Groups of researchers in the USA, China, Korea and Germany have proved the possibility of direct electricity generation at wastewater treatment in anaerobic conditions for the application of microbial

fuel cells (MFC). But now, the development of MFC is still under research (*Kim et al., 1999; Bond et al., 2002*).

It is advisable to also focus on the next aspect of bioelectrochemical way of energy generation. Thus, simultaneously with the electrons those are produced by microorganisms and transmitted to the anode, the protons are formed and involved in redox processes in the MFC cathode. It is also shown that by applying a small additional potential to the MFC it is possible to obtain hydrogen. Electrical energy required to get hydrogen in such way reaches the value of 0.6 kW/m^3 H_2 that is significantly less than for obtaining hydrogen in the process of water electrolysis (4.5–5 kW/m^3) (*Liu et al., 2005*).

A fundamentally new way to overcome the biochemical barrier in producing of 4 mol H_2/mol glucose is the use of modified microbial fuel cells (MMFC) (*Logan, 2004*). For the formation of hydrogen potential of –0.41 V at the cathode should be established. The potential formed at the anode MFC is –0.3 V so, theoretically, it is possible to generate hydrogen at the MFC cathode using the application of additional voltage greater than 0.11 V (potential difference of cathode and bioanode). In MMFC microorganisms which are immobilized on the anode, oxidize the organic matter transferring electrons to the anode and hydrogen protons into the reaction medium. The protons migrate through the proton selective membrane (e.g. Nafion) to the cathode, where, the hydrogen molecules are formed if additional voltage is applied. Whereas during fermentation 2–3 mol H_2/mol glucose is produced and by-products of fermentation (2 mol of acetate from the mol of glucose) in MMFC give 3 mol H_2/mol acetate, so the combination of processes of fermentation and MMFC hydrogen generation enables to receive 9.8 mol H_2/mol glucose. Without additional voltage the electrical energy is directly obtained. Use of wastes as raw materials for the proposed technology will enable them to combine recycling with obtaining electricity and hydrogen.

In recent years BFC has received a new impetus due to growing interest in the production of so-called “green” (or environmentally friendly) electricity because microorganisms convert virtually the wide spectrum of organic compounds, including various and complex wastes. As it has been already noted it is possible to simultaneously solve both environmental and energy problems. Modern devices and approaches for chemical energy conversion into electrical energy in the form of BFC and similar devices are not able to meet the technology of the XXI century, but it should be noted that they point to the fundamental possibility of solving energy problems. And the comparison of bioelectrochemical methods of energy conversion and its electrochemical conversion in handmade fuel cell has found a lot of common and indicates that the chosen direction is very promising for solving the up-to-date energy problems.

Biotechnology based on the use of BESs is a promising field for the production of electricity and hydrogen from wastewater, because it gives a possibility significantly improve the efficiency of the process in comparison with traditional technologies (such as anaerobic digestion). The advantage of electricity production in BESs is in its one-stage process, whereas traditional technologies of electricity production from wastewater include at least three stages: production of biogas in an anaerobic biological reactor, biogas treatment and combustion. In this study we are using the common name BES for bioelectrochemical systems for electricity and hydrogen producing. The system aimed at only electricity production is called MFC.

Using of BESs presupposes that electrons produced by microorganisms are transferred to the electrode in some way. As it has been already noted in the first generation of BESs the soluble mediators are used for electrons transfer. However, the practical limitations on application of such electrons carriers in large-scale electricity production from wastewater prevented from commercialization of this type of BESs.

However, since 1999 the interest in BES biotechnology has been restored when the possibility of extracellular electron transfer without mediators was shown by B. H. Kim and his colleagues (*Kim et al., 1999; Kim et al., 2002*) and others (*Reimers et al., 2001; Lovley et al., 2008*). Although the BES biotechnology has not been commercialized yet, but the significant progress in its R&D was noticed in the last decade. Hence, there are promising prospects for the future.

MFC is a galvanic cell that combines the oxidation of organic matter on the "biological" anode and electron acceptor reduction at the cathode. To ensure the electricity generation the electron acceptor must be reduced with electrode potential higher than the one of bioanode. Oxygen in the air is the perfect final electron acceptor for sustainable production of electricity from wastewater. Theoretically, the maximum voltage (the electromotive force) of the MFC with oxygen cathode is 1.1 V. However, the practical MFC voltage is generally lower than 0.8 V and 0.6 V at open-circuit and operating conditions, respectively, due to various possible losses (*Logan et al., 2006*). In most cases, these potential losses occur with oxygen cathode because electrochemical reactions with oxygen involvement are usually characterized by slow kinetics (*Kinoshita, 1992*).

The most common MFC design is two-compartment cells with the anode and cathode half-cells (Fig. 1.5). Usually, anode is made of conductive and biologically compatible material. Graphite fiber, graphite paper, granular graphite, carbon brashes and graphite foam are the most used ones. The cathode is typically made of graphite with the addition of platinum as a catalyst. The ion-exchange membrane separates two half-cells in the most common MFC configuration (*Kim et al., 1999*). The ion-exchange membrane provides a transfer of protons from the anode chamber

to the cathode compartment to maintain the electroneutrality in the system. The electrons pass through a conductor of external electric circuit and perform useful work in order to reach the cathode.

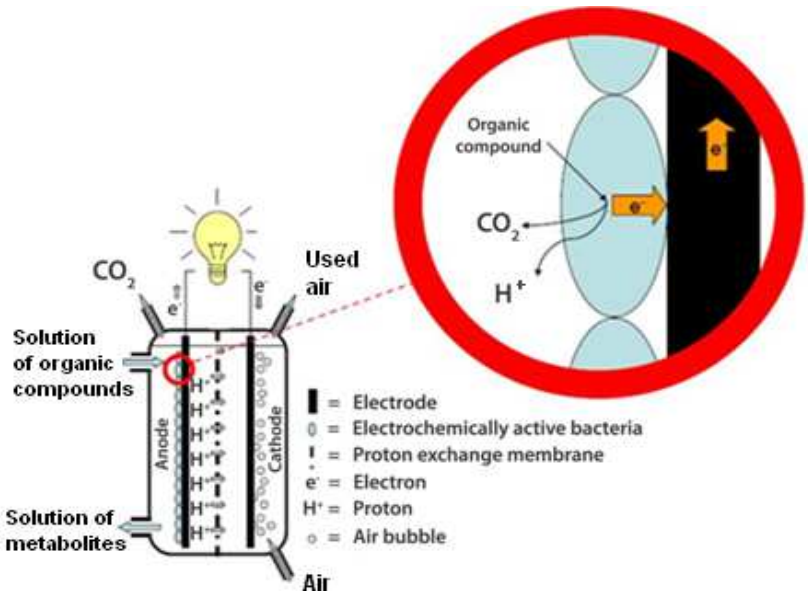


Fig. 1.5 MFC operation scheme, based on (*Logan et al., 2006*)

At the MFC anode the dissolved organic compounds from wastewater are oxidized by electrochemically active microorganisms that transfer electrons from outer membrane to the anode by the mechanism of extracellular electron transfer. Then the electrons are transferred to the cathode through the external electric circuit and consumed in the reaction of oxygen reduction. Gibbs energy of the overall reaction is negative that means that the electrical energy is generated spontaneously and can be removed from the circuit for consumption.

In recent years, the MFC researches have been focused on two main aspects: (I) diminution of material and other operating costs and (II) magnification of the MFC power. In order to reduce the cost of materials a lot of studies devoted to research and development of materials that are alternative to platinum catalysts for oxygen reduction at the cathode have been carried out (*Cheng et al., 2006*). Other researchers have investigated membrane-less MFC in order to diminish the costs of proton-selective membrane (*Jang et al., 2004*). The single-chamber MFC as the low-cost operation BES has been examined as well (*Logan and Regan, 2006*). In single-chamber MFC the air cathode simultaneously serves as the chamber wall and consumes air directly from the environment that results in excluding the costs for aeration. Schematically it is shown on Fig. 1.6.

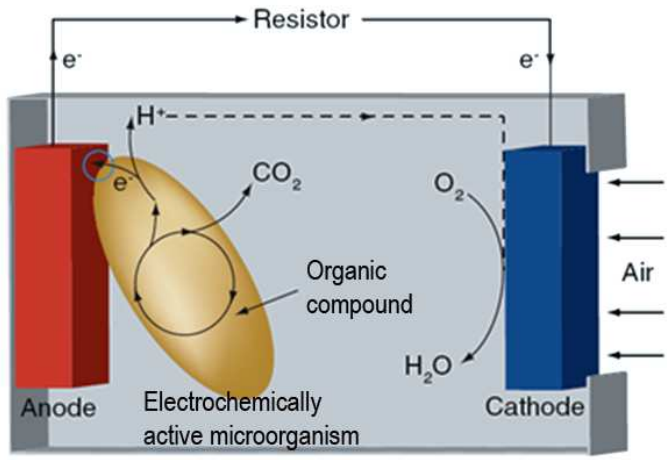


Fig. 1.6 Single-chamber air cathode MFC, based on (*Liu et al., 2010*)

What is more, a lot of studies devoted to enhancement of the MFC power and performance have been performed. These improvements were largely the result of a better understanding of the MFC mechanism. The main result was achieved by reducing the internal ohmic resistance of the system (for example, by reducing the distance between the electrodes), and by increasing the amount of electroactive material at the anode and cathode compartments. The first MFC devices based on mediator-less electrons transfer produced not more than 0.1–1 mW/m^2 of anode surface, but the power of modern MFCs is up to 1000 mW/m^2 of anode surface (*Logan and Regan, 2006*). As a result of this progress the MFC technology has evolved from the level of scientific interest to the promising biotechnology for wastewater treatment. However, it is necessary to continue looking for the way of MFC costs reducing and power increasing to achieve the commercial status of industrial technologies for wastewater treatment. In addition, the MFC needs to be estimated and compared with conventional wastewater treatment systems in terms of efficiency and rate of wastewater purification.

Hydrogen production in MFC is the new and scantily explored process, therefore, different researchers give it its specific name. Thus, B. Logan and his team call this process of hydrogen production as the bioelectrochemically assisted microbial reactor process or BEAMR-process (*Liu et al., 2005*) or/and hydrogen production in microbial electrolysis cells (*Call et al., 2009*). R. Rozendal in his work calls it as biocatalytic electrolysis (*Rozendal et al., 2006*). In this paper we offer such a term as *bioelectrochemical hydrogen generation at BES* for the determination of hydrogen synthesis in this way.

Photoheterotrophic fermentation and dark fermentation are hardly considered to be economically expedient, so there is an urgent demand for alternative technologies of hydrogen production from wastewater. This process should combine the following characteristics:

- ensure the transformation of dark fermentation byproducts that are dissolved organic compounds;
- cope with the endothermic nature of the reactions of various dissolved organic compounds conversion into the hydrogen more than well acquainted sunlight reactions;
- be reliable and flexible because of the application of hydrogen production from wastewater (multicomponent hardly degradable substrate);
- based on a mixed mesophilic cultures isolated in the process of artificial or natural selection and are able to utilize a wide range of organic substrates.

All these requirements are provided in the bioelectrochemical hydrogen production in BES.

The hydrogen generation in BES is the bioelectrochemical process that combines the oxidation of organic substances on bioanode with reduction of protons at the cathode with synthesis of molecular hydrogen. Thus, the transformation of organic matter into hydrogen can be divided into two electrode half-reactions:

Anode: $$CH_3COO^- + 4\,H_2O \rightarrow 2\,HCO_3^- + 9\,H+ + 8\,e^- \quad (1.1)$$

Cathode: $$8\,H^+ + 8\,e^- \rightarrow 4\,H_2 \quad (1.2)$$

Overall reaction: $$CH_3COO^- + 4\,H_2O \rightarrow 2\,HCO_3^- + H^+ + 4\,H_2 \quad (1.3)$$

Theoretical MFC voltage value or MFC EMF that is necessary for biocatalytic electrolysis of organic compounds is about –0.12 V. The negative value of the EMF (ΔG is positive) of this BES demonstrates the need for the introduction of additional energy (electricity) into the system for this bioelectrochemical reaction. This additional electricity is provided by the introduction into the electric circuit supplementary power source. This dissolved organic substrate is transformed into carbonate and hydrogen by electrochemically active microorganisms that act as a catalyst.

This process is similar to the process that occurs in traditional MFC, namely, it is designed of two half-cells that are separated by a proton-exchange membrane. But unlike the traditional MFC the modified MFC are different in their construction: cathode compartment is completely anaerobic; the electrical circuit is connected to an additional source of electrical energy. Schematic representation of the modified MFC is in Fig. 1.7.

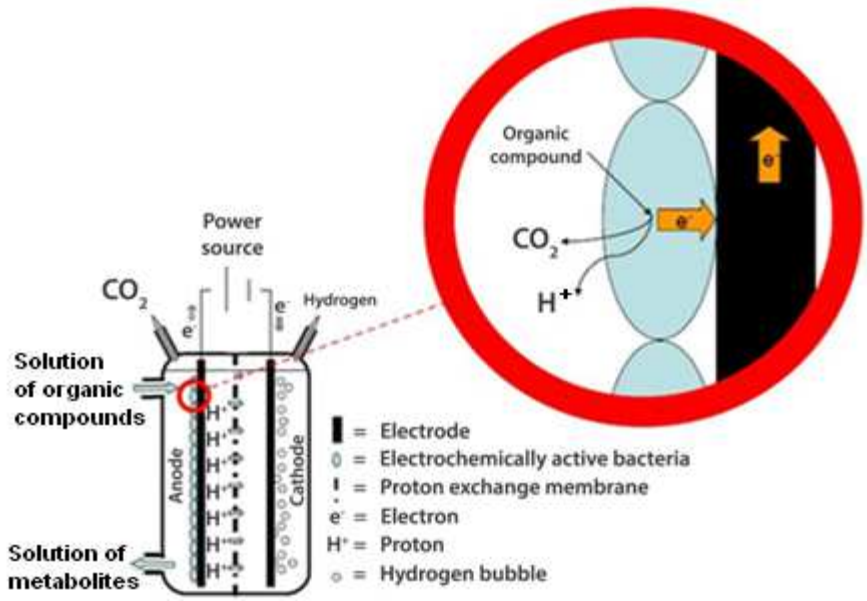


Fig. 1.7 Modified MFC for bioelectrochemical hydrogen production, based on (*Logan et al., 2006*)

At the anode the wastewater dissolved organic compounds are oxidized by electrochemically active microorganisms that transfer electrons to the anode through the mechanisms of extracellular electron transfer. Electrons are transferred through the external electric circuit to the cathode where they are used in the reaction of molecular hydrogen synthesis. System electroneutrality is provided with the cations (protons) transported from the anode compartment to the cathode through the cation-exchange membrane or special salt bridge.

The theoretical value added voltage that is required for this process is about 0.12 V that is equivalent to 0.26 kW·h/m^3 H_2. This value is significantly lower compared with the value of 4.4 kW·h/m^3 H_2 that is necessary for industrial electrolysis of water. Obviously, the metabolic losses of microorganisms as well as other possible losses (including ohmic and activation losses at the electrodes) practically increase the value of the required energy, but it does not exceed the value of 1 kW·h/m^3 H_2. Such negligible additional energy requirements is an important advantage of the process of bioelectrochemical hydrogen production in MFC compared with the industrial electrolysis, because the cost of electricity is crucial in the total costs of hydrogen generation using electrolysis (*Turner, 2004*).

Hydrogen can be removed bioelectrochemically from the by-products of fermentation, thereby, increasing its overall output in combination of fermentation with bioelectrochemical production of hydrogen. It is possible to produce

2–3 moles of hydrogen from one mole of glucose during the fermentation. And the end products of fermentation give 3 mol H_2/mol acetate. As the conclusion the combination of fermentation and bioelectrochemical hydrogen production enables to obtain 8–9 mol H_2/mol glucose.

In comparison with other biological processes of hydrogen production the bioelectrochemical hydrogen in MFC has several important advantages. It is shown that bioanode can operate effectively under unsterile conditions, as electrochemically active association of microorganisms is selected naturally from a wide range of microorganisms. Moreover, this association of microorganisms is capable of utilizing the various substrates (including sugars, fatty acids, proteins) with high efficiency (*Rabaey et al., 2003*; *Rabaey et al., 2005; Heilmann and Logan, 2006*). Other biological ways of hydrogen synthesis are often affected by foreign microorganisms in non-sterile conditions and limited in available substrates for utilization (e.g., dark fermentation). In addition, almost pure hydrogen is produced during the bioelectrochemical hydrogen synthesis in the cathode compartment, unlike the fermentation where the mixture of hydrogen and carbon dioxide is the final product. Thereby the bioelectrochemical process of hydrogen production is not only the next step after fermentation in a flowchart, but it is possible to completely replace the fermentation by BES in certain cases (e.g., wastewater treatment).

The possibility of application of a mixed consortium of electrochemically active microorganisms is determinant to ensure the effective operation of the bioelectrochemical system hydrogen production in BES. Electrochemically active microorganisms are capable of extracellular electron transferring and thus electrons are transported outwards from the cells using the electrode as an electron acceptor for the oxidation of dissolved organic compounds. As the electrochemically active bacteria release electrons on high energy levels low electrode potential is set at the cathode that is used for bioelectrochemical hydrogen production.

In the cathode chamber of BES the hydrogen is formed electrochemically from electrons and protons that are generated by electrochemically active microorganisms in the anode chamber.

Usually the electrolytic hydrogen generation from acidic and alkaline solutions is in a variety of ways (*Antropov, 1984*). The source of hydrogen in acidic solutions is the hydronium ions, the discharge of which at the cathode leads to the formation of hydrogen molecule:

$$2H_3O^+ + 2\,e^- = H_2 + 2H_2O. \tag{1.4}$$

In the alkaline solutions electrons are directly interacted with water molecules with subsequent decay into hydrogen and hydroxyl ions:

$$2H_2O + 2\,e^- = H_2 + 2OH^-. \quad (1.5)$$

Equation (1.4) and (1.5) represent only a general mechanism of the cathodic hydrogen generation process under different conditions of electrolysis. This process consists of a series of successive stages and is run by a variety of ways depending on specific conditions.

The first stage (Stage I) is the delivery of particles that serve as a source of hydrogen formation to the electrode surface. Under the certain conditions this process proceeds without significant restrictions. The next stage (stage II) is the discharge of hydronium ions (or water molecules) to form adsorbed hydrogen atoms:

$$H_3O^+ + e^- = H_{a\partial c.} + H_2O\,, \quad (1.6)$$

or

$$H_2O + e^- = H_{a\partial c.} + OH^-\,. \quad (1.7)$$

Whether in acidic or alkaline medium the discharge occurs and the products of the reaction are the hydrogen atoms adsorbed on the electrode. For a steady going of electrolysis it is necessary to maintain a constant surface concentration of hydrogen atoms, i.e. it is necessary to provide the continuous hydrogen atoms removal from the cathode surface. Hydrogen atoms are removed in three ways (stage III): catalytic recombination, electrochemical desorption and emission. Using the catalytic mechanism the hydrogen atoms removal is due to recombination in the molecules with simultaneous desorption (equation (1.8)), whereby a catalyst performs metal electrode:

$$H_{a\partial c.} + H_{a\partial c.} = H_2 \quad (1.8)$$

The electrochemical desorption is the process of hydrogen atoms removal from the surface of the electrode as the result of the hydrogen ions (or water molecules) discharge on the adsorbed atoms by equation (1.9) or (1.10):

$$H_3O^+ + H_{a\partial c.} + e^- = H_2 + H_2O\,, \quad (1.9)$$

or

$$H_2O + H_{a\partial c.} + e^- = H_2 + OH^-. \quad (1.10)$$

Using the emission mechanism the adsorbed hydrogen atoms are removed from the electrode surface in the form of free atoms (equation (1.11)) with their next bulk recombination in the hydrogen molecule:

$$H_{adc.} = H. \tag{1.11}$$

Hydrogen molecules formed from the adsorbed hydrogen atoms need to be removed from the interphase boundary electrode-electrolyte in the gas phase.

Any of these four stages determine the speed of the whole process of electrolytic hydrogen production and is the limiting stage that is the cause of the overpotentials in the process of hydrogen formation. In this case the braking of the process associated with substances transport (stage I) does not play a significant role. In the process of electrolytic hydrogen production the adsorbed atoms withdrawal are carried out in several ways. If stage (III) is limiting then the rate of the whole process should be determined by the rate of the most effective among the three mechanisms desorption described above. Slow recombination, for example, means that the catalytic formation of hydrogen molecule is more inhibited than discharge stage or transportation and at the same time is much faster than the electrochemical desorption or emission of hydrogen atoms. At the same values of rate constants of parallel stages the hydrogen removal can be carried out simultaneously in several ways.

The cathode material also affects the process of hydrogen production. Primarily this is due to overpotentials of hydrogen electrosynthesis process, which is a significant part of the voltage needed for electrolysis. Therefore, its reduction minimizes the cost of electricity for electrolysis. The overpotential value depends on many factors: the cathode material, current density, cathode surface and pH. It was found out that the higher catalytic activity of metal relative to the recombination reaction of atomic hydrogen is the lower is the hydrogen overpotential value (*Suhotin, 1978*).

catalytic activity →

Pb, Sn, Zn, Cu, Ag, Fe, Ni, W, Pd, Pt

← hydrogen overpotential

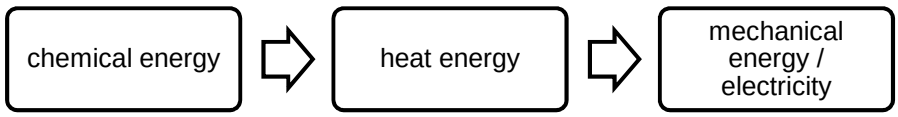
2 Energy-converting ability of bioelectrochemical systems and mathematical modeling of bioelectrogenesis process

Look deep into nature, and then
you will understand everything better.
Albert Einstein

Everything that is created by nature is appropriate and works with maximum efficiency that is why it is not surprising that the fuel and biofuel cells are in the field of science interests.

2.1 Direct conversion of chemical energy into electricity

Today the main part of the energy used by mankind is the chemical energy of the combustion of fossil fuels. The chemical energy of this reaction further is converted into mechanical work (internal combustion engines) or electric energy (thermal power plants) as follows:



In the internal combustion engines the mechanical energy is generated and the electricity is generated on thermal power plants.

The drawback of the existing methods of energy conversion is in their low efficiency (performance index). Especially large energy losses occur on the stage of heat conversion into mechanical work. Thus, heat can be only partially converted into mechanical work, but the most of heat is dissipated in the environment. Therefore, the actual performance is 30–40% for the power plants and 10–15% for the transport systems in urban areas. So, 60–90% of the chemical energy of fuel is needlessly dissipated in the environment. Thus, the direct path of chemical energy conversion into electricity (*chemical energy → electrical energy*) is in a particular interest.

Chemical power sources, fuel or biofuel cells are the electrochemical power sources (PS) in which the chemical energy of fuel oxidation is converted directly into electrical energy. And, therefore, the total reaction in either PS is divided into the following stages:

- anodic fuel oxidation (hydrogen, etc..),
- cathodic oxidant reduction (oxygen, etc.),
- movement of ions in the electrolyte solution,
- movement of electrons in an external electric network from the anode to the cathode (electric current).

The idea of using chemical energy of oxidation (combustion) for the direct electricity generation in the power source had attracted the attention of researchers a long time ago. In this case, in the chain of energy conversion there is no thermal stage and, accordingly, there is no restriction of II principle of thermodynamics. Hence, the efficiency (performance index) of the electricity source can reach of 100% (*Kuzminskiy, 1990*).

Current sources are characterized by the following parameters (data for the chemical current source (CCS) is given as an example) as electromotive force (EMF = 1.5–5.0 V), discharge voltage, current-voltage characteristic (I-V curves) and discharge dependency, capacity (10^2–10^3 A·h), energy density (up to 500 W·h/kg or 300 W·h/L), volume (surface) power density (400 W/kg), resource, service life and shelf life, technical and economic parameters. Comparative characteristics of various CCS are summarized in Table 2.1. (*TU 10MO. 082.058.; TU 10MO. 082.073., GOST 15596-78*).

The data of the electrode potentials that are calculated employing the appropriate equations of electrochemical thermodynamics using thermodynamic functions current-forming reactions (ΔG, ΔH, ΔS), which allows us to estimate the value of the EMF of this or other electrochemical PS couples is shown in the literature (*Sukhotin, 1978*). However, the real electrode potential difference or open-circuit voltage (OCV), as usual, differs from EMF. This phenomenon is due to the fact that because of adverse reactions equilibrium potentials are not established on the electrodes that are determined with main current-forming process for which they are calculated thermodynamically. The PS operation results in changes of potential difference between the poles and decrease of EMF (or OCV). And the potential difference which is established is called discharge voltage of PS.

Table 2.1 Comparative characteristics of various CCS

CCS	Power density, W/kg	Energy density		Service life, years	Number of cycles, depth of discharge – 80%
		W·h/L	W·h/kg		
Landmarks					
Short-term	150	135	80	5	600
Long-term	400	300	200	10	1000
The current status					
Lead-acid	60–138	50–82	18–56	2–3	50–1500
Nickel-iron	70–132	60–115	39–70		440–2000
Nickel-cadmium	60–200	60–115	25–70		800–2000
Nickel-metal hydride	100–200	152–215	54–80	10	500–1000
Sodium-sulfur	90–130	76–120	80–140		250–600
Sodium-nickel-chloride	150	160	100	5	600
Lithium-ion polymer	100	130–280	90–180		300–500

The reasons which cause the difference between EMF and discharge voltage can be divided into two main groups: the electrical phenomena that are not related to changes in the electrode potential, and the phenomena that cause changes in electrode potential or polarization of the electrodes during the operation. Thus, if the PS poles are connected with the conductor the EMF will consist of cathode (ΔE_k) and anode polarization (ΔE_a), the voltage drop because of the resistance of the outer conductor (load) (R_l) and ohmic resistance element (R_o):

$$E = \left(\Delta E_k + \Delta E_{à}\right) + I \cdot R_l + I \cdot R_{i}. \tag{2.1}$$

Value $I \cdot R_н$ in equation (2.1) is the discharge voltage U_p of the external electric circuit and it can be used to perform some of the devices that are powered by this PS. From the equation (2.1) it follows that the discharge voltage is less than its EMF because:

$$U_p = E - U_r = Å - ² \cdot r, \tag{2.2}$$

where U_r is the voltage drop at full current source internal resistance r which includes polarization of cell and the voltage drop at R_O.

So PS voltage is higher if the EMF is higher and the internal resistance is less. In this regard, it is advisable to use electrolytes with high electrical conductivity. Besides, it is useful to make the construction of PS with very small distance between the electrodes, but do not interfere electrode processes with each other.

The electrodes polarization is another value which is necessary to reduce. Electrochemical polarization can be reduced, and the cell voltage, respectively, can be increased by using of catalytically active electrodes with a highly developed surface and by increasing temperature and concentration of reactants. Effect of concentration polarization can be partially corrected by increasing the electrode surface, reagent concentration and temperature. Chemical polarization is conditioned with slowness of chemical stages of the electrode process and can be reduced the same way as electrochemical.

Regarding the evaluation of theoretically possible energy characteristics of biofuel cells and their deviation from the theoretically calculated parameters, i.e., polarization losses, it will be discussed below.

As follows from equation (2.2), the PS discharge voltage also depends on the PS load current I. Typical dependence of the discharge voltage U_p on the discharge current I (I–V curve) is shown at Fig. 2.1.

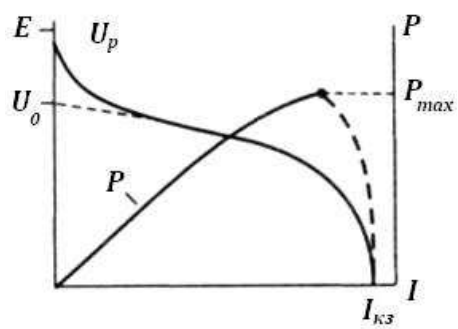


Fig. 2.1 Typical current-voltage curve and the dependence of CPS power P from the load current (*Kuzminskiy et al., 2002*)

In the initial section of the curve it is observed relatively sharp change of cell voltage because of the electrochemical polarization of the electrodes. Further, the voltage changes linearly and the voltage drop is defined both with the ohmic and polarization losses. Finally, the last section of the curve has a sharp voltage drop and current density is close to the limit and called the current limit or short-circuits current I_{sc}. The voltage drop in this region is mainly due to the concentration or chemical polarization of one or both electrodes.

The dependence of the PS discharge voltage on the duration of discharge or the discharge capacity are expressed by discharge curves that shown a current source characteristics for the given conditions of operation.

During the operation PS develops capacity $Ð = U_p \cdot I = {}^{2}{}^{2} \cdot R_l$ (where U_p is an average discharge voltage), which describes the amount of energy that gives current source per time unit. The PS power per unit of weight or volume is called specific. All factors that increase voltage lead to increasing of PS power. To obtain the value effective output P_{max} of the external electrical circuit the external resistance needs to be equal to the internal resistance in PS (Figure 2.1).

The theoretical specific capacity can be calculated by the equation:

$$Q_{th} = \frac{nF}{3600 \cdot G_{eq}}, \tag{2.3}$$

where n is a number of electrons involved in the reaction of current generation; G_{eq} is the equivalent weight of electrode materials.

The energy which is generated by PS is the product of PS capacity on voltage. The actual capacity of the current source is always less than the theoretical one because of the incomplete use of electroactive substances and their losses on other processes.

The literature analysis of the possibilities of forecast estimation of PS energy conversion efficiency revealed that, unlike the thermocouples (*Kuzminskiy et al., 2002*), there are no the value that characterize the dependence of PS power parameters on physico-chemical properties of electroactive components of electrochemical systems for the galvanic, fuel and biofuel cells. Existing criteria for pre-selection of electroactive materials for this PS are confined to the evaluation of the EMF and the theoretical capacity.

2.2 Evaluation of theoretical energy characteristics of microbial fuel cell and mathematical modeling of bioelectrogenesis

Theoretical evaluation of energy performance for new devices that the microbial fuel cells are is crucial because it gives an opportunity to assess and calculate the coefficient of performance for the device and bioelectrogenesis process.

Bioelectrochemical production of electrical energy from high-energy substrates is a new trend in bioenergy, and the complexity of the mathematical model development of the process is determined with the difficulty of adequate description of biological and electrochemical processes and mass transfer processes. Therefore, to simplify the mathematical model of biotechnological electricity production and biofilm formation the glucose and sodium acetate was used as substrate and made an assumption about the presence of the highly efficient chemical or biochemical mediator.

The aim of mathematical modeling of the process is to calculate the values of generated current and voltage. The first task is to define the electrochemical model for electrode reactions that depend on the electrode potential and the concentration of reactants/products in the near-electrode layer. Then through the concentration of chemical components and biomass that are determined with the mass transfer and reactions within the biofilm and in the bulk electrolyte the mathematical model of biofilm formation using mass balance is developed.

The main stationary control parameters and perturbation parameters of the biotechnological production of electric energy process in MFC are determined in the mathematical model. The mathematical model is described in notional arrays taking into account the specifics of the process, namely, the electrochemical parameters and dependence (rate of electrode reactions, charge and current, voltage and overvoltage) and the mathematical description of the near-electrode system “biofilm / volume of fluid” (kinetics of microbial reactions, material balances in the electrolyte bulk, material balances in the biofilm, the calculation of pH). For practical implementation of the mathematical model the generic algorithm is used. An assessment of basic thermodynamic parameters is also proposed, which suggests an inability of process to flow without microorganisms under these conditions and the estimation of limiting stages and losses in MFC.

The general model is implemented through the software written in C/C++. Electrochemical and biochemical reactions are identified using their stoichiometry and speed parameters. One-, two- or three-dimensional description of the biofilm are proposed for the batch mode acetate-fed MFC.

2.2.1 Theoretical evaluation of microbial fuel cell energy characteristics and calculation of losses

Based on the known patterns of cellular metabolism we offer the following bioelectrochemical scheme of the MFC (Fig. 2.2).

The assessment of the theoretically possible energy characteristics of the MFC is usually performed through electromotive force (EMF) assessment that is defined as the potential difference between the cathode and anode. Performed by the MFC work is the multiplication of its EMF on the quantity of electricity which has passed through a solution:

$$A = n \cdot F \cdot E_{EMF} \tag{2.4}$$

where A is the work performed by MFC; n is the number of electrons transferred in one elementary act; F = 96485 C · mol^{-1} is Faraday constant; E_{EMF} is the electromotive force of MFC.

If the entire change in Gibbs energy of reaction can be converted into useful work then we have the following:

$$E_{EMF} = -\frac{\Delta G}{n \cdot F} \tag{2.5}$$

For research of MFC glucose is traditionally used and bioelectrochemical processes can be described by the following overall equation:

Anode: $$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \tag{2.6}$$

Cathode: $$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O. \tag{2.7}$$

Summarizing the equation (2.6) and (2.7) it is obtained the overall reaction of aerobic oxidation of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{electric energy}. \tag{2.8}$$

Table 2.2 shows the main thermodynamic values for some compounds that can be oxidized in MFC.

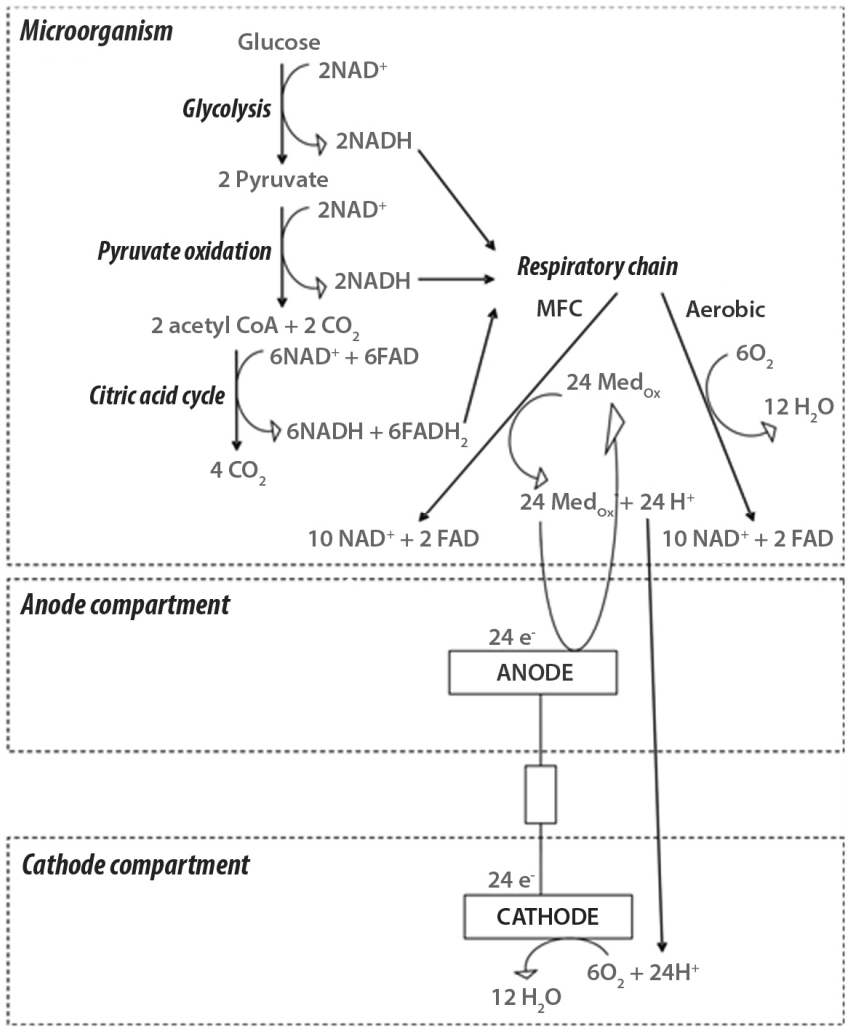


Fig. 2.2 Biochemical scheme of direct MFC (*Kuzminskiy et al., 2008*)

Based on data contained in table 2.2 it the change in enthalpy and entropy in the oxidation of glucose is calculated:

$$\Delta H = 6 \cdot \Delta H_{CO_2} + 6 \cdot \Delta H_{H_2O} - \Delta H_{C_6H_{12}O_6} - 6 \cdot \Delta H_{O_2} = -2801,65 kJ \cdot mol^{-1} \quad (2.9)$$

$$\Delta S = 6 \cdot S_{CO_2} + 6 \cdot S_{H_2O} - S_{C_6H_{12}O_6} - 6 \cdot S_{O_2} = 0,259 kJ \cdot mol^{-1} \cdot K^{-1} \quad (2.10)$$

Table 2.2 Thermodynamic values for some compounds (standard conditions)

Formula and name of the substance	Standard enthalpy of formation – ΔH^{0}_{298}, kJ·mol^{-1}	Entropy S^{0}_{298}, kJ·mol^{-1}·K^{-1}
CH_3COOH (l), acetic acid	484.5	0.160
C_2H_5OH (l), ethanol	277.69	0.161
CH_3OH (l), methanol	238.66	0.127
CH_4 (g), methane	74.81	0.186
$C_{12}H_{22}O_{11}$ (s), saccharose	2222.12	0.360
$C_6H_{12}O_6$ (s), α-D-glucose	1274.45	0.212
CO_2 (g)	393.51	0.214
H_2 (g)	0	0.131
H_2O (l)	285.84	0.070
O_2 (g)	0	0.205
(g) – substance is in the gaseous state; (l) – substance is in the liquid state; (s) – substance is in the solid state		

The calculation of the Gibbs free energy change for the oxidation of glucose (*Kuzminskiy and Holub, 2007*) is the following:

$$\Delta G = \Delta H - T \cdot \Delta S = -2878,83 kJ \cdot mol^{-1} \quad (2.11)$$

Using equation (2.5) we can estimate the maximum value of the EMF for the reaction of complete oxidation of glucose:

$$E_{EMF} = -\frac{\Delta G}{n \cdot F} = -\frac{-2878,83}{24 \cdot 96485} = 1,24V \quad (2.12)$$

Similarly, the value of the EMF for the oxidation of other substrates could be calculated. The calculated values of EMF and free energy change for the oxidation of some organic substances are presented in Table. 2.3.

Table 2.3 Calculated values of the free Gibbs energy and electromotive force for a number of thermodynamically possible reactions of oxidation of organic substances

Reaction	Number of electrons	ΔG, kJ·mol^{-1}	E_{EMF}, V
$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$	24	–2 878.83	1.24
$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5OH$	8	–231.51	0.30
$C_{12}H_{22}O_{11} + 12O_2 \rightarrow 12CO_2 + 11H_2O$	48	–5803.56	1.25
$C_2H_5OH + 3O_2 \rightarrow 2CO_2 + 3H_2O$	12	–1328.50	1.15
$2CH_3OH + 3O_2 \rightarrow 2CO_2 + 4H_2O$	12	–1410.34	1.22
$CH_3COOH + 2O_2 \rightarrow 2CO_2 + 2H_2O$	8	–878.25	1.10
$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	8	–822.58	1.07
$2H_2 + O_2 \rightarrow 2H_2O$	4	–478.05	1.24

In table 2.3 is shown that the oxidation reactions of typical organic substrates are characterized by negative values of the free energy, and hence are thermodynamically possible. Thermodynamically the maximum theoretical value of EMF that can be obtained reaches 1.25 V. But experimental values are expected to be lower due to energy losses.

The value of the EMF can also be calculated from the values of the redox potentials. For example, oxidation of acetate:

$$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O \tag{2.13}$$

at pH = 7 and 5 mmol concentrations of acetate and bicarbonate the potential is determined by the Nernst equation:

$$E_a = 0{,}187 - \frac{8{,}31 \cdot 298}{8 \cdot 96485} \cdot \ln \frac{5 \cdot 10^{-3}}{(5 \cdot 10^{-3})^2 \cdot (10^{-7})^9} = -0{,}296 \tag{2.14}$$

For the reaction of oxygen reduction:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \tag{2.15}$$

at pH = 7 and pO_2 = 0.2 Similarly, it is calculated:

$$E_{к} = 1{,}229 - \frac{8{,}31 \cdot 298}{4 \cdot 96485} \cdot \ln \frac{1}{0{,}2 \cdot (10^{-7})^{4}} = 0{,}805 \tag{2.16}$$

So, the reaction of oxidation of acetate is at the anode because of the lower (more negative) process potential and oxygen is reduced at the cathode and total:

$$CH_3COO^- + 2O_2 \rightarrow 2HCO_3^- + H^+. \tag{2.17}$$

The EMF of acetate reaction with oxygen is equal to the potential difference between cathode and anode:

$$E_{EMF} = E_K - E_a = 0.805 - (-0.296) = 1.1\text{V}. \tag{2.18}$$

Table 2.4 shows the redox potentials of some half-reactions and calculated values by combining them in pairs for possible use in MFC.

Table 2.4 Redox potentials of some half-reactions and calculated values of their pairwise combination (T = 298 K, pH = 7.0, 5 mmol substances concentration)

Microbial fuel cell	Cathodic half-reaction	$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$	$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	$MnO_2(s.) + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$	$Fe^{3+} + e^- \rightarrow Fe^{2+}$	$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$
Anodic half-reaction	Standard electrode potential	0.328	0.361[1]	0.421	0.470[2]	0.771	0.805[3]	1.229[4]
$2H^+ + 2e^- \rightarrow H_2$	–0.420	0.748	0.781	0.841	0.89	1.191	1.225	1.649
$S + 2H^+ + 2e^- \rightarrow H_2S$	–0.274	0.602	0.635	0.695	0.744	1.045	1.079	1.503
$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$	–0.296	0.624	0.657	0.717	0.766	1.067	1.101	1.449
$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$	–0.220	0.548	0.581	0.641	0.69	0.991	1.025	1.525
$Puryvate^{2-} + 2H^+ + 2e^- \rightarrow Lactate^{2-}$	–0.185	0.513	0.546	0.606	0.655	0.956	0.99	1.414
$Fumarate^{2-} + 2H^+ + 2e^- \rightarrow Succinate^{2-}$	+0.031	0.297	0.33	0.39	0.439	0.74	0.774	1.198

At the Table 2.4 used enumeration represents following parameters and values:
[1] $[Fe(CN)_6^{3-}]=[Fe(CN)_6^{4-}]$,
[2] $[Mn^{2+}]$= 5 mmol, pH = 7,
[3] pO_2 =0.2, pH = 7,
[4] pO_2 =0.2, pH = 0,

As follows from these data, the appropriate combination of anodic and cathodic reactions gives certain values of EMF. For example, in addition to the oxygen electrode, the reactions of ferrocyanide or manganese reduction in MFC are applied. The combinations of anodic reactions are purely theoretical because it is not possible to determine a single current generation reactions in MFC because of the complexity of cellular metabolism. Besides, the highest value of EMF is 1.649 V (Table. 2.4). However, this value is observed at sufficiently low (acidic) pH values but these conditions are not suitable for living organisms and thus for practical use of this half-reaction combination. In this case, the pH is chosen as a compromise between the cathode "acidity" and a maximum value of its redox potential. Thus, calculated from the redox potentials values of EMF confirm the above thermodynamic calculations, and therefore it is possible to estimate (theoretically) the maximum possible voltage of MFC.

However, although the above calculated Gibbs free energy change for the oxidation of glucose is –2879 kJ / mol, it is difficult to get all this energy through a series of losses because it is removed in the bacterial metabolism. Therefore, in the MFC as in any real electrochemical or bioelectrochemical system the deviations of energy parameters are observed. This is due to the presence of losses that are divided into the groups listed below.

Activation losses. For the redox reactions it is necessary to overcome the activation energy, so there are activation losses in the electron transfer from (or to) the participants of potential determining reaction. For example, there are losses in the process of oxygen reduction at the cathode or in the electrons transfer to the anode by microorganisms. Low activation losses are achieved (I) using appropriate catalysts (mediators), (II) increasing the temperature of the process and (III) creating enriched biofilm on the surface of the electrodes.

Losses on the electrical resistance. These losses include resistance to movement of electrons through the electrodes, wires and the resistance to movement of ions through catholyte, anolyte or proton selective membrane. These losses can be minimized by (I) decreasing of the electrode compartments, (II) using membranes with low resistance, (III) improvement of wires contacts with electrodes and (VI) increasing the conductivity of solutions to the limit of tolerance of microorganisms.

Concentration losses occur when the electric current generation is limited of the mass transfer rate. They are the most noticeable at high values of current, because then the reaction rate becomes so large that the diffusion rate does not cover the reagents utilization. To increase the rate of diffusion it is desirable to use the additional mixing of electrolyte and others.

Metabolic losses. In direct MFC anode (not oxygen) is the final electron acceptor for microorganisms and its potential is determined by the level of energy gain for cells. High potential difference between the substrate and the anode provides high potential energy gains for microorganisms, but reduces the maximum voltage of MFC. Obviously, to increase the voltage the substrates with the low potential should be used. However, if the potential of the substrate is too low, the anaerobic breathing is inhibited and substituted with more energy beneficial for the microorganisms process of fermentation (if possible). Identification of ways of anode potential reduction and thus maintaining of stable power generation is a challenge for the further investigations.

The total loss of electricity in direct MFC is determined by the sum of all these types of losses. The practical value of the voltage in MBE can be calculated by the formula:

$$U = E_{EMF} - (\sum \eta_a + \sum \eta_m + \sum \eta_c + I \cdot R) \qquad (2.19)$$

where U is the MFC voltage; E_{EMF} is EMF; $\sum \eta_a$ – sum of all activation losses; $\sum \eta_m$ – sum of all metabolic losses; $\sum \eta_c$ – sum of all concentration losses; I – amperage (current); R – total electrical resistance of MFC; IR is the sum of losses in the electrical resistance.

At the figure 2.3 the voltage loss via electron transport from substrate to oxygen in the MFC is schematically shown. The first potential difference drop is due to metabolism and cellular electron transport and has spasmodic character (position 1). Further losses are the result of influence of the electrolyte solution resistance and proportional to the path (distance) of an electron in solution (position 2) and losses at the anode (position 3). That is why at this point the value of the anode potential is determined. The voltage drop between the anode and cathode depends on the resistance of proton selective membrane and applied external load (position 4). Positions 5 and 6 show the losses that occur at the cathode and losses associated with the reduction of electron acceptor (activation losses).

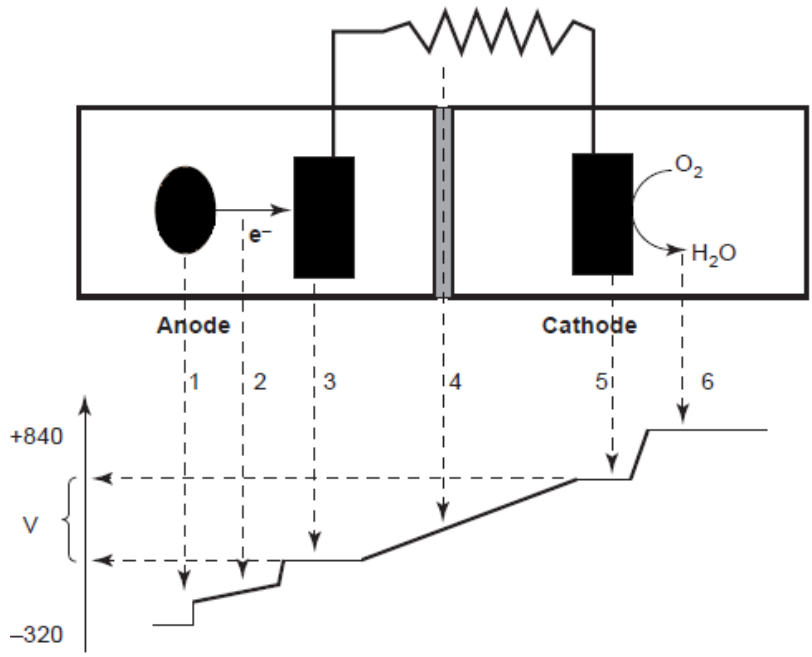


Fig. 2.3 The direct MFC voltage drop at the generation and transfer of electrons, based on (*Rabaey and Verstraete, 2005*)

2.2.2 Electricity and hydrogen production performance indexes in a laboratory bioelectrochemical system

BES is both biotechnological and electrochemical systems, so the key performance indexes of electrical energy and hydrogen production in a laboratory setting can be divided into biotechnological, electrochemical and integral.

The indexes for evaluation of MFC operation are the simplified functions of the mathematical model.

The biotechnological indexes of evaluation of electricity generation in MFC are biomass growth (general and specific), the substrate consumption rate (treatment efficiency using model wastewater or sewage), Coulombic efficiency, COD balance. Growth of biomass is the biotech index which allows estimating the degree of environment suitability in MFC for the maximum electricity generation and optimal growth of bacteria.

Biomass growth yield. Cell growth will reduce total efficiency due to diversion of electrons into biomass (*Logan et al., 2006*). The substrate utilization for growth is measured by the net (or observed) cell yield, *Y*, calculated as:

$$Y = \frac{X}{\Delta COD} \tag{2.20}$$

where *X* is the biomass (g COD) produced over time (hydraulic retention time). An important advantage of an MFC is the lower cell yield compared to aerobic processes. This is caused by the reduced energy available for biomass growth as a significant part of the substrate energy is converted to electrical power. Reported MFC net yields range from 0.07 and 0.22 g biomass COD / g substrate COD, while typical aerobic yields for wastewater treatment are generally around 0.4 g biomass COD / g substrate COD. The growth rate can be measured directly by determining the biomass (g COD) built up on the electrode surface and discharged in the effluent (for continuous operation). The low biomass production in MFCs is an especially attractive benefit since sludge disposal by combustion (becoming the standard technology in Europe) costs approximately 600 Euros per tone.

Substrate consumption rate or loading rate. When examining the use of MFCs for wastewater treatment, it is useful to examine performance achieved with this new technology in terms of loading rates with those typically obtained in conventional treatment systems. To do this, we calculate the loading based on volumetric loading rates as B_v (kg COD m^{-3} d^{-1}). Typical values for B_v achieved to date are up to 3 kg COD $m^{-3}d^{-1}$, compared to values for high-rate anaerobic digestion of 8–20 kg COD m^{-3} d^{-1} or activated sludge processes of 0.5–2 kgCODm^{-3} d^{-1}. These loading rates can be normalized to the total anode volume for comparison with suspended biomass processes (e.g., activated sludge, anaerobic digestion), and to total anode surface area for comparison with biofilm processes. Based on the reported areal short-term peak power productions, the anode surface-specific conversion rates for MFCs are up to 25–35 g COD m^{-2} d^{-1}, which is higher than typical loading rates for rotating biological contactors (RBCs; 10–20 g COD m^{-2} d^{-1};) and comparable to those of high rate aerobic biofilm processes such as the moving bed bioreactors (MBBRs).

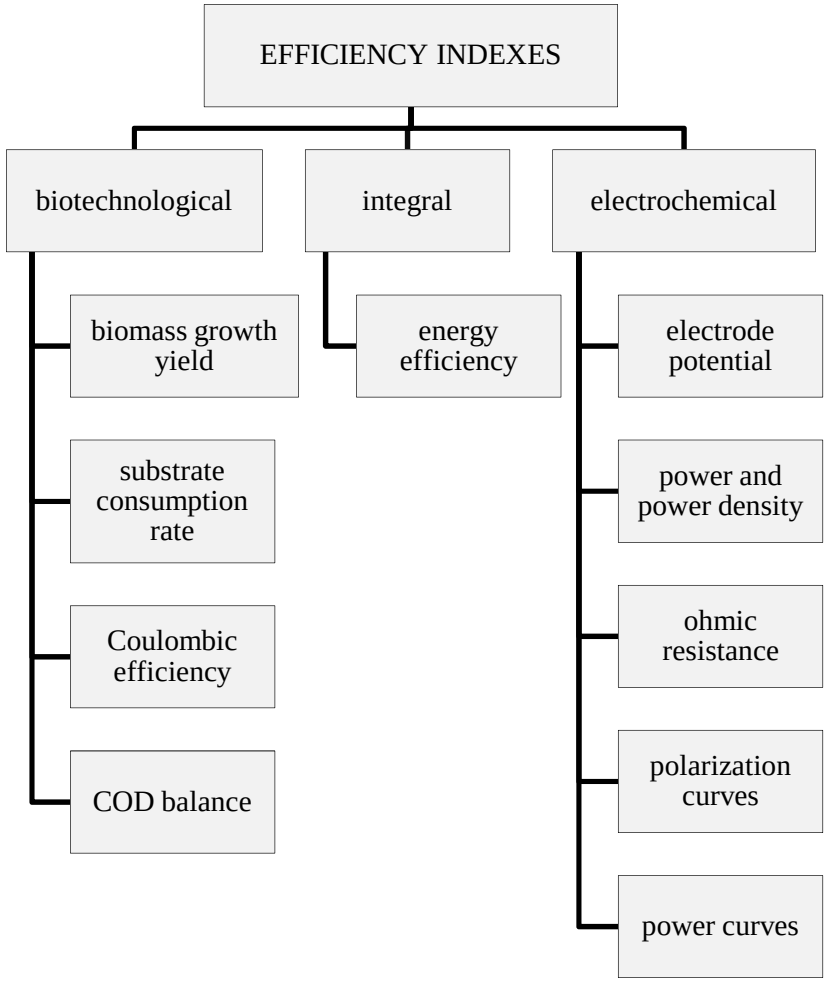


Fig. 2.4 BES efficiency indexes classification

Coulombic Efficiency. The Coulombic efficiency, ϵ_{Cb}, is defined as the ratio of total Coulombs actually transferred to the anode from the substrate, to maximum possible Coulombs if all substrate removal produced current. The total Coulombs obtained is determined by integrating the current over time, so that the Coulombic efficiency for an MFC run in fed-batch mode, ϵ_{Cb}, evaluated over a period of time t_b, is calculated as:

$$\epsilon_{Cb} = \frac{M \int_0^{t_b} I dt}{F b v_{An} \Delta COD} \tag{2.21}$$

where $M = 32$, the molecular weight of oxygen, F is Faraday's constant, $b = 4$ is the number of electrons exchanged per mole of oxygen, v_{An} is the volume of liquid in the anode compartment, and ΔCOD is the change in COD over time t_b.

For continuous flow through the system, we calculate the Coulombic efficiency, ϵ_{Cb}, on the basis of current generated under steady conditions as:

$$\epsilon_{Cb} = \frac{MI}{Fbq\Delta COD} \tag{2.22}$$

where q is the volumetric influent flow rate and ΔCOD is the difference in the influent and effluent COD.

The Coulombic efficiency is diminished by utilization of alternate electron acceptors by the bacteria, either those present in the medium (or wastewater), or those diffusing through the cation exchange membranes (CEM) such as oxygen. Other factors that reduce Coulombic efficiency are competitive processes and bacterial growth. Bacteria unable to utilize the electrode as electron acceptor are likely to use substrate for fermentation and/or methanogenesis. It has been observed that fermentative patterns diminish through time during enrichment of the microbial consortium in the MFC (6). As long as the anode remains attractive enough for the bacteria due to its potential, alternative electron acceptors will not be used. However, high potential compounds such as nitrate (+0.55 V) will almost certainly be used (*Logan et al., 2006*).

COD Balance. Once the efficiencies for electricity and biomass production are completed, the fraction of COD that was removed by unknown processes, φ, can be calculated as

$$\varphi = 1 - \epsilon_{Cb} - Y, \tag{2.23}$$

The electrochemical indexes for evaluation of the basic characteristics of MFC are the electrode potential (measured vs. standard electrodes), power, power density, ohmic resistance, polarization curves, power curves.

Electrode Potential. The potential of an electrode (anode or cathode) can only be determined by measuring the voltage against an electrode with a known potential, i.e., a reference electrode. A reference electrode consists of several phases of constant composition and therefore has a constant potential. The standard hydrogen electrode (SHE) or normal hydrogen electrode (NHE), consisting of a platinum electrode in a hydrogen saturated acidic solution (all components at unit activity), has a potential of 0 V. Because the NHE is not a very practical reference electrode to work with in an experimental setup, other reference electrodes are often used. The most popular reference electrode in MFC experiments is the silver-silver chlo-

ride (Ag/AgCl) reference electrode, because of its simplicity, stability, and nontoxicity. In a saturated potassium chloride solution at 25 °C the Ag/AgCl reference electrode develops a potential of +0.197 V against the NHE. Also practical, but less common in MFC experiments, is the saturated calomel electrode (SCE, 0.242Vagainst the NHE). Electrode potentials are often strongly dependent on the pH in the system and it is therefore important to report the solution pH. Preferably, electrode potentials are reported in the literature back-calculated against the NHE (expressed in V or V vs NHE), but are also often reported as a voltage difference against the reference electrode that was used in the study (e.g., V vs Ag/AgCl). As a consequence of these different methods, the potential of the electrodes appears to vary depending on the electrode used, the pH, and for the cathode the concentration of the electron acceptor. For example, at pH 7 a typical anode potential is –0.20 to –0.28 V (NHE), equivalent to –0.40 to –0.48Vvs Ag/AgCl. At thesamepHa typical cathode potential is 0.30 to 0.10 V (NHE), equivalent to 0.10 to –0.10 V vs Ag/AgCl (*Logan et al., 2006*).

Power. The overall performance of an MFC is evaluated in many ways, but principally through power output and Coulombic efficiency. Power is calculated as:

$$P = \frac{E_{MFC}^2}{R_{ex}} \tag{2.24}$$

Normally the voltage is measured across a fixed external resistor (R_{ext}), while the current is calculated from Ohm's law ($I = E_{cell}/R_{ext}$).

This is the direct measure of the electric power. The maximum power is calculated from the polarization curve (see below).

Power Density. Power is often normalized to some characteristic of the reactor in order to make it possible to compare power output of different systems. The choice of the parameter that is used for normalization depends on application, as many systems are not optimized for power production. The power output is usually normalized to the projected anode surface area because the anode is where the biological reaction occurs. The power density (P_{An}, W/m^2) is therefore calculated on the basis of the area of the anode (A_{An}) as:

$$P_{An} = \frac{E_{MFC}^2}{A_{An} R_{ex}} \tag{2.25}$$

In many instances, however, the cathode reaction is thought to limit overall power generation or the anode consists of a material which can be difficult to express in terms of surface area (i.e., granular material). In such cases the area of the cathode (A_{Cat}) can alternatively be used to obtain a power density (P_{Cat}). The projected surface areas of all components should always be clearly stated, as well as the specific surface area (if known) and the method of its determination.

To perform engineering calculations for size and costing of reactors, and as a useful comparison to chemical fuel cells, the power is normalized to the reactor volume, or:

$$P_v = \frac{E_{MFC}^2}{vR_{ex}} \tag{2.26}$$

where P_v is the volumetric power (W/m^3), and v is the total reactor volume (i.e., the empty bed volume). The use of the total bed reactor volume is consistent with a tradition in environmental engineering to use the total reactor size as a basis for the calculation. A comparison on the basis of total reactor volume, however, is not always level when comparing two- and single-chambered reactors because there is no "second chamber" for an open air cathode. In such cases it is useful to compare reactors on the basis of the total anode compartment volume. If multiple reactors are operated in concert, for example as a series of stacked reactors, the volume used for the air-space for the cathode (or volume for the catholyte) is then included for the overall reactor volume. Thus, the volume used in the calculation should be clearly stated, and volumes of the individual chambers must always be clearly noted.

Ohmic Resistance. The ohmic resistance (R_Ω) of an MFC can be determined using the current interrupt technique by operating the MFC at a current at which no concentration losses occur. Next the electrical circuit is opened (which results in zero current, i.e., an infinite resistance) and a steep initial potential rise is observed, followed by a slower further increase of the potential to the OCV. The determination of the steep potential rise after current interrupting requires the fastest possible recording of the potential. Ohmic losses (IR_Ω) are proportional to the produced current and the ohmic resistance. When the current is interrupted the ohmic losses instantaneously disappear. This results in a steep potential rise in potential that is proportional to the ohmic resistance (R_Ω) and the current (I) produced before the interruption. Using Ohms law, R_Ω is estimated. The slower further increase of the potential to the OCV after the initial steep potential rise gives the electrode overpotentials that occurred during current generation.

Polarization Curves. Polarization curves represent a powerful tool for the analysis and characterization of fuel cells (as discussed above). A polarization curve represents the voltage as a function of the current (density). Polarization curves can be recorded for the anode, the cathode, or for the whole MFC using a potentiostat. If a potentiostat is not available, a variable resistor box can be used to set variable external loads. Using a periodical decrease (or increase, when starting at short circuit) of the load, the voltage is measured and the current is calculated using Ohms law. To separately study the performance of the system in terms of anode or cathode potentials, a reference electrode is used as described above. When a potentiostat is used to record a polarization curve, an appropriate scan rate should be chosen such as 1mV s^{-1}. The polarization curve should be recorded both up and down (i.e., from high to low external resistance) and vice versa. When a variable external resistance is used to obtain a polarization curve, the current and potential values need to be taken only when pseudo-steady-state conditions have been established. The establishment of this pseudo-steady state may take several minutes or more, depending on the system and the external resistance. This condition is only a temporary steady state because over longer times the substrate concentration in the reactor will change due to substrate demand at the anode (unless continuously replenished). This will in turn affect the incidence of substrate/products mass transfer over voltage and current. Care should therefore be taken not to wait too long for the establishment of the pseudo-steady state. Polarization curves can also be obtained over multiple batch cycles, i.e., with one resistor used for the whole cycle, allowing measurement of Coulombic efficiency for each resistor. Long-term recording may risk shifts in the microbial community.

Power Curves. A power curve that describes the power (or power density) as the function of the current (or current density) is calculated from the polarization curve.

Integral indexes of MFC operation is the system effectiveness measure. Energy efficiency or performance index is a key indicator to assess the system's ability to produce electricity. The most important factor for evaluating the performance of an MFC for making electricity, compared to more traditional techniques, is to evaluate the system in terms of the energy recovery. The overall energetic efficiency, ϵ_E, is calculated as the ratio of power produced by the cell over a time interval t to the heat of combustion of the organic substrate added in that time frame, or:

$$\epsilon_E = \frac{\int_0^t E_{MFC} I dt}{\Delta H m_{sub}} \tag{2.27}$$

where ΔH is the heat of combustion (J mol^{-1}) and m_{sub} is the amount (mol) of substrate added. This is usually calculated only for influents with known composition (i.e., for synthetic wastewaters) as ¢ΔH is not known for actual wastewaters. In MFCs, energy efficiencies range from 2% to 50% or more when easily biodegradable substrates are used. As a basis for comparison, the electric energy efficiency for thermal conversion of methane does not exceed 40%.

The effectiveness of the MFC for hydrogen production is assessed by indexes: cathodic hydrogen recombination, Coulomb efficiency and volumetric hydrogen production rate (*Rozendal et al., 2006*).

Total theoretical number of moles of hydrogen that can be isolated from the removed COD is calculated by the equation:

$$v_{th} = \frac{b_{H_2/S} V_L \Delta COD}{M_S} \tag{2.28}$$

where $b_{H2/S}$ = 4 mol/mol is the maximum stoichiometric yield of hydrogen from 1 mol of glucose; ΔCOD is the difference between COD values at the beginning and end of cultivation, g COD/l;V_s is volume of the solution in the chamber, l; M_S is the molecular weight of the substrate, 82 g/mol (for glucose).

The number of moles of recombined hydrogen is calculated from the measured current based on equation (2.29):

$$v_{CE} = \frac{\int_0^t Idt}{2F} \tag{2.29}$$

where $I = V/R_{ex}$ is the amperage calculated from the voltage applied to the resistor (10 ohms), A; F = 96 485 is Faraday constant, C/mol; dt is the intervals of data collection, sec.

Coulomb efficiency (CE) is calculated from equation (2.30):

$$C_E = \frac{v_{CE}}{v_{th}} \tag{2.30}$$

The ratio of the number of moles of hydrogen to the possible number of moles that can be produced (from the measured current) is an index of cathodic hydrogen recombination (r_{Cat}) and calculated by the equation (2.31):

$$r_{Cat} = \frac{v_{H_2}}{v_{CE}} \tag{2.31}$$

where v_{H_2} is the number of moles of hydrogen produced during periodic cultivation, mol.

The overall performance of hydrogen (R_{H_2}) is calculated as follows:

$$R_{H_2} = r_{Cat} C_E \tag{2.32}$$

The maximum volumetric hydrogen production rate (Q) is measured in m^3 H_2/m^3 of reactor per day (m^3 H_2/m^3day) and is defined using the formula (2.33):

$$Q = \frac{43,2 I_v r_{Cat}}{F c_g(T)} \tag{2.33}$$

where I_v is the average volumetric current density, A/m^3; c_g is the calculated concentration of gas at a temperature T (using the ideal gas law) (*Logan et al., 2006*).

2.3 Mathematical modeling of bioelectrogenesis process

For the further optimization of MFC it is necessary to identify the main parameters of the mathematical model development, variation of which is leading to the qualitative and quantitative alteration of the process (Fig. 2.5, A):

- Stationary input $X_{in(i)}$ and output parameters $X_{ex(i)}$ (i = 1, 2, ..., m) are the variables values of which can be measured, but cannot be affected: the cell volume, electrodes surface area, physical, physico-chemical and chemical properties of the substrate and buffer solutions, the internal resistance of the MFC, and, particularly, temperature, pressure, mass transfer.
- Control parameters, input $S_{in(j)}$ and output $S_{ex(j)}$, (j = 1, 2, ..., r) are the variables that can be directly affected in accordance with certain requirements that allow process control. In this case, the following parameters are the composition of the medium, the concentration of the substrate and the external resistance.

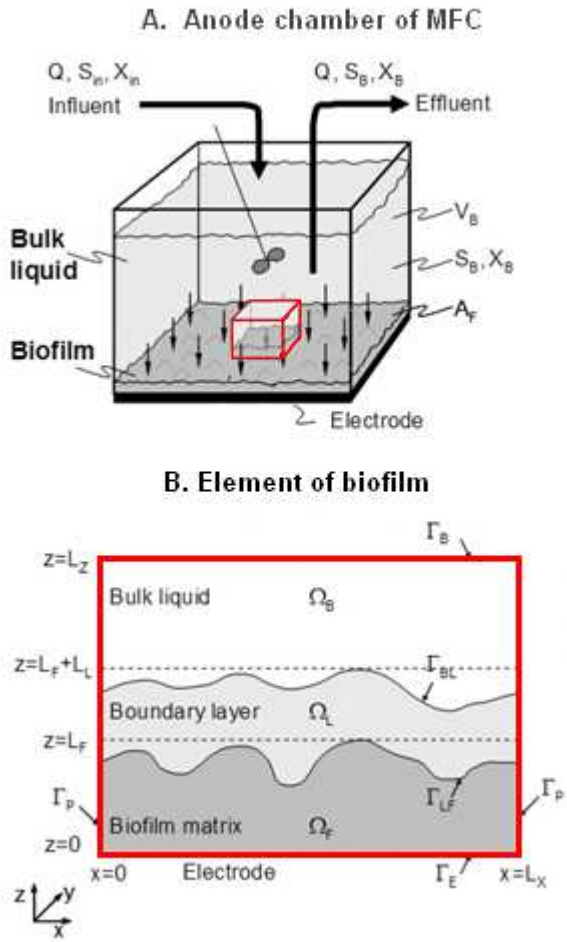


Fig. 2.5 The scheme of system visualization of exoelectrogenesis and mass transfer in MFC: A – Anode chamber of MFC and process parameters, B – Elements of biofilm, based on (*Picioreanu et al., 2004*)

- Perturbation parameter $L_{(k)}$ (k = 1, 2, ..., e) are the variables the values of which randomly change over time and are not impossible to be measured by the available means: impurities in the raw materials, the activity of micro-organisms interactions within populations and others.

Output parameters (stationary and control) describe the state of the process, which is the result of combined effect of input (stationary and control) and perturbation parameters.

For the mathematical description of the processes in the research facility a number of variables, abbreviations and symbols is used, which are presented at the table 2.5.

Table 2.5 Abbreviations and symbols is used For the mathematical description of the processes in the research facility

Nomenclature	Description of variables, unit
A	area, L^2
b	Tafel coefficient, $L^2MT^{-3}I^{-1}(V)$
d	density, ML^{-3}
D	diffusion coefficient, L^2T^{-1}
E	equilibrium potential, $L^2MT^{-3}I^{-1}(V)$
F	Faraday's constant (96 485 C/mol)
ΔG	Gibbs energy, L^2MT^{-2} (kJ)
i	current density, IL^{-2}
I	total current through the MFC, I
K	half-saturation coefficient, NL^{-3} or ML^{-3}
K_a	acidity constant
L	dimension in biofilm domain, L
m	mass of biomass particles, M
n	number of moles, N
P	microbial fuel cell power, L^2MT^{-3} (W)
Q	total charge produced, TI (C)
q	specific rate, $MM^{-1}T^{-1}$
r	net reaction rate at electrode, $ML^{-2}T^{-1}$ or $N\,L^{-2}T^{-1}$ or in bulk or biofilm, $ML^{-3}T^{-1}$ or $N\,L^{-3}T^{-1}$
R	electrical resistance, $L^2MT^{-3}I^{-2}$ (Ω)
S	dissolved chemical component (solute) concentration, NL^{-3} or ML^{-3}
t	time, T
V	voltage (for the MFC) or potential (for the electrodes), $L^2MT^{-3}I^{-1}(V)$
v	volume, L^3
X	biomass component (particulate) concentration, ML^{-3}
x, y, z	coordinates in the biofilm (z perpendicular to the electrode), L
Y	yield, N/N or M/M
γ	degree of reduction for a dissolved or biomass compound (e-/C-mol)
Φ	volumetric flowrate, L^3T^{-1}
η	overpotential (or polarization potential), $L^2MT^{-3}I^{-1}(V)$
ρ	absolute reaction rate, $ML^{-3}T^{-1}$

Note: L, M, T, I and N are the dimensions of the quantities length, mass, time, electric current, and amount of substance, respectively.	
Subscripts	Description
0	Initial
A	Anode
Ac	Acetate
B	bulk liquid
C	Cathode
CAT	catabolic reaction
Car	Carbonate
cell	microbial fuel cell
conc	Concentration
e	calculated per mol electron
E	Electrode
ext	external electrical circuit of MFC
F	Biofilm
G	Gibbs energy
H	hydrogen ion (H^+)
i	index of a solute or biomass component
int	internal electrical circuit of MFC
j	index of a reaction
L	boundary layer
M	mediator (general form)
Met	Methanogen
Mox	oxidized mediator (M)
Mred	reduced mediator (MH_2)
ohm	Ohmic
Q	coulombic (charge)
ref	Reference
S	soluble component
X	Biomass

A – Electrochemical Model

Electrode rates

It is assumed that the transfer of electrons is by means of mediators synthesized by microorganisms, hence, the rate for the electrochemical oxidation of a reduced mediator at the anode surface It is assumed that the transfer of electrons is by means of mediators synthesized by microorganisms, hence, the rate for the electrochemical oxidation of a reduced mediator at the anode surface:

$$M_{red} \leftrightarrow M_{ox} + 2H^+ + 2e^- \tag{2.34}$$

is expressed as a function of current density as:

$$r_{Mred,\mathrm{E}} = -\frac{i}{2F}, r_{Mox,\mathrm{E}} = \frac{i}{2F} \tag{2.35}$$

The current density produced in the electrochemical mediator oxidation is represented by the Butler-Volmer equation (*Newman, 1991*):

$$i = i_{0,ref}\left(\frac{S_{E,Mred}}{S_{ref,Mred}}\right)\left(\frac{S_{E,Mox}}{S_{ref,Mox}}\right)^{-1}\left(\frac{S_{E,H}}{S_{ref,H}}\right)^{-2}\left[exp\left(\frac{2{,}303}{b}\eta_{act}\right) - exp\left(-\frac{2{,}303}{b}\eta_{act}\right)\right] \tag{2.36}$$

All concentrations S_E in Eqation (2.36) are at the electrode surface. These concentrations are also used to calculate the activation overpotential η_{act}.

Current and charge

The current I collected at an electrode is obtained by integration of all possible local current densities i_j over the electrode surface (as in the biofilm-induced biocorrosion model of Picioreanu and Van Loosdrecht, 2002):

$$I = \int_{A_F} \sum_j i_j dA. \tag{2.37}$$

The problem is however complicated by the fact that Eq. (2.23) is implicit in *I*: the activation overpotential, which is the driving force for the current density i_j, depends in turn on the current *I*. A potential balance over the MFC permits calculation of the activation overpotential as is explained further. The charge (Coulombs) produced is calculated from the integration of cell current over time:

$$Q = \int_0^t Idt. \tag{2.38}$$

The coulombic yield (Y_Q) is the ratio between the actual charge produced Q and the maximum theoretical one, Q_{max}, which is the number of electrons (γ) available for a redox reaction in all oxidizable substrates (n_i moles) fed to the fuel cell:

$$Y_Q = \frac{Q}{Q_{\max}} = \frac{Q}{\sum_i \gamma_i n_i}. \tag{2.39}$$

Voltage and overpotential

When the (microbial) fuel cell is connected with an external resistance R_{ext} (load), Ohm's law $V_{cell} = I \times R_{ext}$ gives the microbial fuel cell voltage, V_{cell}. Furthermore, the cell power is $P = V_{cell} \cdot I$. The actual fuel cell voltage is decreased from the equilibrium potential, E_{cell} (the maximum imposed by thermodynamics), by a series of irreversible losses $V_{cell} = E_{cell} - L_{losses}$. The equilibrium cell potential E_{cell} is expressed by the difference between the ideal equilibrium potentials of the cathode and anode, $E_C^{(B)}$ and $E_C^{(B)}$, respectively. $E_C^{(B)}$ and $E_A^{(B)}$ at a moment of time are calculated as a function of the concentrations of reacting chemical species in the bulk liquid, S_B, at that moment. The losses, called overpotential or polarization, originate primarily from three sources: (1) activation overpotential (η_{act}, related to the rates of electrode reactions), (2) ohmic overpotential (η_{ohm}, related to the resistance to the flow of ions in the electrolyte and to flow of electrons through the electrode materials), and (3) concentration overpotential (η_{conc}, related to mass transfer limitations of chemical species transported to or from the electrode). All these overpotentials are by convention here positive values. By summation of all polarization losses, the cell voltage is written as:

$$V_{cell} = (E_C^{(B)} - E_A^{(B)}) - (\eta_{act} + \eta_{ohm} + \eta_{conc}). \tag{2.40}$$

The activation and concentration overpotentials occur separately both at cathode and anode, so that one obtains:

$$V_{cell} = (E_C^{(B)} - \eta_{C,act} - \eta_{C,conc}) - (E_A^{(B)} + \eta_{A,act} + \eta_{A,conc}) - \eta_{ohm}. \tag{2.41}$$

Concentration overpotentials will correct the ideal equilibrium electrode potentials taking into account that the actual concentrations at the electrochemical reaction site are the concentrations S_E at the electrode surface. Concentrations S_E are determined by mass transport and reaction and are variable in time (and for 2d or 3d models also in space). If, for a reactant, the difference in concentration between

bulk and electrode surface creates the concentration polarization $\eta_{conc} = RT/(nF)\ln(S_E/S_B)$, then we can define new equilibrium potentials for the cathode and anode, E_C and E_A, as a function of S_E. If both the electrolyte and fuel cell electrodes obey Ohm's law, then $\eta_{ohm} = I \times R_{int}$. One can therefore write Eq. (2.41) as:

$$V_{cell} = (E_C - \eta_{C,act}) - (E_A + \eta_{A,act}) - IR_{\text{int}}. \tag{2.42}$$

For simplification, because the model focuses on the behavior of the anodic chamber, a constant value for the cathode potential, $V_C = E_C - \eta_{C,act}$, is assumed. Values for the equilibrium potentials of all anodic reactions are needed, as functions of the standard redox potential, amended for the actual concentrations calculated at the electrode surface. For example, the mediator oxidation reaction (2.20) has:

$$E_M = E_M^o + \frac{RT}{2F}\ln\frac{S_{Mox}S_{H^+}^2}{S_{Mred}}. \tag{2.43}$$

Finally, at 25°C, with the internal and external resistances known, with the standard reduction potential E^0_M for the mediator couple, and with $V_{cell} = I \cdot R_{ext}$, the activation overpotential is:

$$\eta_{act} = V_C - I(R_{\text{int}} + R_{ext}) - (E_M^0 - 0,059\,pH + \frac{0,059}{2}\log\frac{S_{E,Mox}}{S_{E,Mred}}). \tag{2.44}$$

Biofilm/bulk liquid model

In general, the reaction stoichiometry of microbial growth relates the amounts of different substrates consumed to the amounts of metabolic products and biomass formed during a certain microbial conversion. In order to construct a realistic model for the biotransformations occurring in the MFC, it is essential to have the reaction stoichiometry based not only on correct elemental and charge balances, but also on the second law of thermodynamics. Microorganisms from MFC extract the large quantities of Gibbs free energy needed to build biomass (the anabolic process) from redox reactions (catabolism) involving an electron donor/electron acceptor couple. We shall assume for the purposes of this example that the MFC bacteria grow anaerobically on acetate as electron donor and carbon source, with oxidized mediator as electron acceptor, and with NH_4^+ as N source. The growth system contains acetate, biomass (with a typical elemental formula $CH_{1.8}O_{0.5}N_{0.2}$), oxidized mediator (M_{ox}=M), reduced mediator (M_{red}=MH_2), HCO_3^-, NH_4^+, H^+ i H_2O as the eight rele-

vant components. The general macro-chemical reaction equation for the production of 1 C-mol biomass can be written as:

$$aC_2H_3O_2^- + fM - fMH_2 + bNH_4^+ + cH^+ \rightarrow H_2O + eHCO_3^- + 1CH_{1.8}O_{0.5}N_{0.2}.$$

The six stoichiometric coefficients (*a–f*) can be calculated from the conservation equations for C, H, O, N, electric charge, and the Gibbs energy balance.

Because we may not always know the Gibbs energy of formation for the mediator species, we adopt the approach of Heijnen (*Heijnen et al., 1999*), based on the Gibbs energy per electron present in the redox couple, ΔG_e^{01}. This has the advantage of being directly related (by $\Delta G_e^{01} = -F\Delta E^{01}$) to the conventional redox potential of redox half reactions, E^{01}, which is normally known for a given mediator. ΔG_e^{01} is calculated at biochemical standard conditions (pH = 7. 298 K, 1mol/L, 1 bar), using HCO_3^-, the most abundant form of carbon dioxide at this pH.

First, the Gibbs energy of the catabolic reaction ΔG_{CAT} is calculated from the Gibbs energies of half-reactions:

$$-\Delta G_{CAT} = \gamma_{Ac}(\Delta G_{e.Ac}^{01} - \Delta G_{e.M}^{01}). \tag{2.45}$$

For the acetate/ HCO_3^- couple (electron donor), $\Delta G_{e,Ac}^{01}$=26.8 kJ/e-mol (*Heijnen et al., 1999*). For the mediator couple (electron acceptor), $\Delta G_{e,M}^{01} = -FE_M^{01}$. Taking thionine as mediator in this example, the reduction potential at biochemical standard is E_M^{01} = 0.064 V (*Heijnen et al., 1979*) (that is, E_M^0 = 0.477 V in standard conditions, vs. standard hydrogen electrode, SHE), thus $\Delta G_{e,M}^{01}$ = 6.175 kJ/e-mol. The reduction degree for acetate is γ_{Ac} = 4 e/C-mol, thus ΔG_{CAT} = 131.9 kJ/C-molAc. Second, the dissipated Gibbs energy can be estimated from a correlation function of the number of carbon atoms in the electron donor and its degree of reduction (*Heijnen et al., 1979*). If maintenance is neglected, the estimated maximum energy dissipation yield is $1/Y_{GX}^{max}$ = 432 kJ/C-mol biomass. Finally, from a balance of degree of reduction (conservation of electrons) and the Gibbs energy balance, and with a degree of reduction for biomass γ_X = 4.2, the maximum yield of C-mol biomass/C-mol acetate, $Y_{Ac,X}^{max}$ is:

$$Y_{Ac,X}^{\max} = \frac{-\Delta G_{CAT}}{\frac{1}{Y_{GX}^{\max}} + \frac{\gamma_X}{\gamma_{Ac}}(-\Delta G_{CAT})} = 0.231 \tag{2.46}$$

which means a stoichiometric coefficient a = –2.163mol acetate/C-mol biomass. In a similar manner, the yield of biomass from mediator (with γ_M = –2 because two electrons are involved per mol thionine) can be calculated as:

$$Y_{MX}^{\max} = \frac{\frac{-\gamma_M}{\gamma_M}(-\Delta G_{CAT})}{\frac{1}{Y_{GX}^{\max}}} = 0.153, \tag{2.47}$$

which gives the stoichiometric coefficient f = 6.553 mol mediator/C-mol biomass. The rest of four stoichiometric coefficients follow from the elemental and charge balances. The calculated molar stoichiometry of microbial growth on acetate with thionine as electron acceptor is:

$$\begin{aligned} &2{,}165C_2H_3O_2^- + 6{,}536M + 0{,}2NH_4^+ + 6{,}16H_2O \rightarrow \\ &\rightarrow 1CH_{1{,}8}O_{0{,}5}N_{0{,}2} + 6{,}536MH_2 + 3{,}33HCO_3^- + 1{,}413H^+. \end{aligned} \tag{2.48}$$

In wastewater treatment, the biomass yield is calculated on chemical oxygen demand (COD) basis (*Henze et al., 2002; Klimiuk and Łebkowska, 2005; Montusiewicz et al., 2010*). In terms of COD, the maximum biomass yield is:

$$Y_X = \frac{\gamma_X}{\gamma_{Ac}} Y_{Ac,X}^{\max}. \tag{2.49}$$

The reaction stoichiometry per gCOD acetate is therefore:

$$\begin{aligned} &C_2H_3O_2^- + Y_{Mox}M + Y_N NH_4^+ + Y_W H_2O \rightarrow \\ &\rightarrow Y_X CH_{1{,}8}O_{0{,}5}N_{0{,}2} + Y_{Mred}MH_2 + Y_{Car}HCO_3^- + Y_H H^+. \end{aligned} \tag{2.50}$$

A hypothetical mediator with a lower reduction potential would theoretically give a higher coulombic yield. However, for microbial growth this is energetically less favorable and less biomass would be produced. For example, one can calculate that for E_M^{01} = –0.200V (that is, E_M^0 = 0.210V in standard conditions, vs. SHE), the Gibbs energy produced in catabolism is only $-\Delta G_{CAT}$ = 30 kJ/C-mol Ac. With this value, one obtains only 0.068gCOD biomass/gCOD acetate. Although in a pure culture, the lower-potential mediator produces more electrons per substrate (has a higher coulombic yield), in a mixed culture this too slow-growing microorganism can potentially not compete with others having a higher growth rate.

Microbial reaction kinetics

The absolute rate of acetate conversion with oxidized mediator leading to microbial growth can be expressed as a double Monod limitation kinetic equation:

$$\rho = q_{Ac,\max} X \frac{S_{Ac}}{K_{Ac} + S_{Ac}} \frac{S_{Mox}}{K_{Mox} + S_{Mox}}. \tag{2.51}$$

The net rates for acetate, biomass, the two mediator forms, bicarbonate and protons are expressed using rate Eq. (2.52) and the stoichiometric Eq. (2.50) as:

$$r_{Ac} = -\rho,\ r_X = Y_X \rho,\ r_{Mred} = Y_M \rho,\ r_{Mox} = -Y_M \rho,$$

$$r_{Car} = Y_{Car} \rho,\ r_H = Y_H \rho. \tag{2.52}$$

The rates (2.52) are applied in mass balances for components in biofilm (with $S_{F,Ac}$, $S_{F,Mox}$, $S_{F,Mred}$, $S_{F,Car}$, $S_{F,H}$ and X_F, all variable in time and space) as well as in the bulk liquid (with $S_{B,Ac}$, $S_{B,Mox}$, $S_{B,Mred}$, $S_{B,Car}$, $S_{B,H}$ and X_B, all variable in time).

Mass balances in bulk liquid

The bulk liquid in the anodic compartment of a MFC will be considered completely mixed so that solute and biomass concentrations, S_B and X_B, (and accordingly their local reaction rates) can be assumed uniform in the whole bulk liquid.

Soluble components: A system of n_S ordinary differential equations representing mass balances of each soluble component (chemical species) in the bulk liquid will be solved:

$$\frac{dS_B}{dt} = \frac{\Phi}{v_B}(S_{in} - S_B) + r_{S,B} + \frac{1}{v_B}\int_{v_F} r_{S,F}\, dv + \frac{1}{v_B}\int_{A_F} r_{S,F}\, dA. \tag{2.53}$$

with a set of specified initial conditions S_B (t = 0) = S_0 for all soluble components. Mass balances (2.39) take into account rates of exchange with the exterior and the rates of reactions in the bulk, in the biofilm and on the electrode. In the model case study chosen as an example, batch operation is assumed (Φ = 0), but continuous operation can also be simulated. The net rates of reaction in the bulk ($r_{S,B}$) and in the biofilm ($r_{S,F}$) are made up of contributions from rates ρ_i of all individual reactions i multiplied by the corresponding stoichiometric coefficients (or "yields" in biotechnological applications). These rates are given by Eq. (2.53). The rate of exchange between the biofilm and bulk liquid can be expressed in one of two ways:

- as the product between an average mass flux of component to/from the biofilm, $j_{S,F}$ (kg/m^{2}s or kmol/m^{2}s), and the surface area of the biofilm A_F (m^2),
- an average rate of reaction in the biofilm $r_{S,F}$ (kg/m^{3}s or kmol/m^{3}s) times the biofilm volume, V_F (m^3),

$$\overline{j}_{S,F} A_F = \overline{r}_{S,F} v_F = \int_{V_F} r_{S,F} dv. \tag{2.54}$$

The second option was preferred, because the change in the volumetric rates of solute components can be easily calculated. A second reason is that in a 2d or 3d model case the biofilm surface is actually not the same as the electrode area on which the biofilm is supported and therefore not easily known. Similarly, the electrochemical rates of solute component change on the electrode surface, $r_{S,E}$ (given by Eq. (2.54)) can be integrated over the electrode surface to give the contribution of the surface reactions to solute accumulation. The solution of Eqs. (2.53) is a set of bulk liquid concentrations needed at each moment in time as boundary conditions for solute mass balances in the biofilm.

Biomass components: Similar to the solute balances, mass balances are also written for all n_X relevant biomass types included in the model:

$$\frac{dX_B}{dt} = \frac{\Phi}{v_B}(X_{in} - X_B) + r_{X,B} + r_{\det} \frac{A_F}{v_B} - r_{ata} \frac{A_F}{v_B}. \tag{2.55}$$

Initial concentrations of all biomass types suspended in the bulk liquid, X_0, need to be assumed in accordance with those used in the experiments. In the batch case exemplified here, $\Phi = 0$. Moreover, for simplicity, the biomass exchange between bulk and biofilm (the surface-based rates of biomass detachment and attachment) has been ignored in the case studies presented here although the general model implementation offers this possibility.

Mass balances in biofilm

Unlike the bulk liquid, the biofilm sub-domain is characterized by spatial concentration gradients both for solutes and for biomass components, $S_F(x,y,z)$ and $X_F(x,y,z)$. Moreover, these concentrations are also time dependent as the biofilm continuously develops over time. Biofilm model equations are presented in detail in other publications (*Picioreanu et al., 2004; Xavier et al., 2005*) therefore only the essential model modifications will be explained here.

Solute components: For any soluble component, a mass balance can be set up by assuming that the transport is only by molecular diffusion and that dissolved components can be produced or consumed in several biotic or abiotic transformation processes with net rate $r_{S,F}$:

$$\frac{\partial S_F}{\partial t} = \frac{\partial}{\partial x}(D\frac{\partial S_F}{\partial x}) + \frac{\partial}{\partial y}(D\frac{\partial S_F}{\partial y}) + \frac{\partial}{\partial z}(D\frac{\partial S_F}{\partial z}) + r_{S,F}. \qquad (2.56)$$

Migration of ions in an electrical potential field is here neglected by assuming that the high medium conductivity will make potential gradients insignificant. Eq. (2.56) is applied on a rectangular computational domain partially filled with biofilm and partially with a mass transfer boundary layer without biofilm. Simplifications are made in 1d and 2d model reductions (d/dx and/or $d/dy = 0$). The boundary conditions needed to solve the mass balance Eq. (2.37) reflect the particular setting of the modeled biofilm system. At $z=L_z$ (top of the biofilm computational domain) (Fig. 2.4, B) bulk concentrations of all soluble components are assumed, $S_F = S_B$. The electrode surface on which the biofilm develops (at $z=0$) is now electrochemically active for certain chemical species (e.g., mediators, protons with $r_{S,E} \neq 0$), but inert and impermeable for others ($r_{S,E} = 0$). In general, the boundary condition at the electrode surface expresses the fact that the rate of superficial production of a species on the electrode surface must equal the flux out by diffusion:

$$D\frac{\partial S_F}{\partial z} + r_{S,E} = 0. \qquad (2.57)$$

The remaining lateral system boundaries are connected and form a so-called "periodic" boundary type (*Xavier et al., 2005*). The bulk/boundary layer and the boundary layer/biofilm interfaces are considered internal boundaries, where conditions of concentration continuity apply. For the initial state at $t = 0$, a uniform distribution of concentrations throughout the whole domain is assumed, with $S_F = S_B$. The solution of solute mass balances in the biofilm, $S_F(x,y,z)$, at a given moment in time is used to calculate:

- the overall biofilm reaction rates needed in bulk liquid mass balances (2.39);
- electrode reaction rates and currents because $S_E = S_F(x,y,0)$;
- biomass growth rates in the biofilm.

Biomass balances: The model for biomass production/consumption and transport within the biofilm closely follows the individual-based modeling approach described and applied in Picioreanu et al. (2004) and Xavier et al. (2005) (*Picioreanu et al., 2004; Xavier et al., 2005*). The relevant parameters are: n_{P0}, initial number of biomass particles, d_X, density of biomass particles, m_0, initial mass of biomass particles and $m_{X,max}$, critical biomass for biomass particle division.

pH calculation

The electrochemical mediator oxidation rate depends on pH, which is in turn altered by both electrode and microbial reactions. The H^+ concentration is calculated from a local charge balance (electroneutrality condition) applied at every location in the biofilm and bulk liquid:

$$S_H - \sum_{i=1}^{n}\left(\frac{K_a S_a}{S_H + K_a}\right) + \sum_{i=1}^{m}\left(\frac{S_H S_C}{S_H + K_a}\right) - \frac{K_w}{S_H} - \sum_{i=1}^{k} S_{Ai} \sum_{i=1}^{p} S_{Ci} = 0 \qquad (2.58)$$

The concentrations of anionic species from weak acids, S_{A^-}, are expressed from the acidity constant K_a combined with the total concentration of that acid, S_A. A similar treatment is applied for cations from weak bases. Cations from strong bases (e.g., Na^+) or anions from strong acids (e.g., Cl^-) are usually also present. OH^- concentration results from the ionic product of water K_w.

Hence, it should be noted that the mathematical model described in notional arrays due to the specifics of the process, namely, it consists of the electrochemical model (rate of electrode reactions, charge and current, voltage and overvoltage) and the mathematical modeling of the near-electrode system "biofilm/volume of fluid" (kinetics of microbial reactions, material balances in the electrolyte bulk, material balances in the biofilm, the calculation of pH). It is reviewed the calculation of basic thermodynamic parameters which suggests inability of the exoelectrogenesis without microorganisms and classified the losses in MFC. Also, it is classified and described mathematically key indicators for evaluation of the MFC performance that are the simplified function of the mathematical model.

3 Exoelectrogenic microorganisms as bioelectrochemical energy converters

Human subtlety...
will never devise an invention more beautiful,
more simple or more direct than does nature,
because in her inventions nothing is lacking,
and nothing is superfluous.
Leonardo da Vinci

An exoelectrogen normally refers to a microorganism that has the ability to transfer electrons extracellularly. While exoelectrogen is the predominant name, other terms have been used: electrochemically active bacteria, anode respiring bacteria, and electricigens (*Logan, 2009*). Electrons exocytosed in this fashion are produced following ATP production using an electron transport chain (ETC) during oxidative phosphorylation. Conventional cellular respiration requires a final electron acceptor to receive these electrons. Cells that use molecular oxygen (O_2) as their final electron acceptor are described as using aerobic respiration, while cells that use other soluble compounds as their final electron acceptor are described as using anaerobic respiration. However, the final electron acceptor of an exoelectrogen is found extracellularly and can be a strong oxidizing agent in aqueous solution or a solid conductor (electron acceptor). Two commonly observed acceptors are iron compounds (specifically Fe(III) oxides) and manganese compounds (specifically Mn(III/IV) oxides) (*Hartshorne et al., 2009*). As oxygen is a strong oxidizer, cells are able to do this strictly in the absence of oxygen.

Investigation of MFC proved the presence of a wide range of microbial communities that were isolated from various natural environments, such as wastewaters, ocean sediments or anaerobic activated sludge from wastewater treatment plants.

For a better understanding of electricity and hydrogen generation in artificial bioelectrochemical systems we should thoroughly know the principles of energy metabolism of exoelectrogens, mechanisms of electron transfer to the anode, and species diversity of the microorganisms. These issues are discussed in this chapter of the monograph.

3.1 Energy metabolism of exoelectrogens

If bacteria want to survive, grow or become dominant within a microbial community, they do not only require substrate and nutrients but they also need the presence of an appropriate electron acceptor. Based on the usage of a final electron acceptor, there are two main modes of microbial energy conservation: respiration and fermentation. These processes are ubiquitous in various natural environments.

Recently, they have been accompanied by a new exciting respiration process occurring in bioelectrochemical systems (BESs): electrogenesis.

Respiration. Microorganisms survive and grow due to the energy they generate by transferring electrons. During respiration, microorganisms liberate electrons from an electron rich substrate at a low redoxpotential and transfer these electrons through a number of electron transport complexes through the cell membrane where a final electron acceptor is reduced (*Schlegel 1992*). Microorganisms do not use the energy produced by the flow of electrons in a direct way, instead, the flow of electrons is used to create a proton gradient across the cell membrane as described by Mitchell (1961) (*Madigan et al., 1997).* The energy released by the inward flux of the protons through a membrane complex (ATP synthase) is used to regenerate energy carrier molecules, such as adenosine triphosphate (ATP). By creating this proton gradient, the potential difference between the electron donor (i.e. the substrate at low potential) and the electron acceptor is translated into a process for the generation of energy. The higher the potential difference between the electron donor and electron acceptor, the higher the proton driven potential difference and the higher the potential amount of ATP which can be refueled. Respiring microorganisms can use a large variety of different electron acceptors, ranging from oxygen, nitrate, iron and manganese oxides to sulfate, but their ability to use the acceptor with the highest redox potential will increase their energy for growth (*Madigan et al., 1997*) and is their incentive to explore alternative electron acceptors.

Fermentation. In many environments, the availability of electron acceptors is limited, which impedes microorganisms from using the respiratory pathway. In these cases, which are abundant in many environmental conditions, fermenting organisms are likely to establish themselves. Fermentation is an ATP-regenerating metabolic process in which degradation products or organic substrates serve as electron donor as well as electron acceptor (*Schlegel, 1992*). The advantage of this pathway is that fermenting organisms are able to grow in numerous environments which are non supportive for organisms that only use the respiratory pathway because suitable electron acceptors are lacking. Fermenting organisms are important within the overall microbial processes in nature for their ability to degrade polymeric compounds into readily degradable monomers. However, fermentation is energetically far less efficient compared to respiration as only 1 to 4 moles of ATP are

formed during the fermentation of glucose where 26 to 38 moles of ATP are formed during the aerobic degradation of glucose. This is also reflected in the Gibbs free energy value, which is a factor 7 lower for the fermentation of glucose compared to the aerobic respiration. The remainder of the Gibbs free energy is not lost but is conserved within the excreted fermentation products such as volatile fatty acids, hydrogen, alcohols and many more. The trade off between their low energetic yield, and their ability to colonize niches devoid of readily available electron donors and acceptors, determines the success of fermenting organisms in many ecosystems.

Electrogenesis. Unlike natural environmental systems, the anode compartment of a BES is an engineered environment in which the availability of soluble electron acceptors is limited. The microbial electricity generation in a BES relies on the drive of bacteria to acquire maximum energy. The main electron acceptor present in a BES, enabling bacteria to use respiratory processes, is a solid conductive electrode. The higher amount of metabolic energy released by transferring electrons to the electrode compared to the use of other electron acceptors is their drive to colonize the electrode and develop electron transfer strategies. The complete web of electron transfer mechanisms is not fully understood yet, probably a complementary range of processes such as direct electron transfer and mediated electron transfer are used. The result is a process in which bacteria serve as a biocatalyst to transform an electron rich substrate i) into electrons, which are transferred to the electrode, ii) into protons which migrate to the cathode and iii) into oxidized products which leave the reactor. The electrons flow through an external electrical circuit towards the cathode electrode where a final electron acceptor is reduced by a chemical (*Zhao et al., 2006*) or microbial catalyst (*Clauwaert et al., 2007*).

In MFC with oxygen cathode oxygen is the final electron acceptor but in anaerobic conditions in the anode chamber anode acts as an intermediate electron acceptor and removes them from the cell surface, without affecting the subsequent metabolic processes.

The excess of protons (formed during proton pump work and wasn't used in the ATPase) diffuses through the proton-selective membrane to the cathode chamber, where connects with oxygen molecules and electrons with the subsequent formation of water (in the case of the oxygen cathode).

Most microorganisms are electrochemically inactive because their proteins associated with the transfer of electrons located in the cell membrane. To facilitate the electrons transfer from the microbial membrane to the electrode of MFC mediators can be used (*Akiba et al., 1987; Kim et al., 2000; Choi et al., 2001; Choi et al., 2003; Schroder et al., 2003; Kuzminskiy et al., 2008a)*. Mediators are recovered during the metabolic oxidation of organic material and reduced form re-oxidized mediator at the working electrode (anode), which occurs at very high electric potential.

Based on the proposed scheme practically any bacterium could be used for electricity production in MFC using mediators. There have been investigated a wide range of artificial mediators for electron transfer between the cell and the anode, including tionin (*Bennetto et al., 1985; Kim et al., 2000),* viologens (*Wilkinson, 2000; Choi et al., 2001*), methylene blue (*Wilkinson, 2000*), 2-hydroxy-1,4-naphthoquinone (*Roller et al., 1984; Clauwaert et al., 2007*) and other hydrophobic compounds (*Akiba et al., 1987, Kim et al., 2000; Roller et al., 1984).* In general, these mediators are toxic for microorganisms at concentrations required for efficient generation of power. This fact combined with high price of mediators makes commercialization of MFC with mediators unlikely.

3.2 Extracellular electron transport by exoelectrogens

Exocellular electron transfer is an important mechanism in anaerobic microbial communities to sustain microbial metabolism when electron donors are physically separated from electron acceptors. This situation commonly occurs in environments that contain large amounts of oxidized forms of iron and manganese, i.e., Fe(III) and Mn(IV). Fe(III)- and Mn(IV)- minerals form an interesting electron acceptor in anaerobic environments, but they are poorly soluble in most natural environments (e.g., near neutral pH conditions). To be able to utilize these insoluble electron acceptors microorganisms have evolved several mechanisms through which electrons can be directed towards these exocellular electron acceptors. These strategies can be subdivided into two main categories:

- Exocellular electron transfer mediated by soluble electron shuttles (redox mediators),
- Exocellular electron transfer through direct contact with insoluble electron acceptors (mediator-less electron transfer) (Figure 3.1).

The first of these categories, i.e., exocellular electron transfer mediated by soluble electron shuttles, involves the redox cycling of the electron shuttles in between the microorganisms and insoluble electron acceptors. During this redox cycling the electron shuttles are continuously reduced by the microorganism and re-oxidized again by the insoluble electron acceptor. The electron shuttles involved in this process can be naturally present redox-active organic compounds, such as humic acids, but are also often produced by the microorganisms themselves, such as quinones. Furthermore, this exocellular electron transfer mechanisms in Fe(III)- and Mn(IV)-respiring microbial communities can also be artificially enhanced by adding artificial electron shuttles, such as thionine, iron chelates, and neutral red.

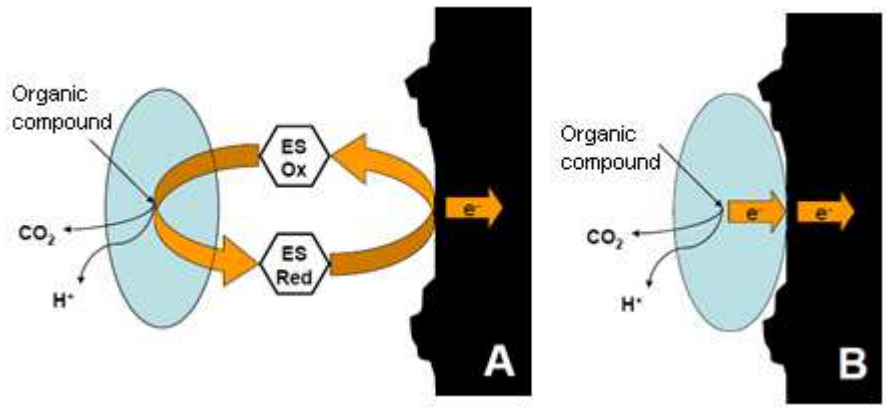


Fig. 3.1 Exocellular electron transfer mechanisms to insoluble electron acceptors: (A) exocellular electron transfer mediated by soluble electron shuttles (ES), and (B) exocellular electron transfer through direct contact with insoluble electron acceptors (mediator-less electron transfer), based on (*Logan et al., 2006*)

As electron shuttles also interact with electrode surfaces, microbial communities that respire according to this exocellular electron transfer mechanism are *electrochemically active*. This has formed the basis of the first generation of microbial fuel cells. Unfortunately, in open environments such as MFCs that are continuously supplied with wastewater, electron shuttles are not retained in the system. This limits the applicability of this first generation of MFCs, as continuous addition of artificial electron shuttles is costly and imposes environmental risks due to the toxic nature of most artificial electron shuttles.

Renewed interest in MFC technology, however, emerged when researchers at the end of the last century realized the implication of the second category of exocellular electron transfer to insoluble electron accepters, i.e., exocellular electron transfer through direct contact. In most other types of microorganisms the non-conductive cell wall of intact microbial cells prevents the contact of insoluble electron-acceptors with the redox proteins present in these cells. However, some Fe(III)- and Mn(IV)- respiring microorganisms have managed to overcome this problem by using membrane-bound redox proteins. These membrane-bound redox proteins include inner-membrane, periplasmic, and outer-membrane redox proteins (i.e., cytochromes), which allow for the transfer of electrons from the inside to the outside of the microorganism (*Logan et al., 2006*).

Graphically mechanisms of electron transfer to the anode of BEC can be represented as shown in Fig. 3.2. The various methods are not necessarily mutually exclusive.

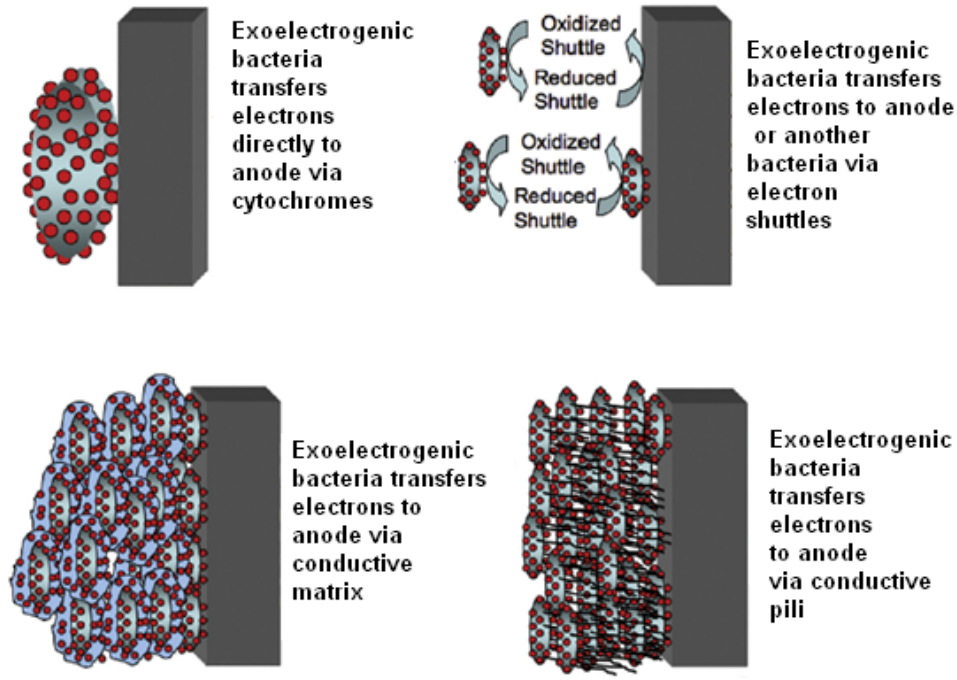


Fig. 3.2 Proposed mechanisms for electron transfer to the anode of microbial fuel cells. Red dots represent outer surface cytochromes, black lines represent nanowires, and the blue clouds represent the possible extracellular matrix which contains c-type cytochromes conferring conductivity, based on (*Lovley, 2008*)

Initial investigations into the mechanisms of microbe-anode interactions have focused on studies with pure culture models because pure cultures can be genetically modified for functional studies and genome-scale investigations on gene expression and proteomics are more readily interpretable with pure cultures. Pure culture studies are likely to have the most relevance to power production in mixed communities if the pure culture: 1) is representative of those that predominate on anodes; 2) is capable of high current densities; and 3) completely oxidizes environmentally relevant organic electron donors, such as acetate. Two cultures, *Rhodopseudomonas palustris* strain DX-1 and *Geobacter sulfurreducens* have been reported to be capable of current densities comparable to mixed communities. Of these two, detailed investigations on mechanisms for electron transfer to anodes have only been reported for *G. sulfurreducens*. Studies on this organism have the additional benefit that it is closely related to organisms that, as noted above, often predominate on anodes and that it is capable of completely oxidizing acetate with an electrode serving as the sole electron acceptor.

On the basis of genome-scale gene expression and genetic studies it has been proposed that cells of *G. sulfurreducens* in direct contact with the anode surface transfer electrons to the anode via c-type cytochromes displayed on the outer cell surface. This hypothesis is supported by sophisticated spectroelectrochemical studies. However, *G. sulfurreducens* can form relatively thick (>50 mm) anode biofilms and cells at this substantial distance from the anode contribute to current production. Gene expression and genetic studies have suggested that, "microbial nanowires" the electrically conductive pili of *G. sulfurreducens*, are important in this long-range electron transfer, but their actual function requires further investigation. A cytochrome that may be easily released into the biofilm matrix may also contribute. The *G. sulfurreducens* biofilm is conductive, in contrast to previously reported microbial biofilms which act as insulators. This is consistent with modeling studies which indicate that the current production observed in *G. sulfurreducens* fuel cells would only be feasible with a conductive biofilm. The *G. sulfurreducens* anode biofilm also has significant capacitance, which has been attributed to the abundant c-type cytochromes which provide substantial electron storage capacity in individual cells.

In contrast, studies with *Shewanella oneidensis*, an intensively studied electrode reducer, have suggested that soluble electron shuttles are the mediators for most of the electron transfer to the anode with this organism. This was most clearly apparent from electrochemical analyses that gave a response for *S. oneidensis* that was significantly different from the response resulting from direct electron transfer to the anode by *G. sulfurreducens*. Riboflavin released from the cells appears to be the source of the shuttle. Therefore, even though *S. oneidensis* produces microbial nanowires, direct wiring to the electrode does not appear to be an important conduit for electron transfer to the anode. Differences between *S. oneidensis* and *G. sulfurreducens* in their ability to interact with smooth gold electrodes further suggest different attachment and/or electron transfer mechanisms. Some of the outer surface c-type cytochromes, known to be important for extracellular electron transfer in *S. oneidensis* are also important for optimal current production, but this may reflect a requirement for the cytochromes for electron shuttle reduction. Many of the cells contributing to power production in *S. oneidensis* fuel cells are planktonic and electron transfer over such long distances is only conceivable with electron shuttles. The closely related *Escherichia coli* may also release metabolites that serve as electron shuttles as can a diversity of other organisms.

Whether the initial extracellular electron acceptor is an electron shuttle or the electrode itself, an often misunderstood point in the microbial fuel cell literature is that these final electron transfer steps are unlikely to directly yield energy for the microorganism. Energy conservation results from electron transfer and associated proton pumping across the inner membrane, but not from any subsequent electron

transfer reactions. Therefore, even though it is often considered that growth yields will be higher with anodes poised at higher potentials, this is unlikely to be the case, just as reduction of Fe(III) forms with different mid-point potentials results in similar growth yields (*Lovley, 2008*).

Thus, exoelectrogens are the catalysts of electrochemical reactions in MFC. Mentioned organisms are natural substitutes of precious metal catalysts that take place in technical fuel cells (such as platinum in hydrogen fuel cells). However, unlike precious metals catalysts, exoelectrogens capable to self reproducing, which is a very significant advantage in such potentially hazardous as waste water

More information about these microorganisms will be described in the next part of the monograph.

3.3 Exoelectrogenic microorganisms

3.3.1 Species diversity of exoelectrogens association

Fuel cells are used to produce electricity electrochemically from many different chemicals, such as hydrogen gas and methanol, through catalytic oxidation of the fuel at the anode and chemical reduction at the cathode. Microbial fuel cells are unique in that they do not require the use of metal catalysts at the anode. Instead, they use microorganisms that biologically oxidize organic matter and transfer electrons to the anode. These electrons flow through a circuit to the cathode, where they combine with protons and a chemical catholyte, such as oxygen. The reduction of oxygen is usually catalyzed by a precious metal catalyst, such as platinum, although non-precious metals can be used. The oxidation of the fuel at the anode in an MFC is not a true catalysis step, as the microorganisms (which contain true catalysts) derive energy from the oxidation of the fuel, creating an overall energy loss. Depending on the energy gain by the bacteria, and energy losses at the cathode, a voltage of 0.3–0.5 V is usually obtained for fuels such as glucose or acetic acid.

Electrical current generation has been shown for four of the five classes of *Proteobacteria,* as well as the *Firmicutes* and *Acidobacteria phyla*. The yeast *Pichia anomala* has redox enzymes on its outer membrane and can produce current in an MFC, and the oxygenic phototrophic cyanobacterium *Synechocystis* sp. PCC 6803 was discovered to produce electrically conductive appendages called nanowires. Although many different types of microorganisms produce electrical current in MFCs, many of these strains exhibit low power densities when grown as pure cultures. It is therefore unclear whether these bacteria exist as exoelectrogenic oligotrophs among faster-growing competitors, or whether a low level of current generation provides some other benefits through interactions. For example, a Gram-

positive bacterium (*Brevibacillus* sp. PTH1) that was abundant in a mixed community in an MFC produced little power as a pure culture unless a *Pseudomonas* sp. was also present or supernatant from an MFC with this bacterium was added.

There are at least three possible reasons why microorganisms can use exocellular electron transfer, which can result in power generation in an MFC. The first and best studied reason is cell respiration using solid metal oxides, such as iron. Many strains of bacteria can release electrons from a terminal oxidize in the respiratory chain to Fe (III) outside the cell, producing soluble Fe (II). Second, it is possible that cells can transfer electrons directly to another cell, without the need for intermediates. The fermentative bacterium *Pelotomaculum thermopropionicum* was observed to be linked to the methanogen *Methanothermobacter thermautotrophicus* by an electrically conductive appendage, which provided the first direct evidence for interspecies electron transfer. Electron transfer directly into bacteria has been indirectly observed in other situations.

Oxygen reduction can be catalyzed by bacteria on the cathode (known as a biocathode) of an MFC, allowing for bacterial growth using electrons produced at the anode from the oxidation of organic matter. Bacteria can derive energy from this reaction because electrons enter the bacteria on the biocathode at a higher potential than that needed for oxygen reduction. Biocathodes have also been used for nitrate reduction and hydrogen evolution. The evidence that electrons can be both released and accepted by microorganisms suggests that electron exchange between cells is a naturally occurring phenomenon in microbial communities.

A third possible reason for exogenous electron transfer, and one that has not yet been examined experimentally, relates to a possible role of electron transfer for cell-cell communication. The finding that bacteria within a biofilm communicate through quorum sensing chemicals is well established. Many bacteria, such as *Pseudomonas aeruginosa*, generate quorum signals with fatty acyl-homoserine lactones (acyl-HSls), and recently it was shown that certain *Rhodopseudomonas palustris* strains produce the quorum signal *p*-coumaroyl-HSl. In humans, dozens of transmitters, such as acetylcholine, are produced by neurons for cell-cell communication. This situation is interesting from an evolutionary perspective in the context of the development of cell-cell communication in organisms. The opportunistic pathogen *P. aeruginosa* produces pyocyanin, a chemical that is a signal for the upregulation of quorum sensing-controlled genes. Pyocyanins also function as electron shuttles, allowing electrical current generation in MFCs. The importance of quorum sensing signals in the context of intraspecies and inter-species electron transfer, as well as microbial pathogenesis, is not well understood. Pyocyanins produced by one bacterium can be used by other microorganisms to produce current in an MFC, but they also function as antibiotics. Another opportunistic pathogen, *Ochrobactrum anthropi*, has been shown to be capable of power generation in an

MFC but, unlike most exoelectrogenic bacteria, does not respire using solid metal iron oxides. The yeast *P. anomala* (Fungi kingdom) is also a pathogen that is capable of power generation in an MFC. Through the study of possible reasons for microbial current generation, as well as the mechanisms of electron transfer, we learn more about bacterial respiration, cell-cell communication and the fundamentals of electron transfer through organic molecules. Such information will not only be scientifically interesting, but could be useful in medical applications, as well as in bioelectricity production using MFCs and hydrogen gas production using microbial electrolysis cells.

Several studies found that MFCs contained diverse microbial communities, which was unexpected given the apparent need for cells to be able to respire using an electrode. Studies that use pure cultures have now confirmed that many different bacteria in anodic biofilms can generate power (*Logan, 2006*).

3.3.2 Morphological, cultural and metabolic characteristics of the most widespread exoelectrogens

For the first time the possibility of electricity production in the absence of exogenous mediators was shown in 1999 using bacteria *Shewanella putrefaciens* (*Kim et al., 1999*). *Shewanella putrefaciens* (*Pseudomonas putrefaciens, Alteromonas putrefaciens*) is Gram-negative marine bacteria, facultative anaerobes with the ability of metabolic reduction of iron (III) and manganese (IV), which are used as the final electrons acceptor in the electron transport chain. They have bacillary form, are catalase- and oxidase- positive, mobile. Metabolites of *S. putrefaciens* cause smell of rotting fish, because these bacteria produce trimethylamine (hence the species name "putrefaciens" – *Lat*. rotten). On the solid and liquid nutrients *S. putrefaciens* is easily recognized by their bright pink colonies. On the solid mediums they form small and medium-sized circular pink, yellow-orange or brown colonies, with a high growth rate. Bacteria are also characterized by rapid growth in liquid media, which gives to liquid a pink hue (*Min et al., 2005*).

The mechanism used by *Shewanella* for electron transfer out of the cell, is not identified. Representatives of the *Shewanella* genus have external membrane cytochromes for direct electron transfer, but they also can form electroconductive nanopili (*Myers and Myers, 1992, El-Naggar et al., 2010*).

Shewanella oneidensis is another electrochemically active representative of the *Shewanella* genus, forms, except nanopili, also flavins, which can function as electron carriers (*Canstein et al., 2008*). *S. oneidensis* is a Gram-negative, facultative anaerobic bacterium of *Shewanella* genus, which is mainly located on the seabed under anaerobic conditions, in sediments and in the soil. They are able to reduce metal compounds. Currently work is underway on using *S. oneidensis* in biosecurity

of metal surfaces from corrosion. *Shewanella oneidensis* was first isolated in 1988 by Professor Ken Nealson from sediments of Lake Oneida in New York. The lake where the bacteria was first discovered accounts for its naming.

Shewanella oneidensis has outer membrane cytochromes (MtrC and OmcA) which reduce Fe(III) during anaerobic respiration; it does this by coupling oxidation of organic carbon to electron acceptors such as Fe(III), oxygen, nitrate, and other metals. *Shewanella oneidensis* has the ability to produce pilus like structures when its immediate environment is low in electron acceptor concentration. These pili help the organism to locate and reduce metals. The main central metabolic pathway for *Shewanella oneidensis MR-1* is the serine isocitrate lyase pathway in which formaldehyde made from pyruvate is reacted with glycine to produce serine.

Clostridium butyricum is a bacilliform, Gram-positive bacteria isolated from soil, water, etc., grow only in the absence of oxygen, making butyric fermentation. Spores are highly resistant to heating and toxic chemicals and detergents. *C. butyricum* size ranges from 0.6 to 7 microns in length. Toxins produced by *C. butyricum* (agent of botulism) are the most powerful known poisons. Exoelectrogenic activity of *C. butyricum* EG3 strain was discovered in 2001 (*Kim et al., 1999*).

Geobacter is a genus of proteobacteria. Geobacter are an anaerobic respiration bacterial species which have capabilities that make them useful in bioremediation. *Geobacter* was found to be the first organism with the ability to oxidize organic compounds and metals, including iron, radioactive metals and petroleum compounds into environmentally benign carbon dioxide while using iron oxide or other available metals as electron acceptor. The first *Geobacter* species (initially designated strain GS-15) was isolated from the Potomac River, just downstream from Washington D.C. in 1987. This organism, known as *Geobacter metallireducens*, was the first organism found to oxidize organic compounds to carbon dioxide with iron oxides as the electron acceptor. *Geobacter* species also have the ability to transfer electrons on to the surface of electrodes (*Bond et al., 2002*).

Table 3.1 Summarizes information about existing studies about exoelectrogens

Microorganism	Comment
Shewanella putrefaciens MR-1	Gammaproteobacteria. During growth in anaerobic conditions bacteria accumulate in the outer membrane cytochrome c-type in large quantities. These outer membrane cytochromes involved in electron transfer to insoluble metal oxides are encoded by gene *omcB*
Clostridium butyricum EG3	Phylum *Firmicutes*. These are the first gram-positive bacteria, which generate current in the MFC. This strict anaerobic bacterium was isolated from a mediator-less MFC, worked on starch processing industry wastewaters. They consume glucose, lactate, formate, butyrat, acetate, CO_2 and H_2
Desulfuromonas acetoxidans	Deltaproteobacteria. Identified in a sediment MFC community and shown to produce power. First MFC was designed with this bacterium. It was shown that adding such exogenous mediator as anthraquinone-2, 6-dysulfonata to anode chamber increased productivity only by 24%
Geobacter metallireducens	Deltaproteobacteria. Shown to generate electricity in a poised potential system. They are capable of producing electricity in MFC by using aromatic compounds as a substrate
Geobacter sulfurreducens	Deltaproteobacteria. The first exoelectrogen that was found in fresh waters. Able to form nanowires for direct electron transfer to metal oxides
Rhodoferax ferrireducens	Betaproteobacteria. A facultative anaerobe (Most Fe(III) reducing microorganisms are strict anaerobes). First time complete oxidation of sugars coupled to Fe(III) reduction has been observed in an organism that grows at a near neutral pH. This bacterium is able to completely oxidize glucose by the equation: $C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$
Aeromonas hydrophila	Deltaproteobacteria. Heterotrophic, Gram-negative, rod shaped bacterium, mainly found in areas with a warm climate. This bacterium can be found in fresh or brackish water. It can survive in aerobic and anaerobic environments.
Pseudomonas aeruginosa	Gammaproteobacteria. Produced low amounts of power through mediators such as pyocyanin.

Microorganism	Comment
Desulfobulbus propionicus	Deltaproteobacteria. Capable of growing in the presence of Fe (III), humic acids or graphite electrode as an electron acceptor. Capable of oxidize elemental sulfur S^0.
Geopsychro-bacter electrodiphilus	Deltaproteobacteria. Psychrotolerant.
Geothrix fermentans	Produced an unidentified mediator (phylum Acidobacteria). Gram-negative strict anaerobes, are able to consume simple organic acids (acetate, propionate, lactate) and fatty acids (palmitic) in the presence of electron acceptor (Fe (III), nitrates, humic acids)
Shewanella oneidensis MR-1	Gammaproteobacteria. Various mutants identified that increase current or lose the ability for current generation.
Escherichia coli	Gammaproteobacteria. Found to produce current after long acclimation times. They can use their metabolites as carriers of electrons.
Pelotomaculum thermo-propionicum	Anaerobic, thermophilic. They metabolize fumarate and pyruvate. The first direct evidence of interspecies electron transfer was received using this bacterium.
Desulfovibrio desulfuricans	Deltaproteobacteria. Reduce sulfate during growth on lactate
Acidiphilium	Alphaproteobacteria. Generate current at low pH and in the presence of oxygen
Pichia anomala	Current generation by yeast (kingdom Fungi).

3.4 Biofilm of microorganisms with exoelectrogenic activity formation

In various literary sources researchers suggest different ways of formation and enrichment of biofilm of microorganisms with exoelectrogenic activity (*Samarukha, 2012*). Thus, inoculum sources, cultivation conditions, inoculator construction and terminal electron acceptors are essential factors of formation and enrichment of electroactive biofilm. There are a lot of experimental data about certain factors impact on biofilm formation process, using different procedures of formation and enrichment of biofilm by electroactive microorganisms, but comparison of these procedures hasn't still been held. Therefore the aim of this subsection is a theoretical comparison of methods of exoelectrogenic anode biofilm formation.

Existing methods of biofilm formation may be divided into 3 types:

- Biofilm formation from pure cultures of microorganisms with exoelectrogenic and destructive activities or from binary culture of destructors and exoelectrogens.
- Selection from different natural or artificial sources (soil, activated sludge of wastewater treatment plants) associations of microorganisms and biofilm formation.
- Biofilm formation by enriching microorganisms-destructors association with exoelectrogens.

Biofilm formation from pure cultures of microorganisms with exoelectrogenic and destructive activities

Using pure cultures of exoelectrogens for biofilm formation in BES is one of the most studied directions because it allows to study bioelectrogenesis process in detail, evaluate losses and optimize MFC design, and may find applications in biosensors and micro-MFC (*Qian et al., 2011*).

Most microorganisms that do not require exogenous mediators addition and are able to oxidize organic substrate to carbon dioxide with electrons transfer to the electrode, belonging to the *Geobacteraceae* (species *Desulfuromonas*, *Geopsychrobacter* and *Geobacter*), *Rhodoferax ferrireducens* and *Geothrix fermentans* (*Bond et al., 2002; Bond and Lovley, 2003; Chaudhuri and Lovley, 2003; Bond and Lovley, 2005*) *G. sulfurreducens* has 111 different genes that encode cytochrome *c*, which significantly exceeds the number of the genes in any other organism (*Busalmen et al., 2008*; *Dumas et al., 2008*). Most of the protein products of these genes are located in periplasmic space and outer membrane of cells where they play an important role in electron transport chain (*Reguera et al., 2006; Dumas et al., 2008; Esteve-Núñez et al., 2008; Yi et al., 2009*). The most important genes that encode cytochrome *c* is *omcB, omcE, omcS* iand *omcZ* (*Nevin et al., 2009*). *G. sulfurreducens* strains, containing deletions in either of these genes, especially gene *omcZ*, show a lower activity of electron transport chain and reduce electricity production compared with wild-type strains (Table 1) (*Nevin et al., 2009*). It proves that the expression of genes that encode cytochrome *c* is essential for electron transport to extracellular acceptors because mutant strains with deleted genes lose the ability to produce high specific current density in MFC, and after repeated injection of the gene value of the produced specific current density that increase.

Table 3.2 Maximal current production of MFC inoculated with wild-type cells of *G. sulfurreducens* or strains with the designated genes deleted or the deleted genes complemented via expression of the designated gene on a plasmid (*Nevin et al., 2009*)

Genetic Manipulation	Maximum Current Production (mA)
Wild Type	14.42 ±0.62
pilA deletion	1.20 ±0.44
pilA complement	14.12±0.14
omcZ deletion	1.3 ±0.36
omcZ complement	13.44±0.37
omcB deletion	12.41 ±0.63
omcE deletion	12.75±0.41
omcS deletion	12.67±0.57
GSU1497 deletion	8.01 ±0.42

The peculiarity of *G. sulfurreducens* is its ability to form a thick biofilm on the electron acceptor surface (biofilm thickness in MFC anode is more than 50 microns) (*Reguera et al., 2006*). Biofilm formation is possible due to the presence of outer membrane structures – pili that are encoded by gene *pilA* of *G. sulfurreducens*. Wild type cells expressing *pilA* gene is able to form biofilm on almost any surface, whereas mutants with deletion of the *pilA* gene can immobilize on surfaces only by adhesion without thick biofilm forming (*Reguera et al., 2006; Reguera et al., 2007*). Wild type *G. sulfurreducens* in MFC generates more electricity than strains with deletion of the *pilA* gene (*Qian et al., 2011*).

For biofilm formation *G. sulfurreducens* should be cultivated out at 30°C under strictly anaerobic conditions in the atmosphere of gas mixture (7% H_2, 10% CO_2, 83% N_2). *G. sulfurreducens* strains are cultivated in two types of mediums: NBAF with the addition of 0.1% yeast extract and cysteine (NBAFYE). NBAF – modified medium (*Richter et al., 2008*), containing 15 mM acetate as the electron donor and 40 mM fumarate as an electron acceptor.

Materials for the anode have a great impact influence on the exoelectrogenesis efficiency of *G. sulfurreducens.* Graphite is the most widespread anode of MFC (*Dumas et al., 2008*), has a developed surface that provides large surface area for cells immobilization. For MFC with *G. sulfurreducens* graphite is not only a surface for immobilization of cells, but also allows bacteria to link with the surface using

their pili. In this case formed biofilm is tightly linked with the anode and is not destroyed under intense mass transfer conditions (*Richter et al., 2008*). Other anodic materials were also tested in MFC with *Geobacter*. Gold is an excellent material for the anode production because of high conductivity and resistance to oxidation. However, experiments held with gold anodes showed that the specific power of such MFC is much lower than specific power of MFC with graphite electrodes. This is because gold anodes have a smooth surface, so the biofilm can not be fixed on the anode (*Richter et al., 2008*). However, experiments also showed the possibility of using gold anodes in microMFC (*Busalmen et al., 2008, Richter et al., 2008*), whereas using of graphite anode in nanoscale is problematic.

Selection from different natural or artificial sources (soil, activated sludge of wastewater treatment plants) associations of microorganisms and biofilm formation

In most modern microbial fuel cell mixed bacterial culture are usually used. This bacterial culture can be extracted from environment (soil or anaerobic activated sludge from wastewater treatment plants). Such microbial associations are accessible, stable, with a wide variety of exoelectrogens and have a great destructive ability for wide range of substrates – organic acids (*Liu et al., 2005*), carbohydrates, including polymeric carbohydrates such as starch, cellulose, (*Zhao et al., 2005; Rismani-Yazdi et al., 2007*), proteins (*Heilmann and Logan, 2006*). Biofilm formation procedure is very simple: inert electrode is immersed in the substrate solution in anaerobic conditions. Then inoculum is added (eg waste water) and a small positive potential (0.2 vs. Ag / AgCl) is applied to the electrode (*Kim et al., 2004*). As a result, electroactive biofilm is formed in a position to use the electrode as a terminal electron acceptor. However, usually, this primary biofilm has a low bioelectrocatalytic activity (*Rabaey et al., 2003*). The reason that the primary inoculum of electroactive bacteria is low compared to the number of electrochemically inactive bacteria, and although the presence of the electrode (and the applied voltage) is a factor of selective pressure for electrochemically active bacteria selection, biofilm consists mainly electrochemically inert microorganisms. To solve this problem, usually complex and time-consuming enrichment procedure is used. It consists of repeated mechanical biofilm removal from the electrode, re-inoculation and further biofilm reorganization (*Kim et al., 2005*). In view of the sensibility of electrochemically active bacteria to oxygen, these steps should be performed in anoxic conditions.

Biofilm formation by enriching microorganisms-destructors association with exoelectrogens

Rich sources of microorganisms, such as wastewater, activated sludge, and sediments, are often used as inocula for MFC, and analyses of anode biofilms in these systems have shown great bacterial diversity in anodophilic communities, but did not reveal specific trends in dominant members. This finding may be due to the large number of non-exoelectrogens in the anode biofilm. It was reported that an anodophilic community can be sustained in MFCs even when the biofilm is enriched through successive transfer and enrichment. Thus, selective pressure in MFC systems may not be sufficient to exclude non-exoelectrogens. Since many of the exoelectrogens detected in MFCs are dissimilatory metal-reducing bacteria (DMRB), a rapid selection method to obtain an anodophilic consortium (AC) through dilution and regrowth of DMRB using poorly crystalline Fe(III)-oxide was developed.

A biofilm sample from a BES was serially diluted up to 10^{-9} in anaerobic phosphate buffer solution and incubated in an Fe(III)-acetate medium, and an Fe(III)- reducing AC was obtained for dilutions up to 10^{-6}. The activity of MFC inoculated with the enrichment AC was compared with those inoculated with original biofilm or activated sludge. The power densities and Coulombic efficiencies of the AC (226 mW/m^2, 34%) were higher than those of the original biofilm (209 mW/m^2, 23%) and activated sludge (192 mW/m^2, 19%). The start-up period of the AC (60 h) was also shorter than those obtained with the other inocula (biofilm, 95 h; activated sludge, 300 h). This indicated that such a strategy is highly efficient for obtaining an anodophilic consortium for improving the performance of an MFC (*Nevin et al., 2008; Wang et al., 2010*).

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