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REVIEW

Electricity from Microorganisms

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Abstract—Over the last ten years, the recently discovered process of direct electron transfer from anaerobically grown microorganisms to an electrode of a fuel cell has been the object of intense study. The microorganisms responsible for such electron transport were termed electrogenic; the devices using them to generate electric current, microbial fuel cells (MFCs). The review discussed the molecular mechanisms of electron transfer to the environment in the case of the two best studied microorganisms, *Shewanella oneidensis* and *Geobacter sulfurreducens*. The discovery of bacterial conducting pili (nanowires) used for electron transfer to the electrode and between bacterial cells was sensational. In the real MFCs, which use complex substrates (industrial liquid waste), microbial associations are active, often as biofilms. The progress in MFCs design and the prospects of their practical application are considered.

Key words: microbial fuel cells, bacteria, generation of electric current.

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The theoretical possibility of the generation of electric current by bacteria has been known for almost 100 years [1]; MFCs (microbial fuel cells), devices for current generation employing bacteria, have been under intense study for about 50 years [2]. However, this research became extensive only recently. Apart from environmental problems of humanity (search for new energy sources and environmental protection), the newfound interest in this issue is caused by an important microbiological discovery, i.e., detection of bacteria performing direct extracellular electron transport in the course of their metabolism. These organisms were termed electrogenic. The rapid progress in MFC design and the possibility to improve electrogenic microorganisms by means of their full genome sequencing and the application of postgenomic technologies make it possible to hope for industrial technologies that generate electricity using microorganisms in the near future.

Microbial Fuel Cells (MFCs)

Bacterial cells gain energy mainly via electron transport phosphorylation. This process is schematically represented on Fig. 1; the processes of aerobic and anaerobic respiration are described in easily available textbooks [3]. The goal of this scheme is to explain the principle of MFC operation.

Microbial fuel cells (MFCs) are devices for generation of electricity coupled to anaerobic oxidation of organic substrates. The design of an MFC with acetic acid as an oxidized organic compound is schematically presented in Fig. 2. Bacteria and the organic compound are confined to the anode chamber of an MFC where anaerobic conditions are maintained. The cathode is maintained under aerobic conditions (aeration). The anode and cathode chambers are separated by an ion-selective membrane; it provides for the transfer of protons and prevents oxygen from entering the anode chamber. As can be seen from the scheme, the only way for electrons to reach the terminal acceptor is via the anode and an electric circuit.

Electron transfer to the electrode is the key point of this technology. From the 1960s until recently, the presence of exogenous low-molecular weight electron transporters (compounds accepting electrons within a cell or on the cell surface and releasing them at the electrode) was considered essential for efficient MFC operation [2]. Such compounds are termed exogenous mediators or shuttles.

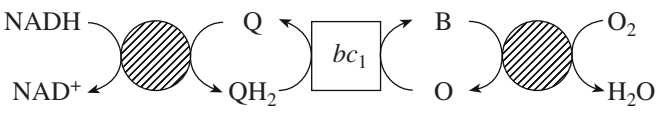

Fig. 1. Schematic representation of a bacterial electron-transport chain. NADH/NAD^+ are the metabolic intermediates transferring reduced equivalents in the cytoplasm;QH_2/Q transfer reduced equivalents in the membrane; bc_1 is the complex containing the periplasmic cytochrome *c* [3].

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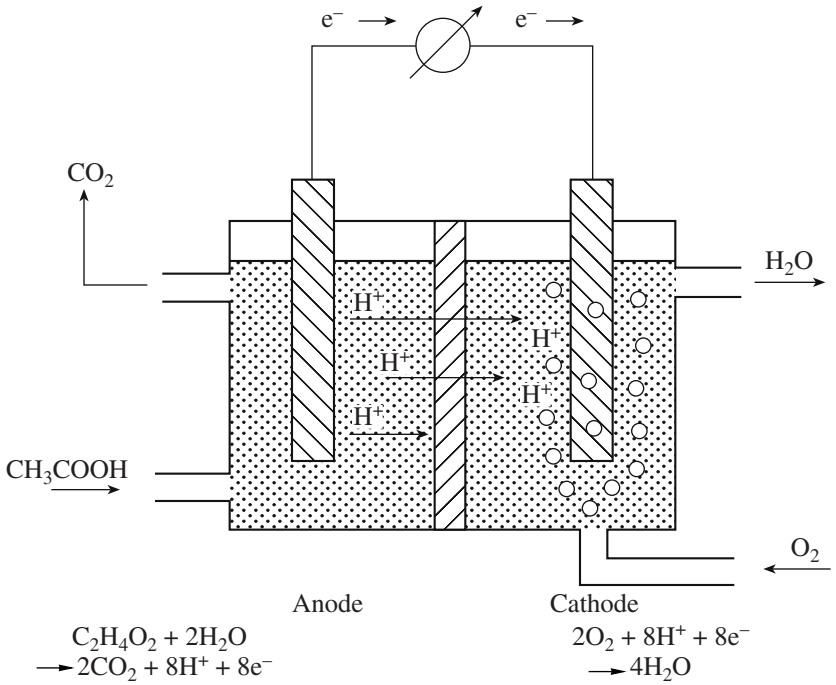

Fig. 2. Microbial fuel cell. The organic substrate (acetic acid) is oxidized in the anaerobic anode chamber; protons and electrons are generated. The electrons moved to the cathode along the conductor; the protons move in the same direction through the liquid and the ion-selective membrane. The cathode chamber is aerated; substrate supply to and CO_2 removal from the anode chamber are arranged. The reactions presented on the figure occur at the anode and cathode in the course of complete acetate oxidation.

It was, however, shown that microbial consortia could efficiently generate electricity without exogenous mediators [4, 5]. It was still unclear whether bacteria can transfer electrons via their direct contact with the electrode or endogenous mediators are synthesized, i.e., compounds transferring electrons from the cell to the electrode. All three variants of electron transport (exogenous mediators, endogenous mediators, and direct contact of bacteria and electrodes) are presently known to occur in nature. Discovery of bacteria capable of complete oxidation of organic compounds and of efficient electron transfer to electrodes via direct contact was a landmark in the history of this research. These organisms were termed electrogenic bacteria.

Electrogenic Bacteria

In 2001 a work was published which described generation of electricity in an ocean coastal zone by means of a specific fuel cell. The anode was submerged into the anaerobic bottom sediment, while the cathode, into seawater containing dissolved oxygen [6]. Simple graphite electrodes were used; the obtained power of 0.01 W/m^2 was sufficient for electronic equipment. Such devices can operate for several years; they can be used to power the equipment located in remote or hard-to-reach sites where battery replacement is difficult, e.g., deep on the seabed.

This system was reproduced under laboratory conditions and thoroughly studied by the group of D. Lovley (United States) in 2002 [7]. The current generation was shown to be related to microbial activity; it ceased immediately when the microorganisms were killed. The authors analyzed the bacterial population attached to the cathode after six months of continuous operation of this MFC. Analysis of 16S rDNA revealed that the population was enriched with δ-proteobacteria. The ratio of δ-proteobacteria in the original sediment and on submerged electrodes not connected to the electrical circuit was 17 ± 4.3%, while it was as high as 71.3 ± 9.6% on active electrodes. A significant portion of this increase (70%) was due to the members of one family, *Geobacteraceae*. These anaerobic microorganisms can couple complete oxidation of organic compounds to reduction of insoluble ferric oxides (Fe^{3+}) to soluble ferrous compounds (Fe^{2+}). The pure culture isolated from the enriched population was identified as *Desulfuromonas acetoxidans*, a marine representative of *Geobacteraceae* [7].

A fuel cell was designed, with a pure culture of *D. acetoxidans* in anaerobic marine water (anode chamber) and aerated seawater (cathode chamber). Addition of acetate to the anode chamber resulted in a current comparable to that obtained in the fuel cells with marine sediments. Addition of an exogenous mediator, anthraquinone-2,6 disulfonate (AQDS) resulted in a weak (24%) increase of production. Bacterial growth

(determined as protein content) occurred in the anode chamber concomitantly with acetate oxidation. It was calculated that in this fuel cell, up to 82% of acetate is oxidized with an electrode as the terminal electron acceptor. Thus, *D. acetoxidans* accumulates energy for growth by electron transfer to the electrodes.

D. acetoxidans was the first electrogenic bacterium described, a microorganism performing complete oxidation of an organic substrate with electron transfer directly to the electrode. Similar results were obtained with other *Geobacteraceae, Geobacter metallireducens*, oxidizing aromatic compounds [7], and the predominantly freshwater *G. sulfurreducens* [8].

Geobacteraceae usually utilize simple organic acids as carbon sources and do not utilize sugars. However, a new electrogenic bacterium *Rhodoferax ferrireducens* was found, with the ability to completely oxidize glucose and transfer electrons to the electrode [9]. This psychrotolerant (optimum at 25°C, growth within the range from 4 to 30°) facultative anaerobe was described as a microorganism reducing Fe^{3+} in bottom sediments [10].

The fuel cell containing glucose and *R. ferrireducens* in the anode chamber acts as a regular electric cell. It operates for 400–500 h and can be recharged simply by changing the glucose solution in the chamber. Of the electrons produced in the course of glucose oxidation ($C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 24H^+ + 24e^-$), 83% pass through the electrical circuit. This MFC with solid graphite electrodes and 10 mM glucose solution creates a current density of ~30 mA/m^2. Application of graphite felt instead of solid graphite electrodes resulted in an increase of the current density up to 74 mA/m^2 [9].

Thus, electrogenic bacteria can be used for construction of fuel cells, sources of direct current operating by oxidation of organic substrates. The efficiency of this process (the ratio of electrons transferred to the external circuit to the total number of electrons produced) is high; further improvements, however, are required for the wide practical application of MFCs.

Mechanism of Electron Transfer to the Electrode

The process of electron transfer from the respiration chain of a microorganism to the electrode is crucial for MFCs operation. These mechanisms are as yet not completely understood. The similarities between microbial reduction of insoluble metal oxides and current production in MFCs are worth consideration. In both cases the electrons are transported to an extracellular solid substrate. This transport can occur either in direct contact between the cell surface and the solid substrate, or indirectly, via the so-called exogenous and endogenous mediators. The best-known *Geobacteraceae* and *Shewanella* species are used for investigation of the mechanisms of electron transfer.

Complete nucleotide sequences of the genomes of these bacteria have been known since 2002 for *S. oneidensis* [11] and since 2003 for *G. sulfurreducens* [12]. Moreover, techniques for genetic exchange and genetic engineering have been developed for these bacteria [13, 14].

For direct electron transfer between bacteria and electrode surface (or metal oxide particles), the electrons should reach the outer membrane of the cell. Unusually high accumulation of a *c* type cytochrome in *S. putrefaciens* MR-1 outer membrane in the course of anaerobic growth was demonstrated as early as 1992 [15]. In the case of aerobically grown gram-negative bacteria, the cytochrome content of the cytoplasmic membrane is usually 10–30 times higher than that of the outer membrane. Although this ratio decreases under anaerobic growth conditions, the cytochromes still remain localized predominantly in the cytoplasmic membrane. Anaerobically grown *S. putrefaciens* MR-1, however, contained 4.4 times more cytochromes in the other membrane than in the cytoplasmic membrane. Subsequent studies revealed that the outer membrane cytochrome involved in the electron transfer to insoluble metal oxides is the protein encoded by the *omcB* gene [16]. Inactivation of this gene resulted in a 45% decrease in ferric oxide reduction [17]. Impaired activity of the *mtrB* gene causes a similar decrease in reducing activity [17]; this gene is required for localization of the OmcB protein in the outer membrane [18]. Thus, the outer membrane cytochrome plays a certain part in reduction of insoluble iron oxides (Fe^{3+}); this is not, however, the only mechanism.

Mutation in the gene *cymA* encoding the cytochrome localized in the cytoplasmic membrane of this bacterium [19] results in an 80% decrease in reducing capacity [17].

When soluble Fe^{3+} compounds (e.g. citrates) are used as terminal electron acceptors, mutations in the *omcB* and *mtrB* genes do not cause a noticeable decrease in substrate reduction, since iron compounds in this case can reach the periplasmic space of the cell.

Mutation in the *menF* gene, which encodes one of the first stages of menaquinone biosynthesis [20], suppresses reduction of insoluble Fe^{3+} compounds almost completely (95%) [17]. Introduction of menaquinone into the medium complements this mutation.

S. oneidensis MR-1 can reduce insoluble metal oxides both in direct contact and at a distance. Immobilization of insoluble oxides in porous spheres was used in order to discriminate between these two capacities. Metal oxides in this system are precipitated both on the surface of the sphere and in internal pores. In the latter case, direct contact with bacteria is impossible; reduction of these oxides occurs indirectly via exogenous or endogenous electron transporters. Alginate was initially used as the material for these spheres [21]; how-

ever, porous glass proved more convenient [17]. Research in this system revealed that *Shewanella* cells deficient in *mtrB* and *omcB* genes lost their capacity for metal reduction in direct contact; those deficient in *menF* and *cymA* genes could not reduce Fe^{3+} either at a distance or in direct contact.

The *S. oneidensis* MR-1 electron transport chain terminates therefore with a *c* type cytochrome (*cymA*) of the cytoplasmic membrane; this cytochrome functions together with a menaquinone. An as of yet unknown mechanism then transfers the electrons via the periplasmic space to the outer membrane cytochrome (OmcB) responsible for iron oxide reduction in direct contact.

Bacteria of the genera *Shewanella* and *Geothrix* reduce insoluble oxides both in direct contact and at a distance [17]; in *Geobacteraceae*, reduction occurs predominantly via direct contact [22].

Discovery of conductive pili termed nanowires first in *Geobacter sulfurreducens* [23] and then in *Shewanella* and other microorganisms [24] is of utmost interest. Deletion of the *pilA* gene, encoding type IV pilin (ORF GSU1496) in *G. sulfurreducens*, inhibited both pili formation and reduction of Fe^{3+} oxides; bacteria, however, were still able to reduce soluble substrates (fumarate of Fe^{3+} citrate). Complementation of the *pilA* deletion with a functional copy of the *pilA* gene in a *trans* position restores the capacity for pili assembling and for reduction of insoluble Fe^{3+} oxides [23]. Force microscopy was used to investigate the conductivity of the pili. In *G. sulfurreducens*, pili formation depended on growth conditions of growth. They formed in the presence of insoluble Fe^{3+} compounds or with fumarate at suboptimal temperature (25°C), but not with fumarate at optimal temperature or in the presence of soluble Fe^{3+} compounds [23]. *S. oneidensis* MR-1 also forms conductive pili when grown under electron acceptor limitations [24]. These pili are 50–150 nm in diameter and tens of micrometers long. A conductive channel (filament) 3–5 nm in diameter is located within each filament [24]. Mutations damaging the cytochromes or the proteins responsible for their localization in the outer membrane make *Shewanella* incapable of oxidation of insoluble Fe^{3+} oxides [24]. The nature of the conductive material within the pili is presently unclear. The mechanism of electron transfer to the pili is also unknown. Bacterial pili are known to be anchored in the periplasm and on the outer membrane of gram-negative bacteria.

Of utmost interest is the discovery of conductive pili in bacterial groups other than reducers of insoluble iron oxide, such as the phototrophic *Synechocystis* PCC6803 and the fermentative bacterium *Pelotomaculum thermopropionicum* [24].

Nanowires, a recently discovered class of bacterial organelles, require further investigation; some hypotheses, however, can already be formulated. Nanowires increase the volume available for the metabolic activity of bacteria reducing insoluble substrates (the length of the nanowires exceeds the size of bacteria proper by an order of magnitude). Moreover, 100-nm pili can penetrate into the soil pores unavailable for direct contact with bacterial surfaces.

The fact that nanowires can connect bacteria of different species is highly intriguing from the point of view of general biology. Such conductive pili have been demonstrated to connect the cells of *P. thermopropionicum* and *Methanothermobacter thermoautotrophicus* when grown together on propionate [25]. Is electron transport possible between bacterial cells of one or several species? What is its biological significance? These issues are yet to be elucidated.

Bacteria have been demonstrated to reduce insoluble metal oxides at a distance, without direct contact [21, 26]; however, the mechanisms of this process and the nature of the endogenous mediators are insufficiently known. The exogenous mediators added to the systems are of no interest for the practical purposes of the generation of electric current.

Quinones [27] and phenazines [28] were suggested as endogenous mediators in some research. Thorough investigation of the *S. oneidensis* MR-1 system [17], however, revealed no real endogenous precursors; their nature still remains an open issue.

Developments in MFCs Design

Among alternative renewable energy sources, MFCs are presently under serious consideration as devices to produce electrical power in the course of treatment of industrial, agricultural, and municipal wastewater. A number of reviews deal with the issues of MFCs investigation and application [29–32]. This interest is due to very high theoretical efficiency of energy generation by MFCs (~80%, i.e., much higher than traditional methanogenesis processes). Such efficiency can probably never be achieved and has certainly not yet been achieved in industrial processes of wastewater treatment.

MFCs operating on waste are open flow systems without exogenous electron transfer mediators [4]. Complex associations of environmental microorganism are active in such systems. Devices for producing electricity from marine bottom sediments [6, 7], presently known as BUGs (Benthic Unattached Generators) [32] have been the prototype of these systems.

The MFCs efficiency is the coulomb efficiency (CE), i.e., the fraction of all the electrons produced in the course of oxidation of organic substrates which is used to generate electric current. Since some energy is required for the maintenance and development of bacterial populations, the coulomb efficiency is always less than 100%. Moreover, in such complex substrates as wastewater, some energy can be spent for reduction of

other electron acceptors (not of the electrode). Oxygen can act as an electron acceptor and decrease CE if it penetrates into the anode chamber. These and other factors limit the real CE in wastewater MFCs within the 0.7–8.1% range [33].

A number of designs have been patented for MFCs operating on wastewater. In one of these designs, the anode chamber is located below the cathode one; in order to prevent mixing of the aerated liquid of the latter with the anaerobic liquid of the former, they are separated by a layer of glass wool. This device does not require agitation. The liquid moves from bottom to top. Multiple electrodes are used; the scheme of their connection enables an increase in the voltage produced. In order to increase the rate of substrate oxidation, platinum-coated electrodes of porous graphite are used. The current in this device is 0.3–0.8 mA [34]. The advantage of such a design is the absence of an ion-selective membrane; such membranes are expensive and may be contaminated when complex substrates are used.

Another industrial device has a cylindrical cathode chamber surrounded by the anode chamber. The chambers are separated by an ion-selective membrane. This MFC also does not require mechanical agitation [35].

High internal resistance and inefficient proton oxidation on the cathode are the general shortcomings of MFCs. As a result of the improvements of the last ten years, the power density of MFCs increased by several orders of magnitude, from 0.1 mW/m^2 to 4.3 W/m^2 [30, 36]. In the latter case, however, the MFC was not a flow system; moreover, ferricyanide was present in the cathode chamber. This device was therefore not a commercial one. However, power densities of 1–1.5 W/m^2 were achieved in a flow system with oxygen in the cathode chamber [37, 38].

Improved contact of microorganisms with the electrode (porous electrodes) and decreased internal resistance (achieved by decreasing the distance between the electrodes) result in increased current density.

The shift from the submerged aerated cathode to the one utilizing the oxygen of the air (a porous cathode with one side submerged into the liquid and the other exposed to air) has been among the major breakthroughs in MFC design. Such MFCs are significantly more efficient than traditional ones [39, 40].

Microorganisms Functioning in Open-System MFCs

The practically developed MFCs utilize organic compounds from the bottom sediments (BUG) or from liquid waste of varied origins. They are open systems, where selection of electrogenic communities occurs to a greater or lesser degree. The anode chamber communities can be expected to be functionally similar to those of methanogenic anaerobic digesters; in the latter communities, however, methanogens play the part of bacteria capable of electron transfer to the electrode. Such communities are termed anodophilic consortia [36].

In BUG systems, 50 to 90% of the anode microorganisms were δ-*Proteobacteria* [7, 8]. *Cytophagales* (up to 33%), *Firmicutes* (11.6%), and γ-*Proteobacteria* (9–10%) were the minor components of anodophilic consortia [41, 42].

A biofilm is usually formed on the anode surfaces of the MFCs operating on liquid waste and other complex substrates; apart from the known electrogenic bacteria (*Geobacter, Shewanella*), they contain a complex microbial association [5, 36]. The range of bacteria capable of electron transfer to the cathode is relatively broad, including the members of the families *Geobacteraceae, Alteromonadaceae*, and *Clostridiaceae* [8, 9, 43, 44]. Some of the members of these associations are possibly symbionts of electrogenic bacteria and do not participate in direct electron transfer to the electrode.

Microbial association in the anode chamber are affected by a number of factors, such as the substrate, cultivation mode (batch of flow), degree of anaerobiosis, and even the conditions within the cathode chamber. Analysis of the populations inhabiting such systems is carried out by means of denaturing gradient gel electrophoresis (DGGE) of the amplified 16S rRNA gene fragments and sequencing of the dominant bands.

For example, inoculation with liquid waste with starch as the substrate in a periodical process with air-aerated cathode resulted in the following composition of the anode chamber community: unidentified clones, 36%; β- and α-*Proteobacteria*, 25 and 20%, respectively; C*ytophaga*, *Flexibacter*, and *Bacteroides*, 19%.

The microbial community of the same system with acetate as the substrate was different: α-, β-, γ-, and δ-*Proteobacteria*, 24, 7, 21, and 21%, respectively; 27% belonged to other types [49]. In a similar system with ferricyanide in the cathode chamber, gram-positive *Brevibacillus agri* (*Fermicutes*) prevailed [30].

The highest power density (4.3 W/m^2) was achieved by a mixed culture in batch mode. In the population, which contained a complex mixture of bacteria (*Firmicutes*, γ-, β-, and α-proteobacteria), high numbers of facultative anaerobes capable of hydrogen production (*Alcaligenes faecalis, Enterococcus gallinarum*) were detected. Colored electron transporters similar to the pyocyanins produced by *Pseudomonas aeruginosa* were also present in the system [36].

The composition of bacterial associations in the anode chamber depends on the composition of the substrates (waste water) and on the symbiotic relationships within the population. Not all bacteria in the population participate in direct electron transfer to the electrode.

Biofilms tens of micrometers thick are formed on the anodes of MFCs. Even within the biofilm, not all bacteria can have direct contact with the electrode. This contact may be achieved indirectly via endogenous electron transporters. Localization of these transporters within the biofilm may result in their higher local concentration and prevent their dilution in the anode chamber, especially in flow systems. Nanowires capable of electron transfer both to the electrode and between bacterial cells are another possible mechanism. Microbial communities revealed in MFCs are reviewed in [30] in more detail.

Hydrogen Production by MFCs

Although the notion of hydrogen energetics is highly popular, in fact this term simply implies that hydrogen is a clean fuel, with water the only combustion product. Hydrogen production still requires some energy source.

Although water seems to be the natural choice of hydrogen source, water electrolysis is energy-consuming and expensive. The energy expenses exceed the energy stored in the hydrogen fuel. Hydrogen production by high-temperature treatment of fossil fuels is presently the cheapest process. Hydrogen production by modified MFCs operating on organic waste may be an interesting alternative. In such devices, anaerobic conditions are maintained in the cathode chamber and additional voltage of ~0.25 V is applied to the cathode. Under such conditions, protons are reduced to hydrogen on the cathode [46]. Such modified MFCs are termed bio-electrochemically assisted microbial reactors (BEAMR) [46].

Although BEAMRs require energy for hydrogen production, the energy expenses are not high (less than 20% of the energy stored in hydrogen fuel). For example, hydrogen production in a BEAMR operating on acetate is ~2.9 mol/mol (the theoretical output is 4.0 mol/mol); the energy requirements are equivalent to burning ~0.5 mol of hydrogen. The total energy gain is therefore more than fivefold [30, 47]. It should be remembered that unlike BEAMR, water electrolysis is an energy-consuming process. Wastewater and diverse soluble organic substrates may be used as fuel for BEAMRs.

Electricity required for hydrogen production in BEAMRs can be obtained from hydrogen-powered fuel cells; these are the most efficient devices, with over 60% conversion to electricity of the energy obtained from hydrogen oxidation. Bacterial enzymes have recently been successfully used in hydrogen fuel cells to replace expensive platinum [48].

Bacteria as Electron Acceptors in Electrobiochemical Processes

The processes described are based on the ability of microorganisms to transfer to an electrode the electrons released in the course of organic matter oxidation. Internal biochemical processes of the cell are thus coupled to external electrical systems. Is the reverse process possible (pumping electrons into the cell)? The answer is yes.

As early as 2004, graphite electrodes were found to act as electron donors for bacterial anaerobic respiration [49], including nitrate reduction [50].

The system for regeneration of the pyridine cofactors NADH and NADPH in MFC-type devices was also described [51].

The practical possibilities of electron transfer to bacteria are presently still obscure; biocatalysis of the oxidation–reduction reactions in chemistry and alteration of bacterial cell metabolism seem, however, the most promising areas.

Prospects

In spite of all the theoretical advantages of MFCs as electric power sources, they are presently used only for such exotic applications as energy supply in hard-to-reach areas [7] or as power sources for implanted devices [52, 53].

For broader, economically acceptable application, improvements in MFC design are required. The material presented above demonstrates that ten years of investigation resulted in a 10000-fold increase in the current density obtained from MFCs [36]. Most of these improvements dealt with MFC design, material of the electrodes, and empirical selection of anodophilic microbial consortia [30].

The power density calculated for the case of bacterial cells closely packed on the anode surface and the average efficiency of microbial anaerobic metabolism is ~2.2 W/m^2 [40]. The cells are, however, known to form more than a single layer on the electrode. Certain mechanisms of electron transfer to some distances exist, and the practically obtained power density is already higher than 4.3 W/m^2 [36]. In fact, we do not know the upper limit for power density in MFCs.

Improvement of MFC design will make other tasks vitally important for their further development. These tasks include better understanding of the nature of electrogenic communities, of the role of individual bacteria in these communities, of the mechanisms of electron transfer to the electrodes and between microbial cells, and of the metabolic pathways and physiology of electrogenic bacteria.

Extensive application of such postgenomic technologies facilitates progress in our understanding of electrogenic bacteria. These approaches include modern

techniques for analysis of potential metabolic characteristics, radioisotope studies of the metabolic pathways [54], full-scale genomic analysis and its experimental verification [55], and mathematical simulation of metabolism [56].

Discovery of nanowires (conductive pili) in electrogenic bacteria may be important, apart from MFCs, also in understanding the functioning of anaerobic microbial communities; they may possibly be applied in nanotechnological devices.

Improved industrial MFC will be used to produce electricity from almost any renewable material, including waste and hydrolysates of plant biomass. This technology is potentially more effective than production of ethanol fuel and methanogenesis; it therefore deserves more attention from both the scientific and the business community.

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