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MICROBIAL X CELLS – INNOVATIVE MULTIPURPOSE BIOELECTROCHEMICAL SYSTEMS

[1]Mario Mitov, [2]Yolina Hubenova
South-West University "Neofit Rilski", Blagoevgrad, Bulgaria
Plovdiv University "Paisii Hilendarski", Plovdiv, Bulgaria

Abstract. This paper reviews the state-of-the art of the bioelectrochemical systems (BESs), utilizing whole living cells as biocatalysts, which has been intensively developed during the last decade. The principles of operation, specificity and potential applications of different BESs, based on microbial fuel cells and microbial electrolysis cells, are summarized and discussed.

Keywords: bioelectrochemical systems, microbial fuel cells, microbial electrolysis cells, practical application

Principles and classification of Microbial X Cells

The bioelectrochemical systems (BESs), employing whole living organisms as biocatalysts, provide a perspective opportunity for realization of the modern concept "waste to energy". Although the concept for current production associated with microbial catabolism was proved over a century ago by Potter (1911), the real research interest to the BESs considerably increased during the last decade (Fig. 1).

All varieties of BESs developed till now share one common principle, based on the unique ability of specific microorganisms, called exoelectrogens, electricigens, or anode-respiring bacteria, to couple the catabolic oxidation of available biodegradable substrates with electrode (anode), used as an external electron acceptor. The electron transfer from the living cells to the electrode is carried out either directly through membrane-bound protein structures, such as pili, c-type cytochromes and filaments (Lovley, 2011), or using soluble electron shuttles as mediators for indirect electron transfer (Babanova et al., 2011).

While the microbial catabolic oxidation in the anode chamber is a shared principle for almost all BESs reactors, any reduction reaction can be realized in the cathode

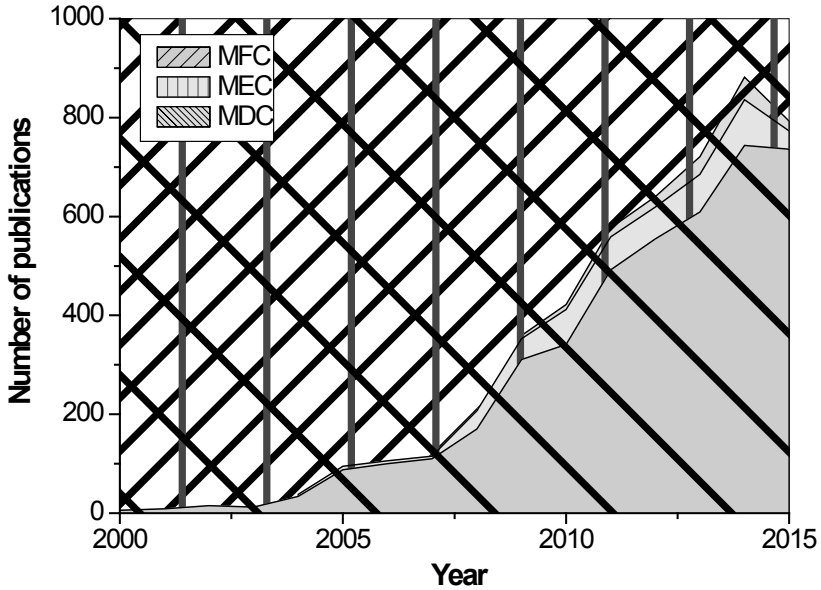

Fig. 1. Number of published papers, concerning the development of the microbial fuel cells, microbial electrolysis cells and microbial desalination cells during last fifteen years (Source: Scopus on 11.05.2015)

chamber, which creates numerous possibilities for different applications. Based on this, about 50 types of BESs with different functions and constructions have been developed mainly in the last decade. The different BESs are often summarized as MXCs, in which the X specifies the main function of a given cell. The large variety of MXCs was systemized for the first time by Wang & Ren (2013), who classified them in five main categories - microbial fuel cells (MFCs), microbial electrolysis cells (MECs), microbial remediation cells (MRCs), microbial electrosynthesis cells (MESCs) and microbial desalination cells (MDCs).

Microbial fuel cells

The microbial fuel cell is the very original type of BES, which main function is an electricity generation. The MFCs are devices similar to the other electrochemical power sources (non-rechargeable and rechargeable batteries, chemical fuel cells), in which the chemical energy stored in biodegradable substrates is converted directly into electrical energy. In contrary to traditional chemical fuel cells, which use chemical,

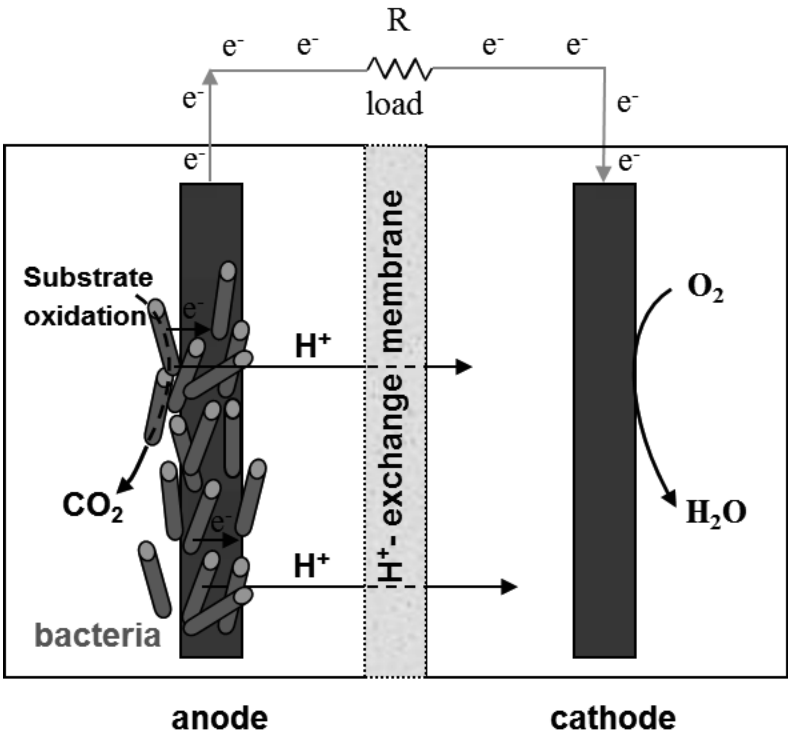

Fig. 2. Principle scheme of microbial fuel cell

predominantly precious metal catalysts, the MFCs utilize low-cost natural available microorganisms to oxidize a variety of electron donors, mainly waste materials, and to donate electrons to the anode (Fig. 2).

Till now, most of the investigations in the field of MFCs have been performed with bacteria (prokaryotes) and only a few papers have reported the utilization of yeast species as biocatalysts (Raghavulu et al., 2011; Prasad et al., 2007; Shkil et al., 2011; Haslett et al., 2011). Recently, we have discovered a new exoelectrogenic yeast strain *Candida melibiosica* 2491 (Hubenova & Mitov, 2010). Using improved construction and nanomodified anodes, we achieved the highest electrical outputs with mediatorless yeast-based biofuel cells until now (Hubenova et al., 2011). Moreover, we demonstrated for the first time that a part of the extracellular transferred electrons from the yeast cells to the anode originates from the aerobic respiration processes, taking

place in the mitochondria (Hubenova & Mitov, 2014a). On this basis, a new mechanism for the extracellular electron transport in the yeast-based biofuel cells was proposed (Hubenova & Mitov, 2015).

Depending on the specific application and construction, the developed MFCs are divided into several sub-categories. Based on different substrates used, there are wastewater MFCs, sediment (or benthic) MFCs, etc. (Liu et al., 2004; Reimers et al., 2001).

In order to evaluate the microbial activity or reactor configurations, in early MFC studies mostly simple substrates as glucose or acetate were used (Liu et al., 2005; Rabaey et al., 2003). The first MFC study that utilized real wastewater as a substrate was reported in 2004 (Liu et al., 2004). Since then numerous papers, reporting electricity generation from different waste streams, have been published (Pant et al., 2010).

Sediment microbial fuel cells

In 2001, the concept for harvesting energy from marine sediment-water interface, based on the principles of MFCs, was introduced (Reimers et al., 2001). In fact, the sediment microbial fuel cells (SMFCs) are devices, mimicking the processes by which exoelectrogenic bacteria, naturally occupying aquatic sediments or soils, couple the degradation of the organic matter with the reduction of available electron acceptors like Fe(III) and Mn(IV) oxides. The same bacteria are also capable of using the SMFC-anode, embedded in the anoxic sediment layer, as an alternative electron acceptor. The naturally occurring potential difference, created by the decreasing oxygen concentration gradient across the depth of water and sediment columns, eliminates the necessity of purging the anodic compartment with inert gas and the use of membrane to separate both compartments, thus simplifying the SMFC construction and reducing significantly the operational costs.

Because of the high salinity, resp. electrical conductivity of the seawater, most SMFCs have been explored in marine environments. However, the number of publications, reporting studies with freshwater SMFCs, has gradually grown recently (Song et al., 2011; Zhou et al., 2014). Applying the robust statistics methods, we evaluated for the first time the performance of large number identical freshwater SMFCs, operating for over 20 months at lab conditions (Mitov et al., 2015). The statistical evaluation of data has shown that the behavior of all SMFCs studied becomes homoscedastic after reaching a steady-state. The high repeatability and reproducibility of the SMFCs' performance has revealed the possibility for their practical application. For this purpose, several power management systems for boosting the output voltage to usable values were developed and multiple SMFCs, connected in series and in parallel, were

examined as autonomous power sources in order to determine the optimal operation mode (Bardarov et al., 2015).

Photosynthetic microbial fuel cells

Taking into consideration the abundance of solar energy reaching the Earth as well as the large variety of photosynthetic living organisms on the planet (Cho et al., 2008), the integration of MFC with photosynthetic processes is another promising strategy for sustainable electricity generation. Utilizing different photosynthetic organisms for solar energy capturing, several varieties of photosynthetic MFCs such as plant-MFCs, phototrophic-MFCs and algae-MFCs have been developed (Deng et al., 2012; He et al., 2009; Strik et al., 2011). The most popular among these BESs are the plant-MFCs, which concept is based on the mutualism between plants and soil microorganisms, in particular those located within plant roots. Harvesting the solar energy and the atmospheric CO_2, the plants produce carbohydrates, which partially are excreted by their roots. Thus available organic substances can be utilized by the microorganisms occupying the rhizosphere for their own growth and development. Placing the plant with its roots in the anode compartment of MFC similar as construction to sediment-MFCs, an electrical current can be generated. Analogous to the other MFC types, the current generation in plant-MFCs is connected with the natural electron-donating properties of exoelectrogenic bacteria, colonizing the rhyzosphere. Till now, a variety of plants as *Oryza sativa, Reed mannagras, Spartina anglica, Arundinella anomala, Dicranum montanum*, etc., were used in plant-MFCs (Helder et al., 2010; Hubenova & Mitov, 2011).

Several years after the proof-of-the principle of plant-MFC, we demonstrated for the first time that higher aquatic plants (duckweeds) can convert the solar energy directly into electricity without any contribution of microorganisms to the current generation (Hubenova & Mitov, 2012). Using a specially designed Direct Photosynthetic Plant Fuel Cell (DPPFC), we have established that the obtained electrical outputs depend on the applied illumination and temperature, thus proving the role of photosynthesis in the electricity generation. Parallel with the plant response to the day/night cycle, it has also been proved that the electricity generation is connected with a duckweed-produced endogenous mediator. Moreover, when grown under polarization conditions in DPPFC the plants intensify their metabolism towards enhanced production of reserve carbohydrates and proteins, which in a practical aspect can be applied as a new approach in the duckweed farming along with the current generation (Hubenova & Mitov, 2014b). Using *Lemna minuta* duckweeds as biocatalysts, we achieved a power density (380±19 mW/m^2) corresponding to 119.83±5.99 GJ/ha.year during DPPFC

operation under natural sunlight illumination, which is the highest reported value in the literature for plant-BESs (Bombelli et al., 2013).

Because of the lower electrical outputs (output voltage, current and power density) compare to the conventional power sources, more attention is recently paid to other possibilities for practical application of the MFCs instead of power generation. For example, if the main function of the system is to use the cathode to reduce oxidized contaminants, such as uranium, perchlorate or chlorinated solvents, the cell is usually referred to as a microbial remediation cell (MRC) (Butler et al., 2010; Gregory & Lovley, 2009).

Microbial desalination cells

A newly-developed technology, integrating the MFC processes and electrodialysis for wastewater treatment, water desalination and production of renewable energy, is the so-called microbial desalination cell (MDC) (Saeed et al., 2015). The basic principle of MDC, introduced by Cao et al. (2009), is to utilize the electrical potential generated

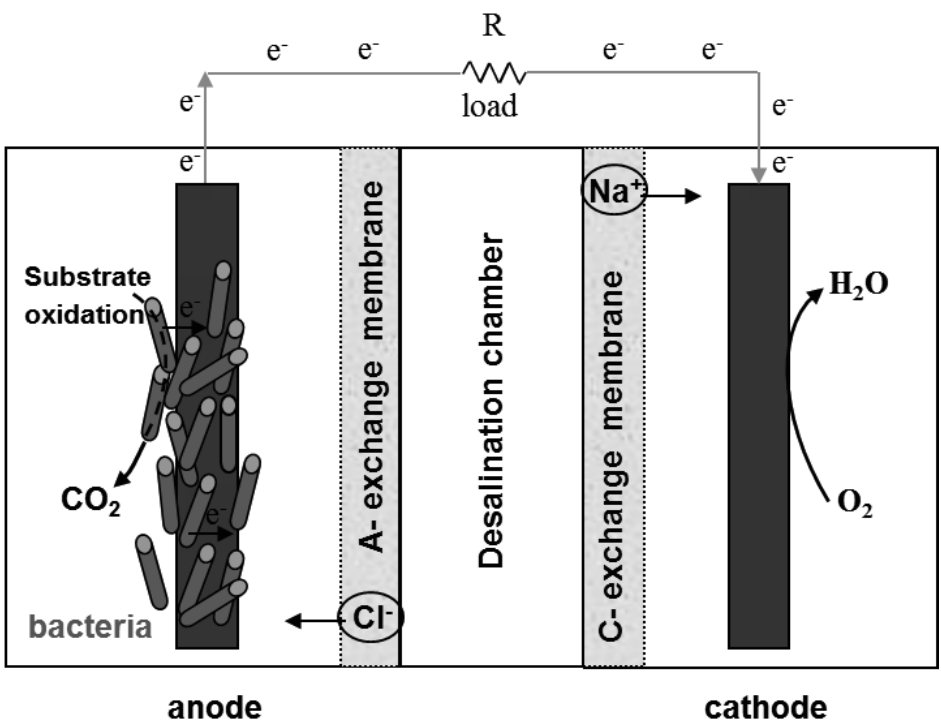

Fig. 3. Basic diagram of microbial desalination cell

across the anode and cathode to drive water desalination. Compare to the other MXCs, MDCs have a third chamber for desalination by inserting an anion exchange membrane (AEM) and a cation exchange membrane (CEM) in between the anode and cathode chambers (Fig. 3).

When bacteria in the anode chamber oxidize biodegradable substrates, generating electrons and protons, the anions in the desalination chamber migrate through the AEM to the anode, while the cations are driven through the CEM to the cathode chamber for charge balance, thus the middle chamber solution is desalinated. The MDC technology can either be used as a stand-alone process, or can be combined with other desalination processes, such as osmosis (OsMDC), capacitive deionization (cMDC), etc. Recently, several different modifications of MDCs have been developed including stacked-MDCs, biocathode-MDCs and recirculation-MDCs (Saeed et al., 2015).

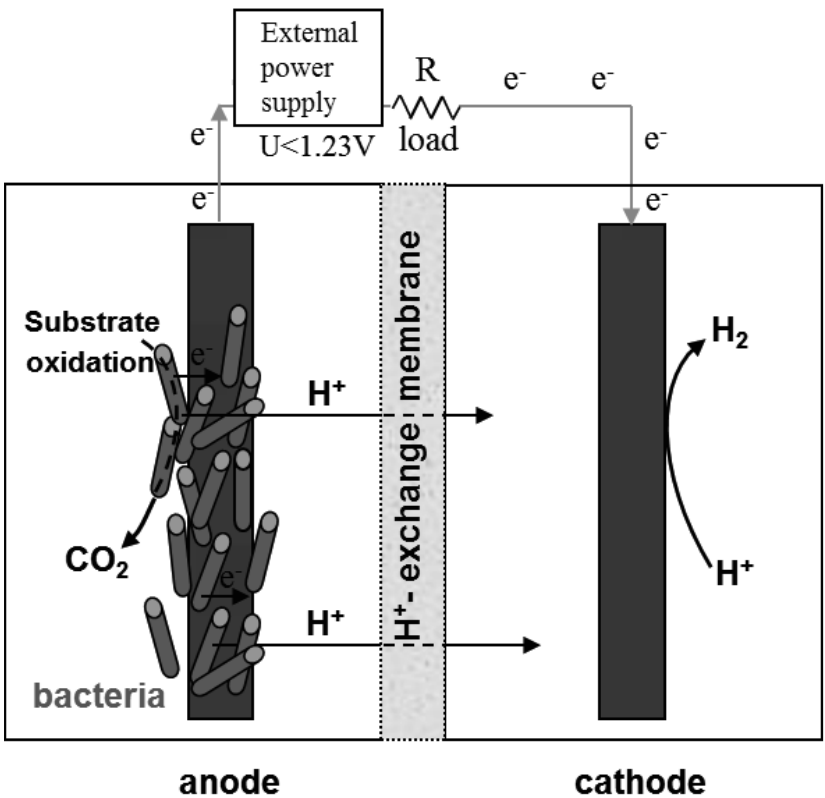

Fig. 4. Schematic diagram of microbial electrolysis cell

Microbial electrolysis cells

In contrary to the upper discussed MXCs, in which spontaneous electrochemical processes take place ($\Delta G<0$), if an external power source is connected to a MFC reactor to reduce the cathode potential, the system converts in a microbial electrolysis cell (MEC), where hydrogen gas or other valuable products can be generated (Fig. 4) (Ditzig et al., 2007; Logan et al., 2008; Rozendal et al., 2009).

The concept of MEC was proved for the first time in 2005 as an approach for hydrogen production from organic matter, including wastewater and other renewable resources (Liu et al., 2005; Rozendal et al., 2006). The main benefit of the microbial electrolysis is that the theoretical voltage needed to generate hydrogen at the cathode is only 0.11 V in comparison with 1.23 V for the water electrolysis. Although the overpotentials increase the required power supply, external voltage ranged from 0.4 to 1.0 V is usually applied, which is much lower than the 1.8–2.0 V used in traditional water electrolysis (Liu et al., 2005; Logan et al., 2008). Other advantages of MEC in respect to the hydrogen production are that renewable and waste materials can be used as substrates and the production rate can exceed 1 $m^3 H_2/day/m^3$ reactor with a yield up to 11 mol H_2/mol glucose, which is more than 3 times higher than dark fermentation (Liu et al., 2010). As far as the MECs use the same bioanodes as MFCs, the crucial challenge for their practical application as a hydrogen producing technology is to find cost effective cathodes for near-neutral pH and ambient temperature, required by the utilization of microorganisms. Following this goal, we have developed numerous novel materials and explored them as electrocatalysts for the hydrogen evolution reaction in neutral electrolytes (Chorbadzhiyska et al., 2013; Chorbadzhiyska et al., 2015; Mitov et al., 2012). Several Ni-based catalysts show a promising performance and have been tested as cathodes in a single-chamber MEC, applying an active sludge from municipality wastewater treatment plant as a biocatalyst.

Except hydrogen, the production of other valuable inorganic chemicals such as hydrogen peroxide, caustic soda, etc., in the cathode chamber of MEC reactors has been also demonstrated (Rozendal et al., 2009; Rabaey et al., 2010).

Another technology, closely related to the MEC, is the microbial electrosynthesis, which concept, introduced in 2009 (Cheng et al., 2009), is based on the use of electrons derived from the cathode to reduce carbon dioxide and other chemicals into a variety of organic compounds (Rabaey & Rozendal, 2010; Rabaey et al., 2011). It has been found that some acetogenic bacteria such as *Clostridium aceticum*, *Moorella thermoacetica*, etc., are able to consume electrical current and produce organic acids (Nevin et al., 2011). A mixed culture from brewery wastewater was reported to generate methane, acetate and hydrogen on a biocathode with addition of CO_2 as the only carbon source (Marshall et al., 2012).

Closing remarks

Despite the remarkable progress in the last decade, most of the introduced MXCs are in an infancy stage of development far from a practical application. In general, the scale-up of technology to usable electrical outputs and production rates is still a challenge. Just a few pilot-scale tests worldwide have been reported till now (Logan, 2010). The first large-scale MFC reactor (1 m^3) has been operated at Foster's brewery in Queensland, Australia. Hydrogen production by microbial electrolysis of winery wastewater has been demonstrated at the Napa Wine Company, Oakville, USA. The robustness of MXCs at long-term operation is also disputable. Several papers, however, have reported over a year stable operation of sediment-MFCs in different environments, which reveals the potential applications of SMFCs as sustainable power sources for electronic devices or sensors, operating in remote areas (Bardarov et al., 2013; Tender et al., 2008). A SMFC-based system for eco-monitoring of aquatic basins is currently under development by our research team.

Despite the complex challenges that should be overcome, we do believe that the Microbial X Cells possess a great potential to become a competitive multipurpose technology for electricity generation, biosynthesis and bioremediation.

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✉ **Prof. Dr. Mario Mitov** (corresponding author)
Department of Chemistry
South-West University "Neofit Rilski",
66, Iban Michailov Str.,
2700 Blagoevgrad, Bulgaria
E-mail: mitovmario@mail.bg