Chapter

Bioelectrochemical Processes in Industrial Biotechnology

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Abstract

Industrial fermentation and biological wastewater treatment are usually based on redox processes taking place in living cells and on enzyme processes. The practical application of these redox processes is usually associated with electricity generation in microbial fuel cells or process enhancement in microbial electrolysis cells. The microbial fuel cell approach leads to straightforward wastewater treatment with less energy demand. Additional advantages of these processes are the direct removal of various pollutants and the avoidance of addition of chemical agents with the resulting waste products of treatment as it is familiar with the traditional chemical methods. Another option for the use of bioelectrochemical processes in practice is the approach of microbial electrolysis cells. The application of electric field on fermentation or microbial wastewater treatment processes might result in different aspects: either in purely electrochemical processes on the electrodes or in different types of bioelectrochemical stimulation of enzyme activity in the living cells. These applications are associated with the combination of enzyme activity with electrochemical processes to produce or remove certain compounds rapidly at high concentrations with no additions of other chemicals. In the present chapter, both approaches (microbial fuel cells and microbial electrolysis cells) are presented and discussed. Some practical applications and experimental examples of such bioelectrochemical redox processes stimulated by constant electric field are demonstrated.

Keywords: redox systems, microbial fuel cells, microbial electrolysis cells, bioelectrochemical oxidation, bioelectrochemical reduction

1. Introduction

The depletion of the traditional energy resources together with the environmental problems caused by the excessive use of fossil fuels and the resulting emissions of greenhouse gases have prompted humanity to replace fossil fuels, at least partially, by renewable energy sources. Besides the well-known hydropower, wind power and solar energy as well as utilization of biomass, there is another option to remedy the problem of energy demand and the resulting pollution. It is based on bioelectrochemical processes.

During the last decades the concept of microbial fuel cells (MFC) is a subject of significant scientific interest [1–4]. This idea seems to be very attractive, because it offers double benefit: first, to generate electricity without air pollution and second, to carry out wastewater treatment to clean water ponds with considerably reduced

energy consumption [3, 4]. Although the electric power density for these devices is rather modest, the generated energy might reduce the energy consumption of a wastewater treatment plant compared to the traditional ones and to open the way to further improvements.

Another option for bioelectrochemical applications in wastewater treatment and industrial biotechnology is the stimulation of microbial redox processes by electric field.

The present work proposes an overview on the principles, achievements and future trends in these two fields of scientific and practical activity: energy generation by microbial fuel cells and enhanced processes in biotechnology and wastewater treatment by microbial electrolysis cells (MEC).

2. Microbial fuel cells

The principle of operation for microbial fuel cells is similar to the one for the traditional fuel cells, cf. **Figure 1**. There are fuels, i.e. reducers, and oxidant, usually oxygen or air. A typical feature of the microbial fuel cells is that, they operate at ambient temperature with a little external energy input to maintain the redox process. The compartment the microbes are placed in depends on their role in the overall process.

In general, the electrochemical reactions taking place on the electrodes are:

On the cathode:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \tag{1}$$

On the anode:

$$\text{Reducers (represented as chemical oxygen demand, COD)} - 4e^- - 4H^+ \rightarrow \text{oxidation products.} \tag{2}$$

Of course, the electron acceptor could be different, e.g. sulfate, nitrate, etc. Thus the beneficial effect could be multiple – cleaning wastewater from excessive COD and removing nitrate from surface or industrial water, for example.

The protons necessary for the cathode reaction are transferred from the anodic space to the cathodic one through a separation proton exchanging membrane (PEM).

Electromotive force ΔU is generated as a result of the two electrode reactions. Any reducer can be used or treated by this method: starting with heavy metal cations to organic substances.

Typical characteristics of the fuel cell are the polarization curves of the dependence of the fuel cell electromotive force on the current density. An example of such polarization curve for any fuel cell is shown in **Figure 2**.

Besides the linear Ohmic range depending on the fuel cell conductivity, there is a sharp drop in voltage at low current densities, associated with the overpotential for overcoming energy barrier (reaction rate losses) [5]. At higher current densities the non-linear course of the curve is due to mass transfer limitations [5]. The polarization curves give information about the difficulties to be overcome. Moreover it is evident, that the losses are bigger for the cathode processes compared to the anodic space and the membrane resistance.

When the target process is removal of reducers, the conditions in the anode space of the MFC must be anaerobic to avoid competitive oxidation of the substrates in the bulk. This is one of the reasons why the MFC efficiencies are rather

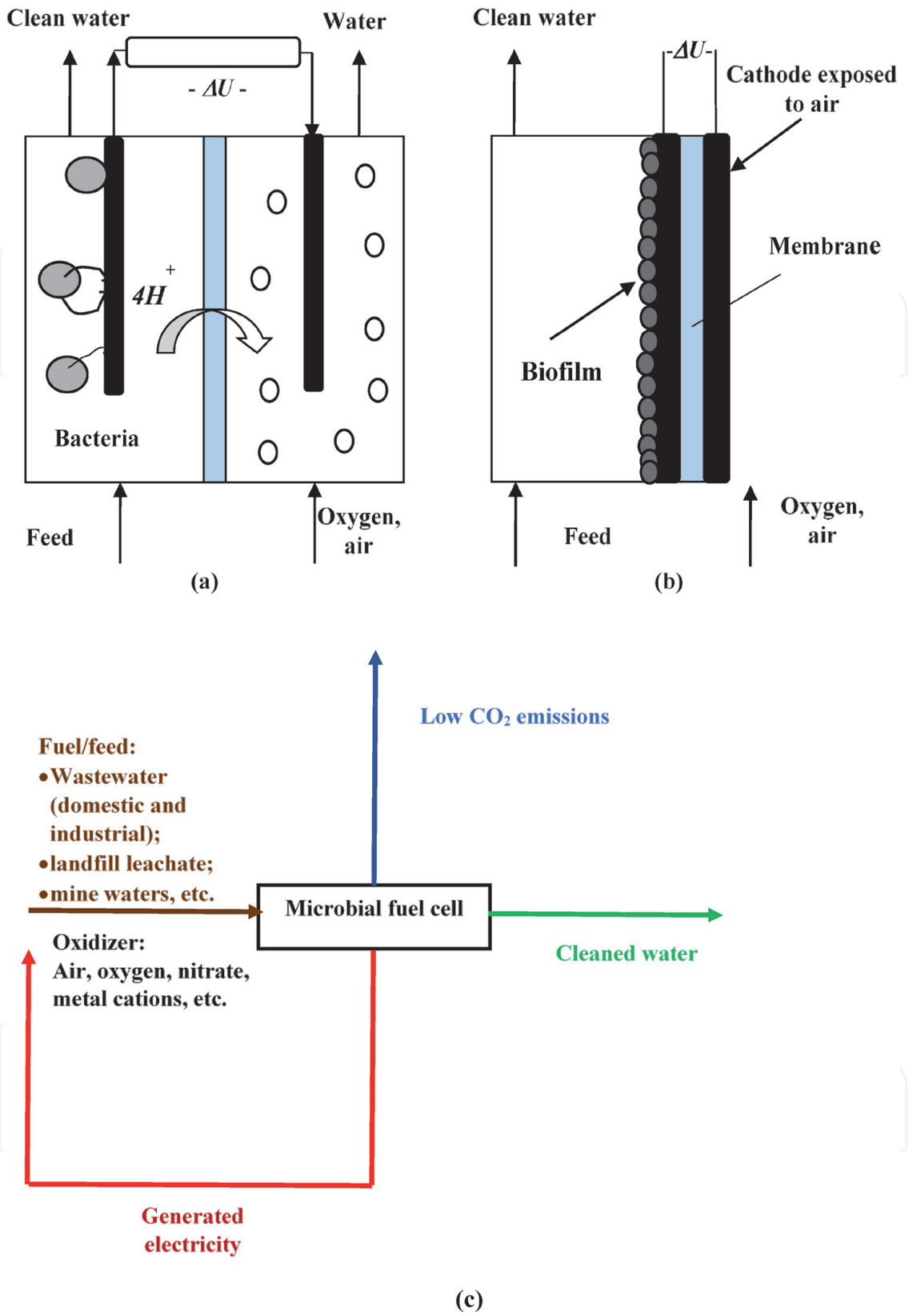

Figure 1.
A general drawing of a microbial fuel cell with oxygen as electron acceptor. Two-chamber set with solution-fed cathode (a); one-chamber set (b). Sketch of the MFC application mode (c).

low. Therefore enhancement of the electron transfer to the electrode is required. It is accomplished by different means: self-produced mediators by the living cells, artificial ones and by direct electron transfer to the anode [6, 7]. It is important to select bacteria that can directly transport electrons outside the cells, so-called "exoelectrogens", cf. Logan et al. [8].

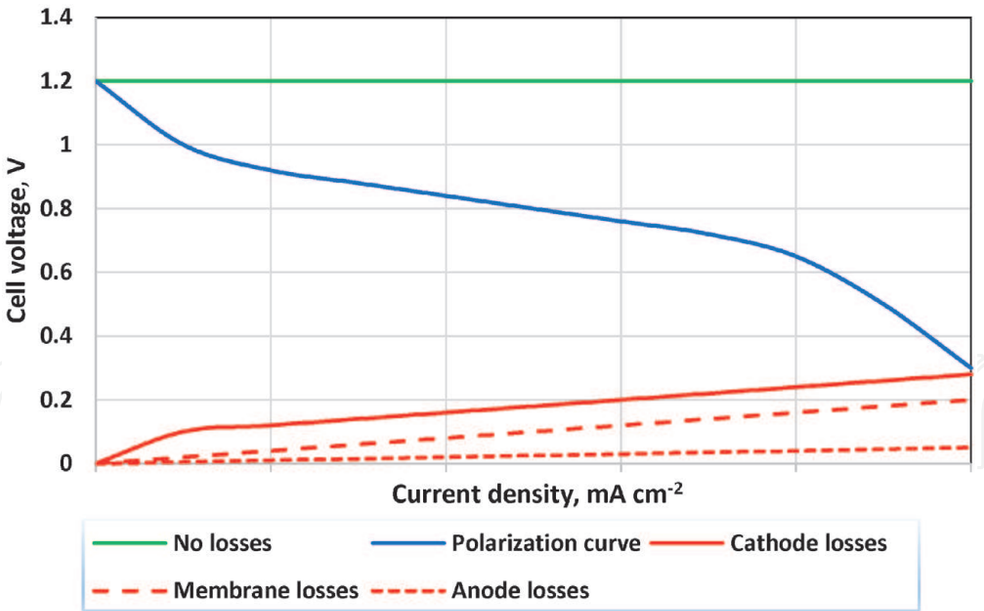

Figure 2.
Typical polarization curve of fuel cell operating in gaseous phase.

Self-produced mediators, e.g. pyocyanin can shuttle electrons and produce electricity. This method was first proposed as a mechanism for electron transfer to Fe^{3+} by Rabaey *et al*. in 2005 [9]. The major advantage of self-produced electron mediators is the long-range interaction between the bacteria and the anode. The microorganisms do not need to be in direct contact with the electrode for the transfer of electrons to occur.

Artificial mediators can cross cell membranes and accept electrons, leaving the cell in a reduced form to transfer the electrons to the electrode [10]. Examples of such artificial mediators are the dye neutral red, iron chelates, various phenazines, 4-napthoquinone, and thionine [9]. There are studies proposing other artificial mediators for electron transfer [11, 12].

In general, there are two basic types of microbial fuel cells depending on the state of the microorganisms: with free microbial cells or with ones attached to solid support and to one of the electrodes as well. The latter approach is preferable for continuous operation also for easier electron transfer toward the electrodes.

Direct electron transfer can occur through direct contact between the microorganisms and the electrode or with the use of nanowires. Direct contact requires that the organisms have membrane bound electron transport protein relays, such as cytochromes, to facilitate the transfer of electrons out of the cell [13]. However, this transfer allows for only one layer of bacteria in direct contact with the electrode. There are bacteria with appendages called nanowires. These appendages that are supposed to carry electrons from the bacterial cell to the surface of the anode. These appendages allow for multiple layers of bacteria on the anode to transfer electrons as well as interspecies transfer and transfer from the bulk liquid to the anode [7]. Such bacteria are *Shewanella oneidensis, Synechocystis* strain PCC 6803, *Geobacter sulfurreducens,* etc. [3, 14]. Nanowires can serve as cell connections of bacteria in a biofilm and can facilitate the transfer of electrons from the outer layers of a biofilm to the electrode [15]. The lack of nanowires may result in decrease of current production by 70% [16].

All these features of electron transfer to the anode accompanied by competitive bulk reactions and fermentation processes inside the bacteria lead to low current and power efficiencies. Moreover, certain overpotentials due to limitations of electron transfer described above are possible.

Different substrates are tested for treatment by microbial fuel cells: lignocellulose materials and waste, landfill leachate, manure, low-grade carbohydrates, starch, domestic wastewater, etc. [17]. There are studies on biodegradation of hydrocarbons, including aromatic, polycyclic and heterocyclic ones by MFC [18, 19].

In some studies sulfate [20–22] or nitrate [23–26] was used as electron acceptor in the cathodic compartment, thus cleaning the water from these pollutants.

There are papers studying the reduction of sulfate to sulfide to remove the cations of heavy metals (copper, lead, zinc, etc.) as insoluble sulfide from mining drainage water [27, 28].

Proper cell design, electrodes, substrates, proton exchange membranes and bacterial species forming biofilms on the electrode are very important to microbial fuel cells [3, 7, 14, 16]. MFC requires selection of appropriate electrode materials with high electric conductivity and good adhesive properties at the same time [29–31].

Another important goal of the electrode composition is the electro-catalytic activity, attained by doping with target catalysts. The energy generation of MFC is influenced by many factors, including the type of electrode material [30–32]. Studies on this subject were made by Hubenova et al. [12] proposing styryl-quinolinum dye as molecular electrocatalyst and Mitov et al. [33], who studied nickel-doped cathode. Another study was dedicated to nano-modified NiFe-and NiFeP-carbon felt as anode electrocatalysts in yeast-biofuel cell [34]. There are also works on the use of Pt-coated carbon felt, cf. Park and Zeikus [30] and graphite electrode in a brush-like shape, cf. Logan et al. [35].

As can be seen in **Figure 2**, the cathode losses in cell voltage are the biggest. There are some efforts to minimize, if not to avoid, those losses. One reason is the limited mass transfer rate of oxygen in dual chamber fuel cell in liquid phase, cf. **Figure 1**. Therefore, the direct contact of oxygen molecules with the cathode is proposed in a single chamber fuel cell, cf. Liu et al. [36, 37]. Another approach is to replace oxygen by ferric or copper cations in aqueous solution, proposed by ter Heijne [38]. Power densities reaching 2 W m^{-2} were attained.

There are different microbial strains used for MFC. The most frequently used ones are from the genera *Geobacter* [16], *Shewanella* [3, 30], *Desulfovibrio* [39, 40], *Pseudomonas* [8, 41, 42], as well as *Clostridium* [43, 44], etc.

2.1 Microbial fuel cells for wastewater treatment (COD removal)

The most widespread application of microbial fuel cells is wastewater treatment, either of municipal wastewater or industrial ones. The pollutants, represented as chemical oxygen demand (COD) are used as a fuel, feeding the anode compartment of the fuel cell. Electromotive force is generated in the process of indirect oxidation and the generated electric energy can be used for maintenance of the wastewater treatment facility.

The MFCs were considered to be used for treating wastewater early in 1991 [45]. The power generated by MFCs in the wastewater treatment process can potentially halve the electricity needed in a conventional treatment process for aerating activated sludge. Later it was proposed to treat the wastewater in anaerobic digester to produce biogas and to yield volatile fatty acids, e.g. acetic, propionic and butyric. These acids feed the MFC to yield CO_2 and H_2O [46, 47].

Wastewater from breweries has been a favorite among researchers as a substrate in MFCs, primarily. The range of 3000–5000 mg of COD/dm^3 is typical for wastewater from breweries and it is approximately 10 times more concentrated than in domestic wastewater [48]. It could also be an ideal substrate for MFCs due to its high carbohydrate content and low ammonium nitrogen concentration [48]. Starch

processing wastewater contains a relatively high content of carbohydrates, which can be potentially converted to useful products [43, 49].

The most spread application of microbial fuel cells is for treatment of domestic wastewater. It is comprehensively considered by Muñoz-Cupa et al. [50]. Considerable attention was paid to the treatment of landfill leachates resulting from the trickling rainwater through the waste layers in a landfill. For example, 242.3 Mt. of municipal solid waste was produced in the European Union in 2015, 62 Mt. of which were discarded in landfills [51]. Landfill leachate usually contain dissolved organic matter, inorganic macrocomponents, heavy metals, and xenobiotic compounds. These wastewater are associated with high organic load of up to 152000 mg COD/dm^3 [52]. Such efforts have been made by Kjeldsen et al. [52], Galvez et al. [53] and Damiano [14]. It was reported that COD removal efficiency can reach more than 80%, with low power density (up to 125 W m^{-3}), cf. [50]. The energy output related to the destroyed COD is less than 0.04 kWh/kg COD. The Coulombic efficiency according to Faraday's law is very low, i.e. less than 15% [51]. However, the energy consumption for biodegradation of 1 kg COD in a traditional wastewater treatment facility is around 0.11 kWh/kg COD. It means that about 40% of the required energy can be supplied to the very process by MFC. The next benefit of MFC is the lower energy consumption for the substrate feed compared to the traditional methods where aeration and mixing are necessary.

There is a paper, reporting relatively high power densities (up to 10 W m^{-3}) in MFC after treatment of food leachate by gradual increase of pH in the feed [54]. At certain pH-values within the range of 6.3 and 7.6 the Coulombic efficiency reached 63%.

A combination of photochemical bioreactor and a fuel cell assembly for dairy waste removal was reported by Bolognesi et al. [55]. The Coulombic efficiencies were up to 21% with power density of 2.8 W m^{-3}.

The reported Coulombic efficiencies for MFC vary within a wide range (from 1 to 85%), depending on different conditions studied. The best current efficiencies found in the literature are for MFC operating with 0.4% sucrose solutions (between 71 and 85%) [56].

As can be seen, the power output and the current efficiency derived at MFC operation for wastewater treatment vary considerably depending on the operating conditions: type of substrate, microbes used (single cultures or consortia), cell immobilization, fuel cell construction, etc.

However, it is important to note, that the energy, consumed for MFC operation for this purpose is much less than the one for traditional aerated equipment, irrespective of the low Coulombic efficiency. Hence, the benefits of MFC use in these cases are multiple: first, it is the wastewater treatment and second, energy saving, avoiding the expensive mixing and aeration, with the use of simpler and cheaper equipment.

2.2 Microbial fuel cells with sulfate reduction

Another opportunity to use the MFC technique is to reduce sulfate ions in mine wastewater. They are usually acidic due to high sulfate content and they contain different heavy metals (copper, zinc, iron). Although they are in huge amounts their concentrations are low which make their chemical removal unfeasible from energy point of view. The large flow rates of the mine waters are an additional difficulty because large facilities are needed for water treatment with large energy demand. It is interesting to use the fuel cell technique to reduce sulfate as oxidizer instead of oxygen thus reducing them to sulfide [20–22]. Then the cations of heavy metals will be easily deposited as insoluble sulfides [27, 28, 57–59]. There is double

effect – removal of heavy metals and sulfate and adjustment of pH for the drained water..The most studied sulfate-reducing bacteria are from the genera *Desulfovibrio, Desulfobacter, Desulfococcus,* etc. [28, 39, 40, 57].

The increase in electricity production in MFCs requires to optimize the inhibition of the metabolic pathways, which compete with electricity production [25].

The presence of sulfate and sulfide ions in anaerobic reactors hampers the growth of methanogenic archaea and justifies the use of sulfate and sulfate-reducing bacteria in the anodic space of MFC. It is important that the sulfate-reducing bacteria are able to transfer directly electrons to solid electrodes, thus reducing the competitive bulk redox processes in the anodic space. This technology associates the removal of both sulfate and the chemical oxygen demand (COD) with the production of electricity [22].

2.3 Microbial fuel cells with nitrate reduction

Nitrogen containing ions such as nitrate and nitrite occur widely in different process streams, coming from the extensive use of fertilizers in agriculture or production of explosives. The main problem is the reduction of nitrate to nitrite, both being toxic because of formation of methemoglobin [59] and carcinogenic N-nitrosamines [60] from nitrite.

The MFC with nitrate reduction differ, as all MFC, by the process involved on the other electrode, the bacterial culture used, and the construction of the cell. In most cases wastewater characterized by COD and nitrate polluted fluxes are treated. Many research efforts are directed to simultaneous nitrification and denitrification processes [23–25].

Systems working with a combination of sulfide and nitrate contaminated fluxes in the anode and cathode space of the fuel cell respectively are interesting for research [61–64] because of the simultaneous removal of two pollutants. Here there can be also nitrification and denitrification processes or only denitrification.

The following biochemical pathway of consecutive steps of nitrate-to-nitrite conversion and nitrite reduction to gaseous nitrogen found [65]:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO(g) \rightarrow N_2O(g) \rightarrow N_2(g) \quad (3)$$

Denitrifying microorganisms are generally facultative anaerobes that could utilize chemically bound oxygen under anoxic conditions, e.g. in the nitrate ion, serving as electron acceptor instead of free oxygen.

It was pointed out by Wang et al. [65] that the microbial communities for denitrification are *Comamonadaceae* phylotypes and that the nitrification process could be explained by the high predominance of ammonia-oxidizing bacteria as *Nitrosomonas.* The existence of other potential heterotrophic denitrifiers as *Burkholderiadaceae, Alcaligenaceae* is also confirmed.

Pseudomonas denitrificans was used in [63, 65].

Pseudomonas sp. C27 is applied a pure culture, an autotrophic denitrifier, using sulfide as the only electron donor in a two-chambered MFC [64].

A technology that is particularly useful for nitrogen removal from wastewaters with low COD/N ratios is proposed in [23] to achieve simultaneous carbon and nitrogen removal from a single wastewater stream. For this purpose, the effluent from an acetate supplied MFC anode is directed to an aerobic stage for ammonium oxidation to nitrate. This stream is subsequently fed to the cathode of the MFC for denitrification. The removal rates are up to 2 kg COD m^{-3} day^{-1} and 0.41 kg NO_3-N m^{-3} day^{-1} with maximum power output of 34.6 W m^{-3} and a maximum current of 133 A m^{-3}.

In [25] a single chamber microbial fuel cell with a rotating biocathode was developed to remove simultaneously chemical oxygen demand (COD) and nitrogen with electric current generation. Carbon felt, on which exoelectrogenic bacteria grew, was used as the anode on the bottom and disks of the same material are the rotating cathode on the top. Under continuous regime with a feeding COD/N ratio of 5:1 the removal efficiencies of total organic carbon (TOC) and total nitrogen (TN) were 85.7 ± 7.4% and 91.5 ± 7.2%, respectively, and maximum power output of 585 mW m^{-3}.

In [63] a MFC that consists of two concentric cylindric compartments with effective volume of 300 ml each was investigated (**Figure 3**). The separating membrane was placed on the bottom of the inner cylinder. Activated and pyrolized paddling was used as anode. Granulated activated carbon was added in the cathode compartment in order to increase the electrode surface. The activated carbon was also used for immobilization of *Pseudomonas denitrificans (NBIMCC 1625*). The results for the power generation in this construction are shown in **Figure 4**. The results are better for lower concentrations of sulfide and nitrate because of the inhibition on the bacterial activity at higher sulfate and nitrite concentrations.

The wetland fuel cells are an interesting solution for low cost wastewater treatment. Ge et al. [66] reported for simultaneous nitrate and phosphorus removal in a pyrite-based wetland-microbial fuel cell.

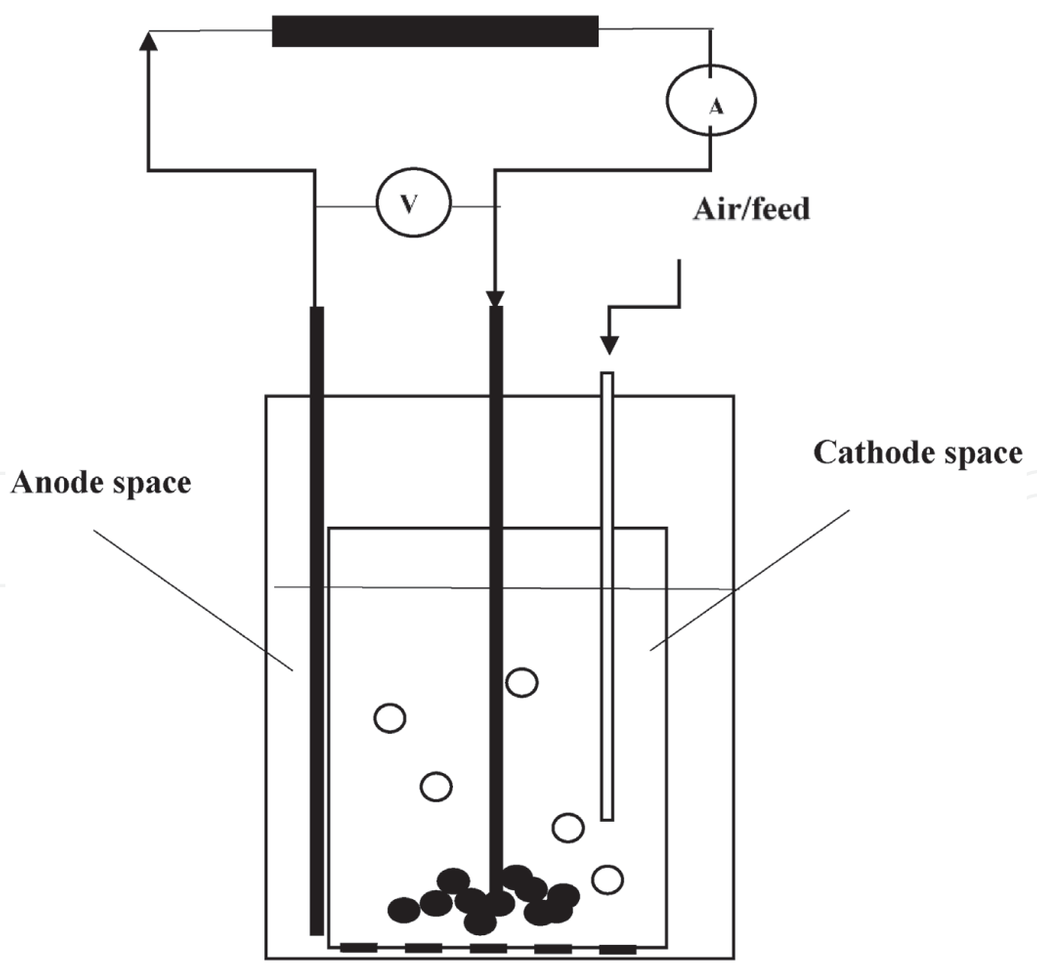

Figure 3.
Drawing of microbial fuel cell with cylindrical assembly of anode and cathode compartments.

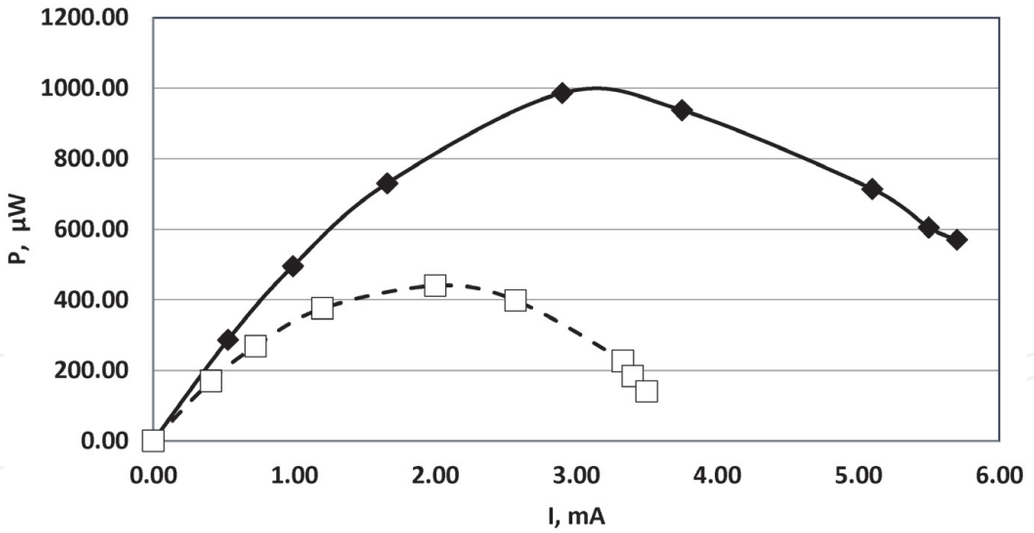

Figure 4.
Profile for power vs. current in cylindrical microbial fuel cell operating with sulfide and nitrate anions. (◆) – 3.5 mM sulfide, 3.5 mM nitrate; (□) – 15.6 mM sulfide, 8 mM nitrate.

3. Microbial electrolysis processes

There is a further development of the concept of microbial fuel cells. The aim is to treat wastewater by electrolysis facilitated by microbial process in the anodic space of electrolyzer with simultaneous production of hydrogen on the cathode [67–71].

Bioelectrochemical systems (BESs) can be regarded as electrochemical systems, in which at least one of the electrode reactions involves electrochemical interactions with electroactive bacteria. Most frequently it is the anodic reaction that requires the presence of certain microorganisms, yielding electrons from a biodegradable substrate to the anode. Some examples for such processes will be described briefly here.

3.1 Hydrogen production in microbial electrolysis cells

In contrast to the electrolytic production of hydrogen MEC require a small additional input of external electrical energy to facilitate hydrogen formation on the cathode [68–70]. The principle of MEC is similar to the traditional electrolysis ones but microbes grow on organic substrate in the anode compartment. Due to vital activity of the bacteria, chemical energy from organic matter in the wastewater is converted into electrical energy. The microorganisms used in MFCs are also applicable to MEC systems due to their similar anodic process [69].

There are papers, claiming to destroy landfill leachate and to produce hydrogen at the same time [71] and the COD removal efficiency is reported to reach 73%, whereas the hydrogen yield was up to 95%.

The voltage produced by the electroactive bacteria is not sufficient for water splitting and hydrogen release. That is why for microbial electrolysis a certain external voltage is applied. For the case of bacterially assisted microbial electrolysis cell (MEC) the hydrogen production increases with the applied voltage (i.e., 0.6–1.0 V) [72], which is much less than the theoretical voltage for abiotic water electrolysis (1.229 V).

3.2 Wastewater treatment by MEC

There are some studies on biological treatment of wastewater, containing recalcitrant organic pollutants, facilitated by electrolysis. There are also studies on the effect of Fenton pre-treatment on the performance of MEC in landfill leachate treatment [73] and the effect of co-substrate [52].

There is also an ambitious project to utilize the energy generated in MFC to treat wastewater in microbial electrolysis [74]. In [74] microbial consortium in activated sludge is used and it is coupled in parallel with MFCs for landfill leachate treatment for power generation. In this paper a 38% efficiency of COD and 90% of ammonia removal are attained. Although the current and power densities are low, this approach might save energy for wastewater treatment. The low voltage efficiencies can be overcome by coupling the MFCs in parallel or consecutively.

3.3 Microbial processes stimulated by constant electric fields

These processes are similar to the concept of MEC, but with the opposite aim. Whereas in the case of hydrogen production microbes facilitate electrolysis making it feasible in terms of energy, here the electric energy facilitates the natural redox processes in fermentation, thus reducing the energy consumption compared to aeration and mixing. Additional advantage could be the electrochemical removal of intermediates inhibiting the overall fermentation process. Some examples of this approach are described below.

3.3.1 Sorbitol-to-sorbose biotransformation

This biotransformation is a step in the traditional technology for L-ascorbic acid production (vitamin C) accomplished with the strain *Gluconobacter oxydans*. It is an aerobic process requiring high aeration rate and energy consumption. There was an effort to minimize energy consumption, applying constant electric field in the fermentation broth [75]. The results were positive: the constant electric field could replace partially intensive aeration and mixing, cf. **Figure 5**. The apparent volumetric mass transfer coefficient k_La was estimated as 6.5 h^{-1}, whereas the attained value at aeration and mixing is about 33 h^{-1}. It is visible from **Figure 5** that the most

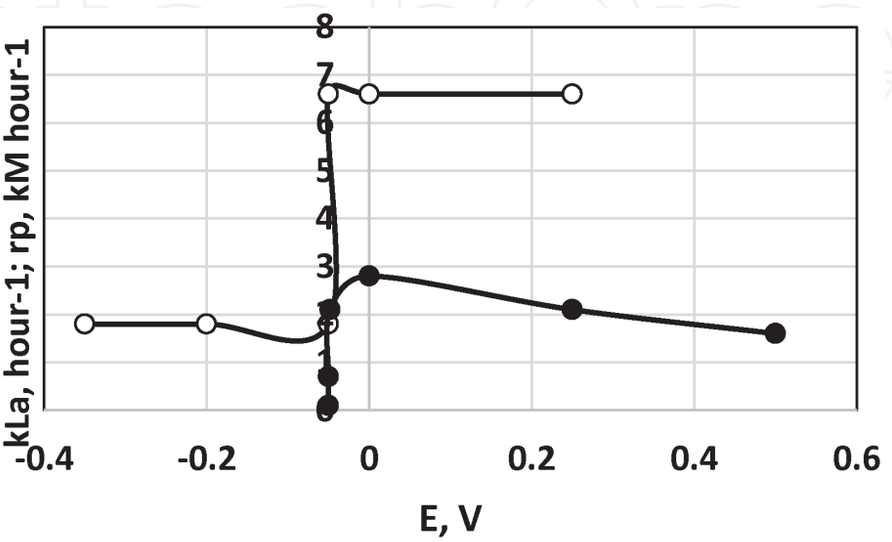

Figure 5.
Dependence of the production rate r_p (•) and the apparent volumetric mass transfer coefficient k_La (o). Initial sorbitol concentration 100 g dm^{-3}.

strongly manisfested effect was around a certain value of anode potential (i.e. – 0.05 V/S.H.E.), following the well-known "windows theory" according to which physical effects (like electric potential, current, temperature, etc.) have maximum impact on biological processes within a certain value range, like in an open window [76]. Calculations of Coulombic efficiency compared to the results of chemical analyses showed that the observed effect cannot be explained by purely electrochemical processes. The current efficiencies calculated by Faraday law were three orders of magnitude less than the expected ones by the chemical analyses. It was explained that the constant electric field affected the active sites of the enzyme sorbitol dehydrogenase. The optimum potential coincides with the standard potential of the co-enzyme couple $NAD^+/NADH^+$ (nicotinamide adenine dinucleotide), allowing speculation about a co-enzyme regeneration. However, this explanation is not convincible because of the very low measured Coulombic efficiency. Similar "window" effect was observed for the same biotransformation but in galvanostatic mode [77].

3.3.2 Biodegradation of 1,2-dichloroethane

This xenobiotic is frequently used in present practice as solvent, precursor for vinyl chloride and polyvinyl chloride production. Its biodegradation is usually accomplished by *Xanthobacter* strains, according to the following oxidative pathway [78]:

$$\underset{\text{1, 2-dichloroethane}}{CH_2Cl-CH_2Cl} \rightarrow \underset{\text{1-chloroethanol}}{H_2CCl-CH_2OH} \rightarrow \underset{\text{chloroactealdehyde}}{H_2CCl-CHO} \rightarrow \underset{\text{chloroacetate}}{H_2CCl-COO^-} \rightarrow \underset{\text{glycolate}}{CH_2OH-COO^-} \quad (4)$$

The ability of microbes to degrade this pollutant is limited at higher concentrations because of substrate inhibition and the accumulation of inhibitory intermediates, i.e. 1-chloroethanol, 1-chloroacetaldehyde and monochloroacetic acid. The idea was to enhance the intermediate reactions of oxidation and hence to remove the possible inhibitors as much as possible. Therefore, constant electric field for anodic oxidation was applied [79].

Experimental results on chloride release with and without application of electric field are shown in **Figure 6**. The results for the reference experiment with no electric field showed, that at higher substrate concentrations only one chlorine atom was released i.e. complete dechlorination was not attained. This fact corresponds to

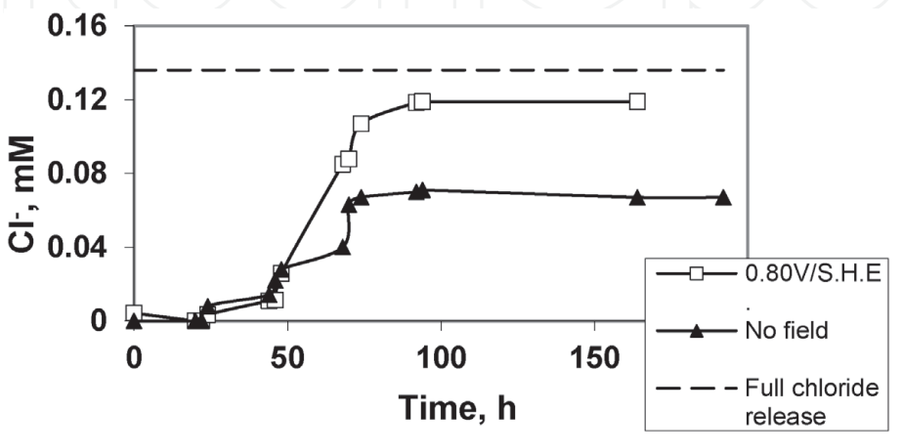

Figure 6.
Time profiles for chloride release for reference experiment and at constant electric field. Initial 1,2-DCE concentration 0.136 M.

the detected accumulation of 1-chloroethanol in the broth. When constant electric field was applied the chloride release was practically complete. In this case 1-chloroethanol was not detected in the broth. The optimum anode potential was 0.80 V/S.H.E. Then, a practically complete biodegradation took place even at high initial 1,2-DCE concentrations, up to 0.14 g dm^{-3}. At higher concentrations, e.g. 0.2 g dm^{-3}, only one chlorine atom was released, even when electric current was applied, possibly due to the dominating inhibition effects.

It was also observed that the effect of the electric field was not associated only with electrochemical process because of the very low Coulombic efficiencies, calculated from the electric current, being microampere-hours of the order of magnitude [80].

3.3.3 Phenol biodegradation

Phenol and its derivatives are considered as some of the most dangerous organic pollutants released into the environment due to human activity. Various methods have been applied to reduce its concentration in the waste streams to acceptable and harmless levels. In microbial degradation of phenol under aerobic conditions, the process is initiated by oxygenation, in which the aromatic ring is initially monohydroxylated at an *ortho*-position to the pre-existing hydroxyl group to form catechol by a monooxygenase phenol hydroxylase. Further, catechol is oxidized with *o*-benzoquinone formation [80] or to a cleavage of the benzene ring.

Depending on the type of strain, the catechol can undergo a ring cleavage that can occur either at *ortho*-position or at *meta*-position thus initiating the *meta*-pathway that leads to the formation of pyruvate and acetaldehyde, as suggested by Sridevi et al. [81].

The effects of constant electric fields on phenol biodegradation were studied by Ailijiang et al. [82], Dehghani et al. [83], Zhou et al. [84] and Beschkov et al. [85]. It was found in [85] that there is an optimum anode potential at which the specific bacterial growth rate for the strain *Pseudomonas putida* and its biodegrading ability are higher at anode potential of 0.8 V/S.H.E. corresponding to the standard redox potential for phenol oxidation [85]. The tests on enzyme activities for phenol hydrolase and 1,2-catechol dioxygenase showed that they are the best at the same potential. These data showed that only the *ortho*-pathway of benzene ring cleavage takes place. Still it appears that the effect of constant electric field is tends to be due to an effect on the enzyme activities rather than to electrochemical oxidation on the anode.

3.3.4 Bacterial denitrification

The essence of electrolytic stimulation of microbial degradation of nitrate consists in the microbial process in an electric field. The assumption was to facilitate the microbial activity by cathode production of hydrogen, which is a strong nitrate reducer [86–88]. Besides the galvanostatic mode of operation, there is a possibility to modify the bacterial activity by electrochemical stimulation under potentiostatic conditions [88]. Some results are shown in **Figure 7**. The advantage of the bioelectric stimulation is obvious. The optimum cathode potential (0.01 V/S.H.E.) coincides with the standard redox potential of the reaction:

$$NO_3^- + H_2O + 2e^- \rightarrow NO_2^- + 2OH^- \quad (5)$$

The reference experimental results showed that there was practically no electrochemical abiotic reduction of nitrate. However, again the Coulombic efficiency

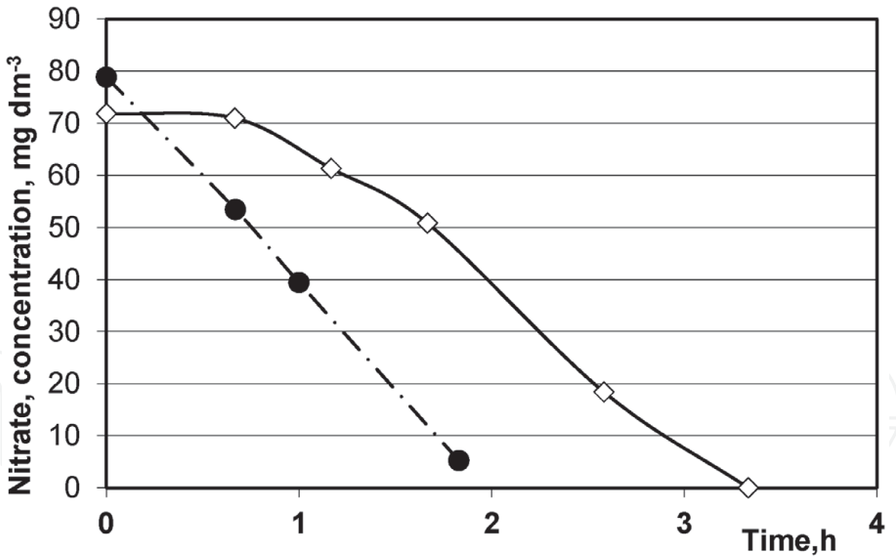

Figure 7.
Comparison of microbial denitrification by Pseudomonas denitrificans *at cathode potential of 0.232 V/S.H.E. with control experiment. (◇) – Control experiment; (•) – Process at constant electric field. Initial nitrate concentration, 80 mg dm^{-3}.*

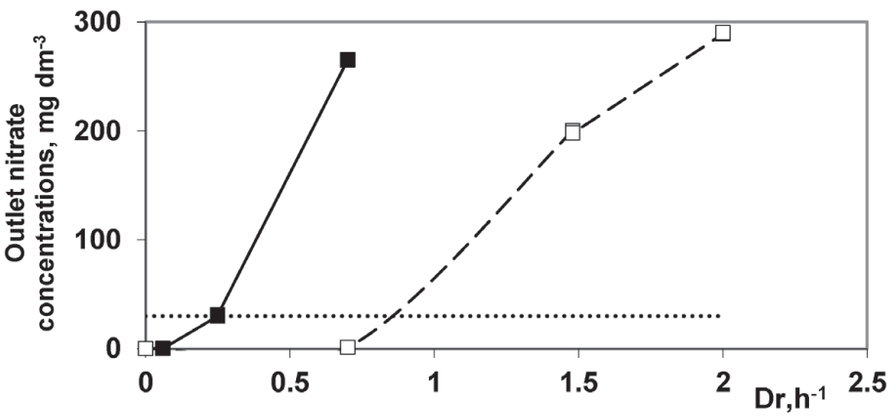

Figure 8.
Comparison of the performance of CSTR for bacterial denitrification by immobilized cells of Pseudomonas denitrificans *with and without constant electric field. Cathode potential 0.01 V/S.H.E. initial nitrate concentration – 300 mg dm^{-3}. Solid line, (■) – no electric field; dashed line (□) – With electric field, E = − 0.08 V/S.H.E. The horizontal dotted line denotes the dilution rates of 90% nitrate reduction.*

of this denitrification process is much lesser than the one corresponding to the chemical analyses. The biochemical stimulation effect of the electric field was confirmed by Field et al. [89] by electrochemical impedance spectroscopy, showing that the effect of enhanced microbial denitrification is the reduced activation energy of the reaction (5), i.e. due to changes in the active site of the enzyme nitrate reductase.

The strong denitrification effect of the constant electric field was confirmed for bacteria of the strain *Pseudomonas denitrificans* immobilized on a support of copolymer of acrylonitrile and acrylamide [90]. The effect of constant electric field is evident in continuous stirred tank reactor with immobilized cells, **Figure 8**. The break through dilution rate Dr = Q/V is more than twice higher than for control experiment without electric field.

One of the most valuable result of these studies is the fact that the bacteria can reduce nitrate to molecular nitrogen at much higher initial concentrations (up to 300 mg dm^{-3}) when electric field is applied, whereas without electric field the concentration upper limit for successful process is below 100 mg dm^{-3}. The

strongest effect on nitrate and nitrite reduction is observed at cathode potential range from −0.1 to 0.1 V/S.H.E. Another important feature is the much higher flow rates of substrate feeds attainable when electric field is applied.

It was confirmed by experiments with cell free extract of *Pseudomonas denitrificans* after ultrasonic cell disintegration, that both nitrate reductase and nitrite reductase are stimulated significantly by the constant electric field at the similar cathode potential, namely 0.01 V/S.H.E., cf. [91].

There are recent studies to indicate the scientific interest toward this bioelectrochemical process [92, 93].

3.3.5 Electrostimulation of carbon dioxide conversion to methane and chemicals

There are efforts to recycle carbon dioxide into methane by MEC process [94–96]. First, carbon dioxide is stripped by alkaline absorption, by sodium hydroxide aqueous solution and afterwards passed through a MEC with methanogenic microbes in the cathode compartment. There bicarbonate ions are converted there into methane by some of the following reactions [97, 98]:

$$HCO_3^- + 9H^+ + 8e^- \rightarrow CH_4 + 3H_2O \quad (6)$$

$$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O \quad (7)$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (8)$$

Various compounds can be used as electron donors, thus allowing to couple carbon dioxide recycling with additional wastewater treatment. Another new approach is to enhance methane production at anaerobic digestion by bioelectrochemical stimulation [94, 98, 99]. The effect consists in the easy removal or degradation of the excessive amounts of volatile fatty acids, e.g. propionic and butyric acids and to attain stable methanogenic process [94].

There are also efforts to reduce bioelectrochemically carbon dioxide to simple organic compounds [100, 101]. However, all these methods require energy input, loading the atmosphere with new amounts of carbon dioxide. An elegant solution will be to recycle carbon dioxide in new fuel cells using CO_2 as oxidizer and aqueous solutions of organic pollutants as a fuel. Hence, the benefit will be triple: removing carbon emissions from air, production of value-added organic compounds, thus saving fossil raw materials and cleaning wastewater at the same time.

All these achievements, as well as many more show serious challenges for further development of bioelectrochemistry in the biorefinery concept and contribute to the remedy the effects of the carbon emissions on climate change.

4. Conclusions

After reviewing the available literature on microbial fuel cells and microbial electrolysis the following conclusions can be drawn.

There are attractive challenges to use microbes to assist electric power generation during wastewater treatment by combining water purification with energy production. Although the power densities are rather low, the process is promising because of the lower energy demand compared to the traditional wastewater treatment technologies. Moreover, the generated energy can partially supply the equipment with energy. The purification rate of wastewater is promising even at low power production and Coulombic efficiency because of the lower energy demand compared to the traditional aerated process.

The microbial activities accompanied by redox processes can be used for energy saving in some electrolytic processes in the so-called microbial electrolysis cells. For example, hydrogen production by water splitting can be assisted by microbial processes with higher efficiency and lower energy consumption compared to traditional electrolysis.

The combination of fermentation redox processes with constant electric field can facilitate various microbial redox processes by stimulating enzyme catalysis under specific conditions, like fixed values of the electrode potential.

Bioelectrochemical effects can make it possible to recycle carbon dioxide to fuels and value-added chemicals.

All these applications will have practical use after increasing the process efficiencies and productivity. Higher power generation for MFC and lower energy consumption for MEC compared to the traditional processes must be attained. For this purpose, different approaches must be tested. On the one hand, suitable electrogenic microbes must be sought and applied for each particular case. On the other hand, highly active catalysts have to be invented and new designs of fuel cells, microbial electrolysis cells and appropriate electrodes must be developed.

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List of abbreviations

BES	bioelectrochemical system
COD	chemical oxygen demand
DCE	dichloroethane
MEC	microbial electrolysis cell
MFC	microbial fuel cell
NAD^+	nicotinamide adenine dinucleotide
S.H.E.	standard hydrogen electrode

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