

Microbial Fuel Cells in Relation to Conventional Anaerobic Digestion Technology

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Conventional anaerobic digestion based bioconversion processes produce biogas and have as such been widely applied for the production of renewable energy so far. An innovative technology, based on the use of microbial fuel cells, is considered as a new pathway for bioconversion processes towards electricity. In comparison with conventional anaerobic digestion, the microbial fuel cell technology holds some specific advantages, such as its applicability for the treatment of low concentration substrates at temperatures below 20 °C, where anaerobic digestion generally fails to function. This provides some specific application niches of the microbial fuel cell technology where it does not compete with but complements the anaerobic digestion technology. However, microbial fuel cells still face important limitations in terms of large-scale application. The limitations involve the investment costs, upscale technical issues and the factors limiting the performance, both in terms of anodic and cathodic electron transfer. Research to render the microbial fuel cell technology more economically feasible and applicable should focus on reactor configuration, power density and the material costs.

1 Introduction

The emerging drive towards a more sustainable society and the high level of energy consumption associated with our existing society constantly increase the need for new sustainable sources of energy. Biomass, especially organic waste, is being considered as a valuable candidate. The use of biomass, in the case of waste organics, is environment-friendly and regarded as a renewable energy source. On average, 1 kg of carbohydrate represents 1.06 kg of chemical oxygen demand (COD), which can be converted to an equivalent power of 4.41 kWh or 13×10^6 coulomb. At present, one can achieve the conversion of 1 kg of carbohydrate to 0.5 L of ethanol or 1.2 m³ of hydrogen, or 0.36 m³ of methane (0.5 L of biogas). On average, these processes yield about 1 kWh of useful energy [1]. In the EU, 1 kWh is worth up to € 0.16. As the production of this 1 kg of sugar costs about € 0.25 and the current market value approximates € 1, using sugar to drive electricity generation is not feasible at present on a large scale [1]. For all bioconversion technologies available at the moment, prime biomass is too costly to convert to “commodity level” energy [2,3]. Therefore the need for economically feasible new technologies is still urgent.

There are several pathways enabling the conversion of biomass to bioenergy. Methanogenic anaerobic digestion based technology, which emerged during the seventies, is now well established. In addition to that, ethanol fermentation and hydrogen fermentation are also approaches for biomass-to-bioenergy conversion [4–7]. Recently, the microbial

fuel cell (MFC) technology has been developed as a novel biotechnology to harvest energy from dissolved biomass [1]. The MFCs produce electricity from organic waste in a direct way, without the need for gas treatment. The conversion can occur at temperatures below 20 °C and at low substrate concentration levels, where anaerobic digestion (AD) generally fails due to low reaction rates and high solubility of the methane produced [3]. However, controversy exists regarding the efficiency, the applicability and obviously the future of the MFC technology in the context of bioconversion.

In this review, a critical comparison of the conventional AD technology and the MFC technology is presented. The potential of the MFC technology, particularly as a complement to the AD technology, will be discussed.

2 Bioconversions

In the presence of oxygen, biomass can be converted through an oxidative metabolism. Eq. (1) illustrates the oxidative conversion of glucose as the most common form of biomass.



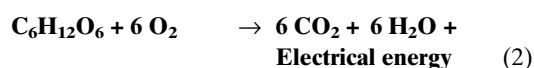
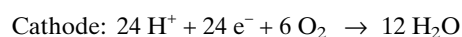
This energy is difficult to harvest as it is captured within the microbial metabolism and the end products (water and CO₂) contain no useful energy. Anaerobic bioconversion of biomass produces methane (methane fermentation) and/or hydrogen (hydrogen fermentation). Both can be combusted with oxygen or converted in a fuel cell, yielding electrical energy at an efficiency of 35 % and above 90 %, respectively [8]. However, the fuel cell based conversion is yet in a developmental phase.

The methane is normally formed both at the glucose level ($\Delta G^\circ = -404 \text{ kJ/mol}$) and the acetate level ($\Delta G^\circ = -20$ to -25 kJ/mol). During hydrogen fermentation, hydrogen is

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mainly produced at the glucose level, but only $\frac{1}{3}$ of the energy available is converted to hydrogen, the other $\frac{2}{3}$ remains occluded in the form of fatty acids. It has been reported that under nitrogen limiting conditions and abundant availability of light, these fatty acids are convertible to hydrogen at a considerably high rate by the activity of photoheterotrophs, such as *Rhodospseudomonas* species [9].

In a microbial fuel cell, substrate (organic matter or biomass) is oxidized at the anode producing carbon dioxide and protons as well as electrons, which are transferred to the electrode [1]. Microorganisms here fulfill the role of biocatalysts in analogy to chemical fuel cells. The electrons and the protons produced in the anode end up in the cathode, via the external electrical circuit and the exchange membrane, respectively. In this cathode, an oxidant (normally oxygen) is being reduced. Eq. (2) illustrates the basic process occurring in MFCs, in the case of a glucose fed system.



(theoretically approaching -2840 kJ/mol)

3 Biocatalysis

Microorganisms play a key role in the anaerobic bioconversion of substrate to energy. In conventional AD, a complex “food chain” type microbial consortium, often in a physically structured configuration, catalyzes the process [2]. The activity of strict anaerobes, e.g. methanogens, is particularly important [10]. For more in-depth-information on the microbiology of AD, the readers are referred to [2] and [11].

In MFCs, depending on the configuration as well as the aim of application, the microbial catalysts can be an axenic culture or a mixed culture. There have been a number of studies on MFCs operated with axenic cultures, among which *Shewanella putrefaciens*, *Pseudomonas aeruginosa*, *Geobacter* sp., *Rhodospirillum rubrum* [12–17]. MFCs also operated at high temperature using a thermophilic bacterium, such as *Bacillus licheniformis* or *Bacillus thermoglucosidasius*, have been described [18]. While in axenic culture MFCs, the bioelectrocatalysis is attributed to the activity of only one bacterial culture, in MFCs operated with a mixed culture, it is determined by the interaction of the whole microbial community, the so-called electrochemically active consortium. These electrochemically active consortia are enriched either from sediment (both marine and lake sediment) [16, 19] or activated sludge from wastewater treatment plants [15, 16, 19–23].

By means of molecular analysis, electrochemically active species of *Geobacter* sp., *Desulfuromonas* sp., *Alcaligenes*

faecalis, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Clostridium* sp., *Bacteroides* sp., *Aeromonas* sp. and *Brevibacillus* sp. were detected in the above-mentioned studies. In comparison with MFCs operated with pure cultures, MFCs that make use of mixed bacterial cultures have some important advantages: a higher resistance against process disturbances, a larger substrate versatility and a higher power output [1, 24]. Noticeably, the mixed culture MFC developed by Rabaey et al. [25] can produce a power density up to 216 W/m^3 , which is the highest value reported so far.

Several hypotheses about electron transfer catalyzed by microorganisms in an MFC were proposed. These include the conventional concept of membrane-associated direct electron transfer [13] and mediator-associated electron transfer [26–30]. Interestingly, it has recently been described that bacteria in a microbial fuel cell can produce mediators themselves [15]. Hence, the principle of an MFC and the extracellular electron transfer from the bacteria to the electrode can be schematized as in Fig. 1. Finally, the possible mediation by so-called nanowires has recently been proposed [31].

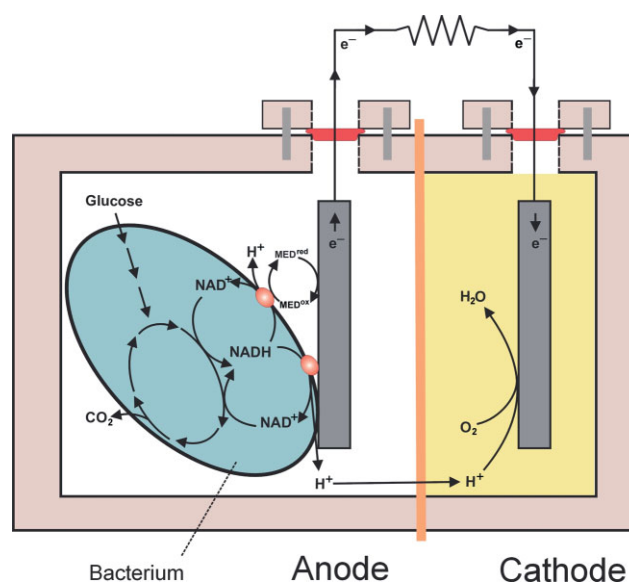


Figure 1. Working principle of a microbial fuel cell [1].

4 Power

Power Input

Anaerobic digestion allows for the intake of both high and low concentration COD biomass, whereas carbohydrates are particularly well suited. Almost any type of bioavailable substrate is accessible to AD [2]. However, AD requires meso- to thermophilic temperatures to achieve sufficient turnover and limited methane solubility. Microbial fuel cells use rather low strength influents containing glucose, sucrose or acetate, at this moment. Also, more complex compounds

such as starch and cellulose have been used to generate electricity in MFCs [32,33]. Even light is a potential candidate, as shown in photobiological fuel cell systems [34–37].

Power Output

(i) Power Output Types

Anaerobic digestion produces methane or hydrogen. The gases are used directly as fuel for combustion. They also can be used in chemical fuel cells generating electricity. Usually $\frac{1}{3}$ of the biogas produced is converted with a high energy level (producing 220 Volt electricity) and the remaining $\frac{2}{3}$ with a low energy level (producing 60–80 °C heat), which can be used to heat the digester. Contrarily, MFCs convert energy available in biomass directly to electricity. The energy produced by single MFCs is at a low level since the voltage generated per MFC amounted to approx. 0.5–0.7 Volt. However, upon stacking the MFCs this voltage can be multiplied by the number of MFCs, which creates the opportunity to generate 100 V or more.

(ii) Power Output Units

In practice, anaerobic digestion allows 1 kg of COD to be converted to an energy amount of roughly 1 kWh and on average, the power density obtained is about 400 W/m³ when the technology is applied to treat about 5 to 25 kg of COD per m³ of the reactor per day. In the case of MFCs, theoretically, 1 kg of COD can be converted to 4 kWh of electrical energy. However, the current generated by MFCs, until now, has not exceeded 0.1 A. The average power density of MFCs is about 40 W/m³. Recently, stacked configurations of MFCs have reached power densities of 250 W/m³ [23], implying that an improvement of MFC performance is underway.

5 Configuration

The anaerobic digestion technology has been established in terms of performance and is technically and economically feasible. An important breakthrough occurred about 30 years ago, with the development of the Upflow Anaerobic Sludge Blanket (UASB) reactor [38], which efficiently retains the complex microbial consortium without the need for immobilization on a carrier material (for example, as a biofilm) contrary to conventional activated sludge systems. This is possible through the formation of biological granules (i.e., granulation, self-

immobilization) with good settling characteristics. Approximately 60 % of the anaerobic full-scale treatment facilities worldwide are now UASB design based [2]. Also, a horizontal-flow bioreactor incorporating a migrating blanket within a compartmentalized reactor (a multivessel), the so-called Anaerobic Migrating Blanket Reactor (AMBR), has been designed [2]. In order to overcome the limitation of UASB reactors, which is related to the interference of suspended solids in the incoming wastewater, other high-rate systems, such as the Expanded Granular Sludge Bed (EGSB) [39] and Internal Circulator (IC) reactors [40] were developed. Moreover, “dry” anaerobic digestion based systems were developed to treat solid organic waste without the requirement of dilution [41]. Having been well optimized, AD reactors are now treating at full scale various kinds of waste [2].

The configuration of microbial fuel cells has been continuously optimized since the first invention with the two-chamber design. As this conventional two-chamber system does not allow for much inner volume and does not allow users to easily carry out manipulation, several researchers altered the design into a two-bottle system with a connecting tube [42]. However, the two-bottle system still showed limitations in terms of power output due to the high internal resistance of the system. Later on, MFCs, in which either the cathode compartment consists of a (catalyzed) electrode that is open to the air or both the anode and the cathode are present in one unit, were developed to allow oxygen from the air to be directly used as the electron acceptor [43,44]. In addition, cylindrical reactors were designed for wastewater treatment [45,46]. A recently developed reactor, a tubular system with outer cathode and inner packed bed anode (see Fig. 2.I),

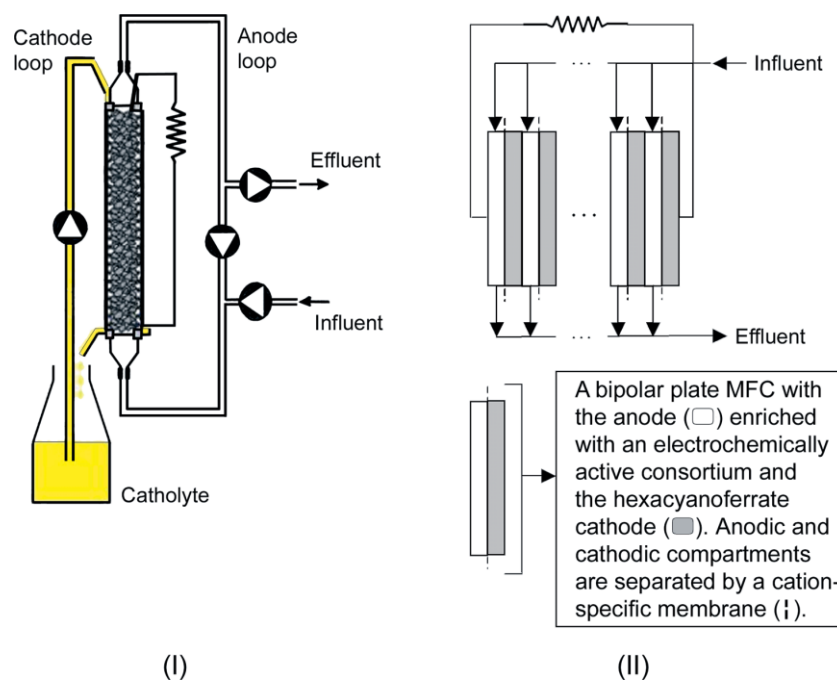


Figure 2. (I): Tubular MFC reactor [47]; (II): Stacked MFC [23].

which uses hexacyanoferrate as the catholyte, demonstrated a significantly higher power output [47]. The average power density obtained was about 50 W/m^3 of the reactor with glucose or acetate as substrates and the maximum coulombic efficiency was about 90%. This reactor design also integrates the advantages of operational principles of upflow granular bed reactors. In order to increase the attainable voltage and current with MFC systems, several MFCs can be joined in a stack and become connected (see Fig. 2.II) [23].

Specific limitations of existing MFC technologies are the costs of materials for the construction of MFCs and the as yet low power output compared to other bioconversion technologies. The proton exchange membrane (PEM), which is widely used in MFCs, represents a considerable cost (Nafion™ costs approximately $\$500/\text{m}^2$) [48] and increases the internal resistance of the MFC. Attempts were made to replace or remove the PEM but low coulombic efficiency due to oxygen influx [44] and high internal resistance [49] still remain problematic. Overall, major technical hurdles will need to be overcome in order to upscale MFCs from the present liter scale to the cubic meter scale required for practical application.

6 Limiting Factors

In methanogenic anaerobic digestion systems, growth of the biocatalyst, gas/liquid/solid phase separation and temperature are the main factors limiting the bioconversion efficiency. Regarding the growth of the biocatalyst, low cell yield and the interference of suspended solids in the incoming wastewater with the formation of granular biocatalysts decrease the performance of UASB reactors [50]. An upflow rate ranging from 1 to 5 m/h and an average gas flux velocity of about $0.5 \text{ m}^3/\text{m}^2$ cross section per hour does facilitate a good separation of gas, liquid and solid phases but may limit the performance. As investigated by Kalogo et al. [51], an UASB reactor treating domestic sewage can achieve a total COD removal efficiency of 80% but 68% of the total COD removed is due to the above-mentioned factors. In addition, a temperature higher than 30°C is generally required for a good performance of the methanogens in the systems.

In MFC systems, the activity of biocatalysts, electron transfer between the bacteria and the anode, internal resistance and overpotentials at both electrodes are the main limiting factors.

The Activity of Biocatalysts

The microbial communities and the microbial activity in MFCs are not well understood. There exists a lack of information about the structure of the microbial communities and the roles of the members of each community in the catalysis. Thus, the research question on how to control the growth and activity of a microbial consortium in an MFC in

order to drive it to a desired performance needs to be addressed. Some research elucidates that biofilms are a common structure of microbial communities in MFCs [20, 16, 47] and that facultative anaerobes are usually present in electrochemically active consortia [20, 15, 52]. Both aspects can be regarded as positive towards practical implementation, as biofilm systems demonstrate higher stability and the facultative anaerobes generally demonstrate high specific growth rates though the diffusivity rate might be a disadvantage of biofilm systems, limiting the biofilm thickness and the maximum turnover rate. Nevertheless, substantially more knowledge of this domain is still needed and more intensive work should be done.

Electron Transfer

The mechanism of bacterial electron transfer to the anodic electrode and the issue of how to improve the electron transfer have been and are still the focus of much controversy. As mentioned earlier, a hypothesis exists describing a direct electron transfer in which some outer-membrane bound proteins, such as cytochromes [16, 13], play the role of transferring electrons to the electrode. Another hypothesis concerns the electron transfer with the help of external or self-produced mediators. Recently, a new finding suggested that bacteria are able to form nanowires contacting the electrode, through which electrons are conducted [31]. Whether the electrons are transferred directly by membrane-bound proteins, by external or self-produced mediators, by nanowire formation or by a combination of several systems should be clarified. Based on that, good approaches could be established to improve the performance of MFCs upon improving the electron transfer.

Internal Resistance

This is a common problem that MFC designers face. A high internal resistance causes a considerable potential drop due to ohmic losses [53]. With or without the PEM, the internal resistance still remains a limiting factor [44, 49]. Several researchers tried to determine the optimum distance between the anode and the cathode for a lowest internal resistance [44]. Modification of the configuration of the MFC is necessary to decrease the internal resistance. This aspect is one of the crucial bottlenecks hampering the upscale of the present technology. Upon increasing the MFC size, the internal resistance will at best remain at the same level, while the current flowing through the system increases and causes considerable potential losses. As such, the size of the upscaled MFC will be limited.

Cathode Reaction

The cathode reaction is considered to be one of the key factors limiting the performance of an MFC [54, 52]. In many

MFC systems, oxygen is the cathodic electron acceptor but usually the poor contact between gaseous oxygen and the cathode, and the imperfect catalysis of the reaction limits the turnover rate. In addition, oxygen leaking to the anode can occur in such systems reducing electricity generation efficiency. The leaking should not be significant, yet still providing high rate electrode reactions. Moreover, to overcome this limitation, platinum has been used as the catalyst [52, 54] and gas diffusion layers were installed. However, the disadvantage of platinum is that it is not only expensive but it also suffers from sulfide diffusion through the PEM towards the cathode, which poisons the catalyst. Hexacyanoferrate has, in many cases, been used as the catholyte in a circulative way facilitating a good contact between the cathode and the catholyte. Such an approach of using hexacyanoferrate resulted in a better cathode reaction and a better performance of the MFCs [47]. However, hexacyanoferrate is not sustainable due to its toxicity, and the fact that it is not fully re-oxidized in the air hinders the application of the technology on a field scale [47]. Oxygen is still the only good final oxidant candidate to ensure the sustainability of MFCs. In order to improve the cathode-oxygen contact, the use of some electro-catalytic metals in integration with carbon as the electrode material for “open-air” cathodes is now a promising approach for the improvement of the cathode reaction [55–57]. Alternatively, the cathode can also be employed to generate hydrogen gas; however, this requires a surplus investment of energy into the system to decrease the cathode potential to the low level, at which hydrogen generation is feasible [58].

7 Maximizing Performance

Approaches to maximize the performance of the AD systems, i.e., UASB reactors, involve solutions to provide more readily available soluble COD in the reactors (concerning the pretreatment of the substrates) and to optimize the mass transfer inside the reactors. In order to optimize this mass transfer in the reactors, several technologies were proposed, including the application of fluidized bed reactors and Internal Circulator (IC) reactors [40, 59]. The IC reactors do not only allow for an efficient mass transfer and a good granulation [59] but also for an excellent separation of gas/liquid/solid phases. The self-regulating internal circulation offers considerable advantages in the operation of the system, leading towards reduced operational costs, increased productivity and reliability on anaerobic treatment [60, 61].

For a maximized performance of MFCs, readily available soluble COD is also required. This demands solutions for the pretreatment of the organics to be used as fuel for bacteria, provided a considerable fraction is not readily biodegradable. In addition to that, better proton selective membranes, optimum mass transfer and better cathodes are also needed to overcome factors limiting MFC performance. Whether to use a membrane or not is now under discussion.

To improve the cathodic performance, some metal oxides combined with carbon [57, 62, 63] or some special materials, such as fullerenes [64], are proposed as good candidates for the construction of the cathode.

8 Disadvantages

Both the AD technology and the MFC technology have disadvantages. Regarding AD, the first disadvantage is that biogas is difficult to store. In addition, the quality of the biogas produced is often suboptimal. The obtained biogas normally contains H₂S, the removal of which is costly. Capital investment for the AD technology is also a matter to be considered. Approximately €100,000 is needed per ton of COD treated per day. A major limitation to widen the application of the bioenergy producing technologies based on AD is the relative low cost of the current non-renewable energy sources [2].

For MFCs, the dominant bottleneck at the moment is the limited effectivity of the open-air cathodes. Catalysts that directly reduce oxygen without unacceptable activation losses need to be found. Limited electrochemical COD removal efficiency is also one of the current disadvantages of MFCs. At present, the COD removal efficiency of MFCs for sewage as the substrate is only about 20 % [1].

Finally, at this moment, maintenance and material costs of MFCs are considerable. Based on the material costs, presented by Tsuchiya and Kobayashi [48], Rabaey and Verstraete [1] estimated that – assuming the costs of €4000 per m³ of the electrode compartment – the costs of 1 kW power output per m³ anode produced by an MFC are higher by a factor of approx. 10 compared to the equivalent production costs for conventional processes.

9 Application Niches

When comparing the different aspects of the two technologies, one can assume that they are competitive. However, considering the aspects carefully and exploring application niches of the technologies thoroughly, it appears that they do not conflict but complement each other. Anaerobic digestion can be applied to treat high strength substrate (with more than 1 g COD per liter). Industrial scale feasibility, high throughput and relatively low cost are the noticeable advantages of this technology in terms of application prospects. Moreover, its bioconversion efficiency is remarkable, often surpassing 90 %. The AD technology is a good candidate to produce “green” energy for a sustainable society in the future, when the non-renewable energy sources are consumed [2].

In the case of MFCs, application niches can be found in the area of treating low concentration COD substrates and at low temperatures (10–20 °C), i.e., where AD does not function well. MFCs can also be used to treat high concen-

tration COD substrates. Carbohydrate based electricity generated by MFCs is reliable. The technology can be implemented at a small scale and the generated electricity can be used as an alternative energy source. A unique niche of the MFC technology could be the combination of energy generation, water production and possibly hydrogen production.

Since the AD and the MFC technologies are not competitive but complementary to each other, they can be integrated in waste treatment processes for a more efficient and thorough bioconversion. The concept of integration of the two technologies for achieving optimal sustainability is illustrated in Fig. 3 with some wastewater treatment models proposed.

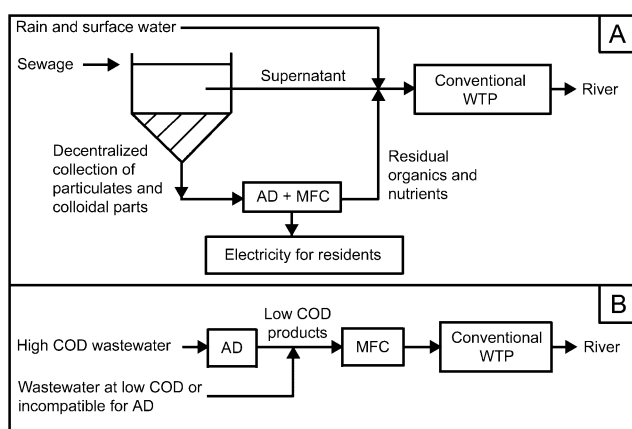


Figure 3. Proposed models for the integration of anaerobic digestion and microbial fuel cells for the treatment of wastewaters. ((A) For domestic wastewater, (B) For industrial wastewater. Note: AD: Anaerobic digestion; MFC: Microbial fuel cell; WTP: Wastewater treatment process).

10 Opportunities

Anaerobic Digestion Based Technology

The in-depth studies over the past decades on anaerobic microbial communities and their delicate interactions and balances have given rise to a number of unexpected findings. The advances in methanogenesis studies have created the potential to control methanogens in the rumen, the colon or in rice paddies, etc. [3]. A second novel spin-off of AD is the halorespiration based technology. Indeed, the interesting discovery of the unique capability of many anaerobes to rapidly and efficiently use chlorinated organics as electron acceptors created a new technology: anaerobic dechlorination. This technology is the default technology at the present time to clean polluted sediments, soils and other organic slurries [65, 66].

Microbial Fuel Cell Based Technology

Recent findings broaden the application area of MFCs. The bioelectrochemically assisted microbial reactor (BEAMR), presented in Fig. 4, was developed by Liu et al. [58]. In this types of reactors, by investing additional energy, hydrogen is produced at the cathode of the MFC. This results in higher hydrogen yields for the organic matter converted in the anode compared to a conventional process, with a limited surplus energy investment. For example, by using acetate about 2.9 mol H₂/mol acetate were produced (from the maximally possible 4 H₂/mol acetate).

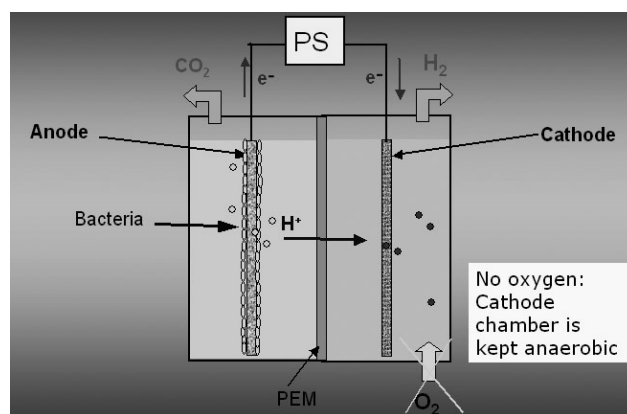


Figure 4. Operational scheme of a bioelectrochemically assisted microbial reactor (BEAMR) [58]. (Note: PS: Power).

There are several bottlenecks to this approach. The hydrogen produced needs to be sufficiently pure to be economically valuable. Also, the production quantities should be sufficient to warrant the investment costs. Finally, one needs to address the question of what is preferred, electricity or hydrogen. Alternatively, one can envisage a two-step process, in which the first phase consists of a “conventional” acid hydrogen fermentation out of diverse substrates, after which an MFC converts the residual volatile fatty acids to electricity during a second step.

In addition to the above-mentioned process, other perspectives can be found in the domains of biosensor and remote sensor development [67], removal of other pollutants than carbohydrates, and sustainable biorefinery development. The use of MFCs as BOD sensors has been reported earlier [54, 68]. Using MFCs for the treatment of more complex substances such as cellulose was shown to be feasible [33]. This approach is, in view of the fact that normally oxygen is the final electron acceptor, also promising and warrants more R&D investment. Besides, the concept of coupling photobiological hydrogen production with the oxidation of microbial hydrogen while simultaneously generating electricity, opens an opportunity to convert solar energy directly to electricity using MFCs [34, 35].

11 Conclusions

Conventional AD and MFC technologies can be regarded as complementary technologies. The combination of the two technologies allows for broadening the spectrum of the bio-conversion technology. While conventional AD can be applied on an industrial scale to treat high strength substrates at temperatures above 30 °C, the niche applications of MFCs are to be sought in low concentrated substrates and low temperature conversions. A number of factors still limit the application spectrum of MFCs. In order to overcome the limitations of MFCs, making the technology practical and economically feasible as well as sustainable, the key research and development features for the future are: (i) New materials for better configurations of MFCs, particularly dry cathodes that have a high affinity to oxygen and use gaseous oxygen directly from the air; (ii) Low capex, meaning low material costs as well as low operational costs and (iii) A reliable output of “non-commodity” electricity produced by MFCs.

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References

[1] K. Rabaey, W. Verstraete, Microbial fuel cells: novel biotechnology for energy generation, *Trends Biotechnol.* **2005**, *23*, 291–298.
[2] L. T. Angenent, K. Karim, M. Al-Dahhan, B. A. Wrenn, R. Domínguez-Espinosa, Production of bioenergy and biochemicals from industrial and agricultural wastewater, *Trends Biotechnol.* **2004**, *22* (9), 477–485.
[3] W. Verstraete, F. Morgan-Sagastume, S. Aiyuk, K. Rabaey, M. Waweru, G. Lissens, Anaerobic digestion as a core technology in sustainable management of organic matter, *Water Sci. Technol.* **2005**, *52*, 59–66.
[4] L. Segers, W. Verstraete, Conversion of organic acids to H₂ by Rhodospirillaceae grown with glutamate or dinitrogen as nitrogen source, *Biotechnol. Bioeng.* **1983**, *25*, 2843–2853.
[5] L. Segers, W. Verstraete, Hydrogen accumulation by H₂-uptake negative strains of *Rhizobium*, *Plant Soil* **1985**, *85*, 77–84.
[6] B. E. Logan, Extracting hydrogen electricity from renewable resources, *Environ. Sci. Technol.* **2004**, *38*, 160A–167A.
[7] A. C. van Haandel, Integrated energy production and reduction of the environmental impact at alcohol distillery plants, *Water Sci. Technol.* **2005**, *52* (1–2), 49–57.
[8] A. J. Bard, L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd ed., John Wiley & Sons, New York **2001**.
[9] L. Segers, W. Verstraete, Ammonium as an alternative nitrogen source for hydrogen producing photobacteria., *J. Appl. Bacteriol.* **1985**, *58*, 7–11.
[10] D. De Beer, V. O’Flaherty, J. Thaveesri, P. Lens, W. Verstraete, Distribution of extracellular polysaccharides and flotation of anaerobic sludge, *Appl. Microbiol. Biotechnol.* **1996**, *46*, 197–201.
[11] L. T. Angenent, S. Sung, L. Raskinet, Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste, *Water Res.* **2002**, *36* (18), 4648–4654.
[12] B. H. Kim, T. Ikeda, H. S. Park, H. J. Kim, M. S. Hyun, K. Kano et al., Electrochemical activity of an Fe(III)-reducing bacterium, *Shewanella putrefaciens* IR-1, in the presence of alternative electron acceptors, *Biotechnol. Tech.* **1999**, *13*, 475–478.

[13] B. H. Kim, H. J. Kim, M. S. Hyun, D. H. Park, Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*, *J. Microbiol. Biotechnol.* **1999**, *9*, 127–131.
[14] H. J. Kim, H. S. Park, M. S. Hyun, I. S. Chang, M. Kim, B. H. Kim, A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*, *Enzyme Microb. Technol.* **2002**, *30*, 145–152.
[15] K. Rabaey, N. Boon, S. D. Siciliano, M. Verhaege, W. Verstraete, Biofuel cells select for microbial consortia that self-mediate electron transfer, *Appl. Environ. Microbiol.* **2004**, *70*, 5373–5382.
[16] D. R. Bond, D. R. Lovley, Electricity production by *Geobacter sulfurreducens* attached to electrodes, *Appl. Environ. Microbiol.* **2003**, *69*, 1548–1555.
[17] S. K. Chaudhuri, D. R. Lovley, Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells, *Nat. Biotechnol.* **2003**, *21*, 1229–1232.
[18] Y. Choi, E. Jung, H. Park, S. R. Paik, S. Jung, S. Kim, Construction of microbial fuel cells using thermophilic microorganisms, *Bacillus licheniformis* and *Bacillus thermoglucosidasius*, *Bull. Korean Chem. Soc.* **2004**, *25*, 813–818.
[19] L. M. Tender, C. E. Reimers, H. A. Stecher, D. E. Holmes, D. R. Bond, D. A. Lowy et al., Harnessing microbially generated power on the seafloor, *Nat. Biotechnol.* **2002**, *20*, 821–825.
[20] B. H. Kim, H. S. Park, H. J. Kim, G. T. Kim, I. S. Chang, J. Lee et al., Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell, *Appl. Microbiol. Biotechnol.* **2004**, *63*, 672–681.
[21] A. K. Lee, D. K. Newman, Microbial iron respiration: impacts on corrosion processes, *Appl. Microbiol. Biotechnol.* **2003**, *62*, 134–139.
[22] H. S. Park, B. H. Kim, H. S. Kim, H. J. Kim, G. T. Kim, M. Kim et al., A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum* isolated from a microbial fuel cell, *Anaerobe* **2001**, *7*, 297–306.
[23] P. Aelterman, K. Rabaey, T. H. Pham, N. Boon, W. Verstraete, Continuous electricity generation at high voltages and currents using stacked microbial fuel cells, *Environ. Sci. Technol.* **2005**, accepted for publication..
[24] K. Rabaey, W. Ossieur, M. Verhaege, W. Verstraete, Continuous microbial fuel cells convert carbohydrates to electricity, *Water Sci. Technol.* **2005**, *52*, 515–523.
[25] K. Rabaey, G. Lissens, S. D. Siciliano, W. Verstraete, A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency, *Biotechnol. Lett.* **2003**, *25*, 1531–1535.
[26] D. H. Park, M. Laivenieks, M. V. Guettler, M. K. Jain, J. G. Zeikus, Microbial utilization of electrically reduced neutral red as the sole electron donor for growth and metabolite production, *Appl. Environ. Microbiol.* **1999**, *65*, 2912–2917.
[27] D. H. Park, J. G. Zeikus, Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: Physiological function of neutral red in membrane-driven fumarate reduction and energy conservation, *J. Bacteriol.* **1999**, *181*, 2403–2410.
[28] Y. Choi, N. Kim, S. Kim, S. Jung, Dynamic behaviors of redox mediators within the hydrophobic layers as an important factor for effective microbial fuel cell operation, *Bull. Korean Chem. Soc.* **2003**, *24*, 437–440.
[29] A. M. Lithgow, L. Romero, I. C. Sanchez, F. A. Souto, C. A. Vega, Interception of the electron-transport chain in bacteria with hydrophilic redox mediators: Selective improvement of the performance of biofuel cells with 2,6-disulfonated thionine as mediator (Part 1), *J. Chem. Res.* **1986**, *5*, 178–179.
[30] S. D. Roller, H. P. Bennetto, G. M. Delaney, J. R. Mason, J. L. Stirling, C. F. Thurston, Electron-transfer coupling in microbial fuel cells: Comparison of redox-mediator reduction rates and respiratory rates of bacteria (Part 2), *J. Chem. Technol. Biotechnol.* **1984**, *34*, 3–12.
[31] G. Reguera, K. D. McCarthy, T. Mehta, J. S. Nicoll, M. T. Tuominen, D. R. Lovley, Extracellular electron transfer via microbial nanowires, *Nature* **2005**, *435*, 1098–1101.
[32] G. C. Gil, I. S. Chang, B. H. Kim, M. Kim, J. K. Jang, H. S. Park et al., Operational parameters affecting the performance of a mediator-less microbial fuel cell, *Biosens. Bioelectron.* **2003**, *18*, 327–334.
[33] J. Niessen, U. Schroder, F. Harnisch, F. Scholz, Gaining electricity from in situ oxidation of hydrogen produced by fermentative cellulose degradation, *Let. Appl. Microbiol.* **2005**, *41*, 286–290.
[34] M. Rosenbaum, U. Schroder, F. Scholz, Utilizing the green alga *Chlamydomonas reinhardtii* for microbial electricity generation: a living solar cell, *Appl. Microbiol. Biotechnol.* **2005**, *68*, 753–756.
[35] M. Rosenbaum, U. Schroder, F. Scholz, In situ electrooxidation of photobiological hydrogen in a photobioelectrochemical fuel cell based on *Rhodobacter sphaeroides*, *Environ. Sci. Technol.* **2005**, *39*, 6328–6333.

- [36] T. Yagishita, S. Sawayama, K. Tsukahara, T. Ogi, Photosynthetic bio-fuel cells using cyanobacteria, *Renew. Energy* **1996**, *9*, 958–961.
- [37] T. Yagishita, S. Sawayama, K. Tsukahara, T. Ogi, Effects of glucose addition and light on current outputs in photosynthetic electrochemical cells using *Synechocystis* sp PCC6714, *J. Biosci. Bioeng.* **1999**, *88*, 210–214.
- [38] G. Lettinga, A. F. M. Vanvelsen, S. W. Hobma, W. Dezeew, A. Klapwijk, Use of the upflow sludge blanket (USB) reactor concept for biological wastewater-treatment, especially for anaerobic treatment, *Biotechnol. Bioeng.* **1980**, *22*, 699–734.
- [39] G. R. Zoutberg, P. de Been, The biobed EGSB (expanded granular sludge bed) system covers shortcomings of the upflow anaerobic sludge blanket reactor in the chemical industry, *Water Sci. Technol.* **1997**, *35* (10), 183–188.
- [40] E. A. Stadlbauer, R. Achenbach, D. Doll, B. Jehle, B. Kufner, L. Oey et al., Design and performance of pulsed anaerobic digesters, *Water Sci. Technol.* **1992**, *25* (7), 351–360.
- [41] G. Lissens, P. Vandevivere, L. De Baere, E. M. Biey, W. Verstraete, Solid waste digestors: process performance and practice for municipal solid waste digestion, *Water Sci. Technol.* **2001**, *44* (8), 91–102.
- [42] M. Chiao, K. B. Lam, L. Lin, Micromachined microbial fuel cells, in *Proc. of the 16th IEEE International MEMS Conference*, Kyoto (Japan) **2003**.
- [43] D. H. Park, J. G. Zeikus, Improved fuel cell and electrode designs for producing electricity from microbial degradation, *Biotechnol. Bioeng.* **2003**, *81*, 348–355.
- [44] H. Liu, B. E. Logan, Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, *Environ. Sci. Technol.* **2004**, *38*, 4040–4046.
- [45] W. Habermann, E. H. Pommer, Biological fuel cells with sulphide storage capacity, *Appl. Microbiol. Biotechnol.* **1991**, *35*, 128–133.
- [46] H. Liu, R. Ramnarayanan, B. E. Logan, Production of electricity during wastewater treatment using a single chamber microbial fuel cell, *Environ. Sci. Technol.* **2004**, *38*, 2281–2285.
- [47] K. Rabaey, P. Clauwaert, P. Aelterman, W. Verstraete, Tubular microbial fuel cell for efficient energy generation, *Environ. Sci. Technol.* **2005**, *39*, 8077–8082.
- [48] H. Tsuchiya, O. Kobayashi, Mass production cost of PEM fuel cell by learning curve, *Int. J. Hydrogen Energy* **2004**, *29*, 985–990.
- [49] J. K. Jang, T. H. Pham, I. S. Chang, K. H. Kang, H. Moon, K. S. Cho et al., Construction and operation of a novel mediator- and membrane-less microbial fuel cell, *Process Biochem.* **2004**, *39*, 1007–1012.
- [50] Y. Kalogo, W. Verstraete, Development of anaerobic sludge bed (ASB) reactor technologies for domestic wastewater treatment: motives and perspectives, *World J. Microbiol. Biotechnol.* **1999**, *15*, 523–534.
- [51] Y. Kalogo, J. H. MBouche, W. Verstraete, Physical and biological performance of self-inoculated UASB reactor treating raw domestic sewage, *J. Environ. Eng. – ASCE* **2001**, *127* (2), 179–183.
- [52] T. H. Pham, J. K. Jang, I. S. Chang, B. H. Kim, Improvement of cathode reaction of a mediatorless microbial fuel cell, *J. Microbiol. Biotechnol.* **2004**, *14*, 324–329.
- [53] J. Larminie, A. Dicks, *Fuel Cell Systems Explained*, John Wiley & Sons, Chichester **2000**, 37–60.
- [54] K. H. Kang, J. K. Jang, T. H. Pham, H. Moon, I. S. Chang, B. H. Kim, A microbial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor, *Biotechnol. Lett.* **2003**, *25* (16), 1357–1361.
- [55] F. H. Lima, B. Giz, M. Janete, E. A. Ticianelli, Electrochemical performance of dispersed Pt-M (M = V, Cr and Co) nanoparticles for the oxygen reduction electrocatalysis, *J. Braz. Chem. Soc.* **2005**, *16*, 328–336.
- [56] S. Baranton, C. Coutanceau, C. Roux, F. Hahn, J. M. Léger, Oxygen reduction reaction in acid medium at iron phthalocyanine dispersed on high surface area carbon substrate: tolerance to methanol, stability and kinetics, *J. Electroanal. Chem.* **2005**, *577*, 223–234.
- [57] S. D. Song, Z. Y. Tang, L. Z. Pan, J. M. Nan, Study on the electrochemical properties of $\text{La}_{1-x}\text{Sr}_x\text{Ni}_{1-y}\text{Fe}_y\text{O}_3$ bifunctional oxygen electrodes, *Acta Chim. Sin.* **2005**, *63* (5), 363–371.
- [58] H. Liu, S. Grot, B. E. Logan, Electrochemically assisted microbial production of hydrogen from acetate, *Environ. Sci. Technol.* **2005**, *39*, 4317–4320.
- [59] J. H. F. Pereboom, T. L. F. M. Vereijken, Methanogenic granule development in full-scale internal circulation reactors, *Water Sci. Technol.* **1994**, *30* (8), 9–21.
- [60] L. H. A. Habets, A. J. H. H. Engelaar, N. Groeneveld, Anaerobic treatment of in-line effluent in an internal circulation reactor, *Water Sci. Technol.* **1997**, *35* (10), 189–197.
- [61] Z. A. Kassam, L. Yerushalmi, S. R. Guiot, A market study on the anaerobic wastewater treatment systems, *Water Air Soil Pollut.* **2003**, *143*, 179–192.
- [62] K. Arihara, M. Lanqun, A. L. Paul, M. Ernesto, A. L. Moore, T. Imase, Electrocatalytic reduction of oxygen in a novel catalytic system with cobalt phthalocyanines and manganese oxide, *J. Electrochem. Soc.* **2004**, *151* (12), A2047–A2052.
- [63] B. Klapste, J. Vondrak, J. Velicka, MnO_x/C composites as electrode materials: Reduction of oxygen on bifunctional catalysts based on manganese oxides (Part II), *Electrochim. Acta* **2002**, *47*, 2365–2369.
- [64] Y. X. Liu, Developing tendency of new materials in electrochemistry and electrochemical engineering, *Rare Metal Mater. Eng.* **2001**, *30*, 507–513.
- [65] S. De Wildeman, G. Diekert, H. Van Langenhove, W. Verstraete, Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes, *Appl. Environ. Microbiol.* **2003**, *69* (9), 5643–5647.
- [66] F. Aulenta, S. Rossetti, M. Majone, V. Tandoi, Detection and quantitative estimation of *Dehalococcoides* spp. in a dechlorinating bioreactor by a combination of fluorescent in situ hybridization (FISH) and kinetic analysis, *Appl. Microbiol. Biotechnol.* **2004**, *64* (2), 206–212.
- [67] A. Shantaram, H. Beyenal, R. Raajan, A. Veluchamy, Z. Lewandowski, Wireless sensors powered by microbial fuel cells, *Environ. Sci. Technol.* **2005**, *39*, 5037–5042.
- [68] I. S. Chang, J. K. Jang, G. C. Gil, M. Kim, H. J. Kim, B. W. Cho et al., Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor, *Biosens. Bioelectron.* **2004**, *19*, 607–613.