Perspectives of biofuels production from renewable resources with bioelectrochemical systems

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ABSTRACT: Bioelectrochemical systems (BESs) are an emerging technology that uses solid-state electrodes to stimulate microbial metabolism (either for substrate degradation or for products formation). Because of their versatility and unmatched level of control over the biological reactions, BESs hold a great potential for application in industrial and environmental bioprocesses. Among them, the bioelectrochemical production of renewable and carbon-neutral energy carriers, such as hydrogen and methane, at the cathode of bioelectrochemical systems is recently attracting considerable attention. While exciting as a concept, the performance of the process seems to be still primarily limited by the low kinetics and efficiencies of the cathodic reactions. In this review, key opportunities for gaseous biofuels production with bioelectrochemical systems are addressed and compared with existing biotechnological approaches such as anaerobic digestion and dark fermentation. The major bottlenecks and challenges that still need to be faced to make this novel technology practical are presented and critically discussed. © 2012 Curtin University of Technology and John Wiley & Sons, Ltd.

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INTRODUCTION

At present, the annual world’s energy demand is estimated to be 13 terawatts (TW), and an addition of 10 TW will be needed by 2050, to maintain current lifestyles.1] Approximately 80% of this energy requirement is provided by fossil sources, 7% by nuclear energy, and 13% by renewable sources.2] The rapidly increasing global demand for the limited fossil fuel reserves and the need to control greenhouse gas effects due to carbon dioxide emissions resulting from their usage are driving the search for sustainable and carbon-free or carbon-neutral (with no addition of new CO2 to the atmosphere) energy carriers.3] However, a large-scale renewable energy production relying on available technologies is not yet competitive with fossil-based energy production because of the substantially higher costs of production. In the European Union, as an example, the costs for electricity production from various renewable sources are: 0.30–0.80 euro per kWh from photovoltaic solar cells, 0.04–0.25 euro per kWh from hydropower, 0.07–0.19 euro per kWh from biomass, or 0.04–0.08 euro per kWh from wind turbines.4] The cost from fossil fuels ranges between 0.03 and 0.05 euro per kWh. Even though the cost of renewable energy is at least two to three times higher than that of fossil energy, a clear downward trend over the past decades is being observed, also because of quick technological improvements. In this context, biotechnological processes catalyzed by microorganisms are receiving increasing attention for the production of renewable liquid and gaseous biofuels. Among the latter, biomethane and biohydrogen hold a great potential because they are energy carriers that can be stored and used for several applications, including transportation, heating, electricity, or even chemicals production.

In terms of economics, hydrogen gas ($6/kg) is more valuable than methane ($0.43/kg), and it can be directly converted into electricity in fuel cells producing only water as waste product, whereas methane requires a preliminary reforming step that would reduce the amount of electricity that can be generated. Hydrogen could become an important energy vector in the next decade or two, both in the transportation sector (fuel cell vehicles) and for decentralized electricity generation,5] provided that cost effective and safer fuel cell technologies, hydrogen storage systems, and related infrastructures are developed. Methane, on the other hand, can be stored and distributed in the existing natural gas infrastructures, and its utilization in gas engines for production of electricity and recoverable heat is an already mature technology. The use of methane as a transportation fuel also holds a significant potential because it would drastically reduce NOx, particulate, and other emissions that are derived from the use of fossil fuels other than natural gas.
On the basis of these considerations, much research efforts are currently being dedicated to optimize existing bioprocesses for hydrogen and methane production to make them more and more affordable. In parallel, novel technologies, such as the bioelectrochemical systems (BESs), are being developed, and this review aims at giving an overview over the different approaches with a main focus on the emerging BES processes.

### Anaerobic digestion: an established technology for biomethane production

Biomethane is currently produced through the anaerobic digestion (AD) process that is an established technology for the treatment of wastewater and solid residues, including sewage sludge, manure, and the organic fraction of municipal wastes. The final product of AD is ‘biogas’ that mainly consists of methane (55–75 vol%) and carbon dioxide (25–45 vol%), whose relative amounts largely depend on the substrate used.

The AD is a multistep biological process involving series and parallel reactions, each one brought about by a characteristic group of microorganisms. These different microorganisms work closely together while metabolizing organic material in an oxygen-free environment.

The first step, hydrolysis, is the conversion of nonsoluble biopolymers (proteins, carbohydrates, and lipids) to their soluble monomers (amino acids, sugars, glycerol, and long chain fatty acids), and it is generally the rate-limiting step of the overall process.[7] The hydrolysis is catalyzed by extracellular enzymes excreted by fermentative bacteria. Soluble amino acids, sugars, and alcohols are then fermented into a variety of products (such as ethanol, butyrate, propionate, acetate, hydrogen, etc) by the fermentative bacteria, without an external electron acceptor. The final fermentation products depend on both the initial substrate and the environmental conditions.

The degradation of long chain fatty acids proceeds via a β-oxidation that requires the hydrogen ion H⁺ acting as an external electron acceptor. Anaerobic β-oxidation consists of the subsequent removal of acetic acid molecules from the fatty acid chain until acetic acid (in the case of an even number of carbon atoms) or propionic acid (in the case of an odd number) remain. Molecular hydrogen is the final sink for electrons, and it must be kept at low partial pressure (10⁻⁴–10⁻⁵ atm) for the oxidation reaction to be thermodynamically feasible. The microbial group responsible for this metabolism is referred to as obligate hydrogen-producing acetogens (OPHA). In the AD, these bacteria must co-exist in syntrophic relation with hydrogen-utilizing methanogens to allow an efficient removal of the produced hydrogen and maintain hydrogen at the needed low level.[8] Hydrogen-utilizing methanogens are responsible for the hydrogenotrophic methanogenesis, an anaerobic respiration in which hydrogen and carbon dioxide serve as electron donor and acceptor, respectively, causing the formation of methane gas. Another metabolic pathway leading to the formation of methane is the acetoclastic methanogenesis in which the C-C carbon bond of acetate is cleaved to form methane (from reduction of the methyl group) and carbon dioxide (from oxidation of the carboxylic group). Methanogens are strictly anaerobic microorganisms that belong to the domain of Archaea which substantially differ from Bacteria in terms of membrane structure and cell wall chemistry.[9]

Finally, the conversion of hydrogen and carbon dioxide into acetate as end product could also occur. This reaction, known as homoacetogenesis, is catalyzed by homoacetogenic bacteria that are characterized by a low affinity for hydrogen and, therefore, are often outcompeted by hydrogen-utilizing methanogens.

A simplified scheme of the AD process is given in Fig. 1, which also incorporates the steps of dark fermentation (see the following section).

The coexistence of different microorganisms, characterized by distinct kinetic traits, in the same reaction environment makes the AD process strongly affected by operational parameters (e.g. pH, temperature, organic load rate, hydraulic and biomass retention time, hydrogen partial pressure, etc). To operate the AD stably, it is vital that the various biological reactions remain sufficiently coupled during the process to prevent the accumulation of intermediate compounds. Generally, hydrolysis and acidogenesis are operated by bacteria that grow faster and are more tolerant to fluctuations of operating conditions than syntrophic acetogens and methanogens. For example, methanogenesis is adversely affected by halogenated compounds, heavy metals, xenobiotics, and pH values higher than 7.5 or lower than 6.5. Therefore, an accumulation of volatile fatty acids, for example, due to a sudden increase of the organic load, will result in a decrease of pH under which conditions methanogenesis cannot occur anymore, and as a consequence, the hydrogen partial pressure will rise. If the hydrogen partial pressure becomes too high, volatile fatty acids longer than acetate (i.e. the direct precursor of methane via acetoclastic methanogenesis) accumulate,[10] with a further inhibition of methanogens.

In spite of that, the simplest full-scale AD processes comprise of a single-stage digester in which all the phases of the process take place. Moreover, in general, AD processes can be broadly divided in ‘low-rate’ systems, with hydraulic retention times (HRT) between 30 and 60 days, and ‘high-rate’ systems, in which the HRT is relatively short (few hours). Additionally, the HRT in low-rate systems is equal to the sludge retention time (SRT), whereas high-rate systems are operated at HRT much lower than SRT. To accomplish the latter objective, all high-rate processes have a mechanism.
either to retain microorganisms in the reactor or to separate bacterial sludge from the effluent and return it to the reactor. High-rate systems are therefore divided into systems with fixed bacterial biofilms on solid surfaces or systems with a suspended bacterial mass where the high SRT is achieved through external or internal settling. Examples of high-rate systems are: contact process, anaerobic filter, fluidized bed, anaerobic sequencing batch reactors, and upflow anaerobic sludge blanket reactors (UASB). The UASB technology efficiently retains anaerobic sludge in the reactor by the formation of biological granules with good settling properties and it is the most common AD technology used worldwide. More complex configurations, which are modifications of the UASB design, are the internal circulation reactor and the anaerobic hybrid reactor.

Although single-stage reactors are simpler to construct and operate, it is also possible to carry out the AD process in two-stage systems (Fig. 2) consisting of two separate reactor vessels that run sequentially. A main advantage of this configuration is the possibility to separately control the operating conditions of each reactor whereby hydrolysis and acidogenesis mostly occur in the first reactor, and the syntrophic acetogenesis and methanogenesis mostly occur in the second reactor; thus, both reactors can be optimized with respect to the feeding rate, HRT, SRT, pH, and temperature. As a result, two-stage digesters have an enhanced stability, increased methane production, and reduced reactor volumes that can be sufficient to justify the higher costs of construction and operation.

Regardless of reactor configurations, AD generally takes place at either mesophilic or thermophilic temperatures with optima at 35°C and 55°C, respectively. However, even though the rate of methane production typically increases with temperature, the optimum temperature for methanogens may not necessarily be the optimum for the other microorganisms involved in the process. In large-scale AD reactors, a part of the energy contained in the produced biogas is utilized on site to provide the energy requirement of the plant (i.e. digester heating, pumps, mixers, etc), and the balance between energy requirement and biogas yield is a primary factor to determine the choice of the working temperature.
Established bioprocesses for hydrogen production

Biological hydrogen production can be achieved via different processes, including biophotolysis (direct or indirect), photofermentation, and dark fermentation.

In direct biophotolysis, microalgae split water into hydrogen and oxygen in the presence of light as energy source (2 H₂O + light → 2 H₂ + O₂). A main drawback of this process is the strong inhibition of hydrogen-producing enzymes (hydrogenases) by the simultaneously evolved oxygen. This problem can be potentially circumvented with the indirect biophotolysis approach, which involves two separate stages. The first one is the light-driven water oxidation into oxygen and the carbon dioxide fixation into organic substrates (6 H₂O + 6 CO₂ + light → C₆H₁₂O₆ + 6 O₂); the second stage involves the further light-driven conversion of organic substrates into hydrogen (C₆H₁₂O₆ + 6 H₂O + light → 12 H₂ + 6 CO₂). The overall reaction yields up to 12 mol of H₂. Another route of photobiological hydrogen production is photofermentation that is carried out predominately by nitrogenase-possessing photosynthetic bacteria. Under nitrogen-deficient conditions, these bacteria convert organic acids and other substrates into hydrogen and carbon dioxide using light energy to drive the otherwise energetically unfavorable reaction (CH₃COOH + 2 H₂O + light → 4 H₂ + 2 CO₂). The large costs of photobioreactors and the low efficiency with which light energy is used to produce hydrogen (the so-called photobiological efficiency) represent the two major bottlenecks of photobiological hydrogen production processes.¹⁵,¹⁶

The most promising among currently used technologies for biohydrogen production is dark fermentation. This process, also referred to as fermentative hydrogen production, is carried out by a wide variety of microorganisms, such as Clostridiaceae and Enterobacteriaceae,¹⁷,¹⁸ in the absence of light. In nature, hydrogen production is a strategy that these microorganisms use to dispose of the excess reducing equivalents (e.g. in the reduced form of nicotinamide adenine dinucleotide, NADH) resulting from their primary metabolism under anaerobic conditions. The production of hydrogen is generally associated with the presence in the microorganism of an iron-sulfur protein called ferredoxin, a very low potential electron carrier. The transfer of electrons from ferredoxin to H⁺ is then catalyzed by the enzyme hydrogenase.¹⁹,²⁰

Even though many organic compounds enable the production of hydrogen during dark fermentation, carbohydrates are the preferred carbon sources.²¹ The complete oxidation of glucose to hydrogen and carbon dioxide would yield a stoichiometry of 12 mol of hydrogen per mol of glucose, but under standard conditions and neutral pH, this reaction is energetically unfavorable (ΔG⁰ = +3.2 kJ/mol).²² The maximum achievable hydrogen yield, by all known microbiological routes, is only 4 mol of H₂ per mol of glucose, and it is obtained when acetic acid is the by-product (C₆H₁₂O₆ + 2 H₂O → 4 H₂ + 2 CH₃COOH + 2 CO₂; ΔG⁰ = −206.3 kJ/mol). If other fermentation products more reduced than acetate (such as lactate, ethanol, butyrate, or butanol) are produced, the hydrogen yield is limited to a maximum of 2 mol H₂ per mol of glucose. The steps involved in the dark fermentation are highlighted in Fig. 1.

However, actual hydrogen yields may be even lower because of consumption of the produced H₂ by methanogens and homoacetogens.²³ Several approaches have been studied to increase hydrogen yields of dark fermentation, such as the inhibition of hydrogen-consuming microorganisms that is commonly accomplished by operating reactors at low pH²⁴ or short hydraulic retention time.²⁵ This latter concept could be, for example, implemented in the two-stage AD process (Fig. 2), whereby the first reactor is much smaller than the second one. The H₂ would be generated in the first reactor, mainly from the acidogenic conversion of soluble starches and sugars contained in the influent stream, whereas the second larger reactor would produce methane gas.

A two-stage process that combines dark fermentation and photofermentation has been also investigated.²⁶ In the first stage (dark fermentation), carbohydrates are converted to hydrogen, carbon dioxide, and organic acids that are further converted into hydrogen in the second stage (photofermentation). However, this process could become promising only if cost-effective photobioreactors will be developed.²⁷

Biological hydrogen production shares many common features with methanogenic AD, especially the relative ease with which the two gaseous products can be separated from the treated wastewater. On the other hand, biological hydrogen production requires hydrogen-producing microorganisms to prevail over hydrogen-consuming microorganisms, whereas successful biological methane production requires the presence of various and closely cooperating groups of microorganisms.

BIOELECTROCHEMICAL SYSTEMS: FUNDAMENTALS AND APPLICATIONS

Bioelectrochemical systems (BESs) employ solid-state electrodes to directly or indirectly stimulate and control microbial metabolism. Stimulation originates from the ability of ‘electro-active bacteria’ to exchange electrons with the electrodes, which accordingly serve as electron acceptors or donors in their energy metabolism.²⁸ In a BES, at least one of the anodic or cathodic reactions is microbially catalyzed; for the anodic reaction, the electrode serves as an electron acceptor for the oxidation of a reduced substrate, whereas for the cathodic reaction, the electrode serves as an electron donor for the reduction of an oxidized substrate (Fig. 3). A key
A feature of electro-active bacteria is their ability to transport electrons inside and outside the cell, which permits them to function in BESs. At least two mechanisms of electron transfer can be discerned: these are direct and indirect (or mediated) transfers (Fig. 3).

The first mechanism relies on physical contact between the bacterial cell and the electrode. To establish this electrochemical connection, microorganisms utilize cytochromes or other redox active components (such as pili or nanowires) located on the outer membrane. The indirect mechanism involves the redox cycling of electron shuttles (also known as redox mediators) between the microorganisms and the electrode. These electron shuttles can be exogenous or endogenous. Exogenous mediators can be either naturally present organic or inorganic compounds, such as humic acids and sulfur species, or externally added, such as viologens and quinones. Endogenous electron shuttles are produced as secondary metabolites by microorganisms, such as flavins and phenazines.

Extensive research has been performed to elucidate both mechanisms, in many cases using iron-reducing bacteria such as *Shewanella* spp. and *Geobacter* spp. as model microorganisms. However, an increasing number of publications show that a wide variety of bacteria (other than iron reducers) can also participate in extracellular electron transfer processes. Indeed, the analysis of mixed microbial communities in BESs revealed a high degree of diversity and was hinted at microbial interactions driving the electron flow. As an example, recent analysis of the community profiles of electro-active microbial consortia in BESs fed with different fermentable substrates (e.g. ethanol, glucose, cellulose, and wastewater) revealed the existence of syntrophic partnerships among fermentative bacteria converting the organic substrates and electro-active bacteria (typically *Geobacter* species) oxidizing fermentation end products (with an anode serving as electron acceptor) and, by so doing, relieving feedback inhibition for the fermentative consortia and allowing a rapid conversion of organics.

Overall, owing to their versatility, unmatched level of control over the biological reactions, and capacity to sustain a wide range of (bio)chemical processes, BESs hold a great potential for application in the broad field of industrial and environmental biotechnologies and particularly for bioenergy generation.

### Bioelectrochemical system for electricity generation: microbial fuel cell

The BES archetype is the microbial fuel cell (MFC) that is commonly regarded as a sustainable technology for electricity generation and simultaneous wastewater treatment. Similar to any other bioelectrochemical system, an MFC consists of an anode and a cathode, typically separated by an ion exchange membrane. At the anode (negative terminal), microorganisms catalyze the oxidation of organic or inorganic substrates by using the electrode as the electron acceptor. The electrons flow from the anode to the cathode (positive terminal) through an external electrical circuit containing a resistor or a load (i.e. the device being powered). Generally, the electrons that reach the cathode combine with protons that diffuse from the anode through the membrane and oxygen provided from air; the resulting product is water (Fig. 4). Oxygen is the most sustainable electron acceptor in MFCs because of its availability in the environment and its high redox potential; nevertheless, to achieve a sufficiently high oxygen reduction rate at the cathode,

![Figure 3. Direct or mediated extracellular electron transfer mechanisms at the cathode of a bioelectrochemical system.](image)

![Figure 4. Schematics of a microbial fuel cell: at the anode, organic material from the wastewater is oxidized by electrochemically active microorganisms that transfer the gained electrons to the electrode. Via an external circuit, the electrons are transported to the cathode, where they are consumed for oxygen reduction.](image)
platinum-based or cobalt-based and iron-based materials are commonly used as catalysts.\cite{46,47}

To date, many substrates have been investigated as possible energy sources to generate electrical power in MFCs. These include carbohydrates (e.g. glucose, sucrose, cellulose, and starch), volatile fatty acids (e.g. formate, acetate, and butyrate), alcohols (e.g. ethanol and methanol), amino acids, proteins, and even inorganic components such as sulfides or acid mine drainage.\cite{48–51} Although the use of a pure or single substrate allows the study of the metabolic processes and conversion products during the microbial conversion, it is certainly not feasible to power full-scale MFCs with pure substrates from an economical point of view. In this perspective, the use of organic waste streams is highly attractive because it allows combining the actual treatment of the waste stream with the generation of valuable and renewable energy. A range of more complex organics, containing a large variety of different readily and non-readily degradable molecules such as domestic wastewater, brewery wastewater, paper recycling wastewater, or the effluent of anaerobic digesters have also been demonstrated to sustain electrical power generation in MFCs.\cite{52–54} Nevertheless, the nature of the substrate affects both the composition of the bacterial community in the anode biofilm and the MFC performance including the power density and coulombic efficiency (i.e. the relative amount of electrons recovered from the substrate as electric current).\cite{55} Moreover, the power outputs achieved using wastewater are often much lower compared with those attainable with pure substrates.

To assess the practical viability of MFCs, their performance in terms of both wastewater treatment capacity (i.e. substrate conversion rate) and bioenergy generation potential (i.e. electrical power generation) can be compared with that of conventional AD systems. For instance, high-rate anaerobic reactors can be operated at organic loading rates as high as 25 kg COD/m³ day; in an MFC, the same substrate conversion rate would correspond to a volumetric current density of around 3500 A/m³ (considering that 1 kg COD can be converted into approximately 12 × 10⁹ Coulombs). So far, the highest current density achieved with state-of-the-art BES under sustained operation is 595 A/m³.\cite{56}

To calculate power generation of AD, it should be first considered that approximately 1 kWh of usable electrical energy is obtained from the conversion of 1 kg COD into methane. During the conversion of biogas into electricity via cogeneration, up to other 3 kWh are typically recovered as heat and used to warm up the digester or get lost.\cite{57,58} By considering a high-rate digester that operates at an organic loading rate of 25 kg COD/m³ day, the resulting volumetric power density would be around 1 kW/m³. So far, in spite of the great scientific advancement that have been made during the last decade, the maximum reported power densities of MFC typically stall around 0.1 kW/m³.\cite{57} largely due to various losses mainly deriving from mass transport and activation limitations, which limit the energy efficiency of the system. Notwithstanding the existing limitations and the currently higher installation costs, the MFC technology holds some specific advantages over AD, such as: the applicability for the treatment of dilute wastewater, the possibility to operate at low temperatures, no need for gas handling and/or cleaning, and the greater control over biochemical conversion processes. All these features make the technology attractive for specific application niches.\cite{58}

Moreover, it should be noted that electrical power may not be the only product MFC systems can generate. Indeed, the electrons released from organic carbon oxidation can potentially be exploited at the cathode for the generation of reduced value-added products such as hydrogen, as it happens in microbial electrolysis cells. An increasing number of publications show that producing a chemical product would provide considerably larger economical and environmental benefits compared with generating electric power.\cite{59}

Bioelectrochemical systems for hydrogen generation: microbial electrolysis cell

In the last years, much research effort is being dedicated to the development of BESs for H₂ production (based on the MFC technology). The process, named microbial electrolysis cell (MEC), differs from a conventional MFC for having an anaerobic cathode where the electrons (released at the anode from the microbial oxidation of organic substrates) reduce protons to H₂ [E°⁺ = −0.414 V vs standard hydrogen electrode (SHE)] in the presence of a suitable catalyst (Fig. 5). MECs are similar in the anode design to MFCs, but there are some important differences in the cathode design. Indeed, because the final product is hydrogen rather than electricity, the architecture must be modified for collecting the produced gas.\cite{60}

In most cases, to drive H₂ production at the cathode of an MEC, the voltage generated from the microbial oxidation of a substrate at the anode needs to be boosted with an external power supply. In theory, when acetate is used as substrate, an applied voltage of 0.14 V is needed, under standard conditions, to make H₂ production energetically favorable. In practice, larger voltages (0.2–1.0 V) must be applied because of the occurrence of different potential losses that decrease the energy that can be gained from the reaction.\cite{61,62} The required voltage, however, is substantially lower than that needed for H₂ production from water via electrolysis, which is typically in the range 1.6–2.0 V.\cite{63}
fermentative H2 production, i.e. the need of using only the potential to overcome the two major bottlenecks of are physically separated from the electron-consuming releasing reactions (oxidation of organic waste materials) low external voltage. Moreover, because the electron-generate hydrogen gas using BESs that require a organic matter instead of water, it is possible to directly produce hydrogen gas at the cathode. Thus, by deriving the protons and electrons from organic matter instead of water, it is possible to directly generate hydrogen gas using BESs that require a low external voltage. Moreover, because the electron-releasing reactions (oxidation of organic waste materials) are physically separated from the electron-consuming H2-producing reaction, the MEC approach has the potential to overcome the two major bottlenecks of fermentative H2 production, i.e. the need of using only carbohydrate-rich substrates and the low H2 yields due to the accumulation of side products (such as acetate and butyrate) which otherwise would not be further converted into H2 (unless at very low H2 partial pressure). In this context, it has also been proposed to operate an MEC in series to a dark-fermentative hydrogen production reactor to produce additional hydrogen gas from the organic acids not converted during dark fermentation. Similarly, an MEC could also operate in series to a conventional anaerobic digester by removing the residual organic matter contained in the digestate, which would otherwise represent a disposal burden and a waste of energy. The use of MEC in series to fermentative or methanogenic bioprocesses is particularly effective because volatile fatty acids are ideal substrates for electro-active bacteria being metabolized at high rates and efficiencies. However, in the current state, the design of integrated systems is complex, and no cost-effective approaches have been developed yet.

Moreover, MEC is a relatively new technology, and many of its potential advantages still need to be experimentally confirmed. The process needs to be thoroughly examined and progress evaluated on the basis of performance indicators; such as applied voltage, overall process efficiency, energy requirement, and hydrogen production rate. Furthermore, also the use of materials as cathode catalysts needs to be critically assessed and optimized. Indeed, noble metal (e.g. Pt-based) catalysts are generally used on the cathode of MECs to enhance the rate and efficiency of hydrogen production. These noble metal-based catalysts are expensive and susceptible to poisoning. Various low-cost materials have been also investigated, but they typically exhibit insufficient chemical stability and/or reactivity at neutral pH for efficient MEC operation. Among non-noble metal cathode catalysts, Ni-based ones hold a great potential, being characterized by a relatively low overpotential for hydrogen evolution, low cost, and a high chemical stability. In a recent study, a high H2 volumetric production rate (up to 50 m3/m3 day) was obtained in an MEC equipped with a high specific surface area Ni foam cathode, operated at an applied voltage of 1.0 V.

More recently, the use of hydrogenase-possessing microorganisms has been proposed as a more sustainable alternative to metal catalysts for H2 production. Because of their metabolic versatility, microbial biocathodes offer the potential to produce a variety of value-added products (other than hydrogen), as discussed in the following section.

Microbial biocathodes: novel catalysts for biofuels production

In conventional BESs, a biocatalyzed anode is combined with an abiotic cathode containing a suitable catalyst for the target reaction. Platinum is generally used as catalyst for both oxygen reduction and hydrogen generation at the cathode of MFCs or MECs, respectively. However, the production of platinum is an environmentally detrimental process that directly conflicts with the sustainable nature of MFCs and MECs. Other metals have also been tested, but recently, there has been an increasing interest in replacing chemical cathodes with biological cathodes in which biological agents catalyze the target reaction. Two types of biocathodes can be distinguished: enzymatic biocathodes or microbial biocathodes. Several enzymes can be effectively ‘wired’ to solid electrodes; as an example, laccases have been used for the oxygen reduction reaction in MFCs, whereas hydrogenases and nitrogenases have been tested as hydrogen evolution electrocatalysts in MECs. The high costs of enzymes isolation, difficulties in attaching these delicate molecules onto electrocatalytic surfaces while protecting their fragile active sites from inactivation, have however greatly hampered their practical application.

The recent discovery that living microorganisms can directly or indirectly (via redox mediators) consume electric current, thereby wiring their metabolism to cathodes,
has resulted in an increased scientific interest towards the development of microbial biocathodes. At least in principle, microbial biocathodes are inexpensive, self-regenerating, can effectively operate under neutral pH conditions and are not susceptible to corrosion.

The versatility of microbial respiration pathways makes microbial biocathodes a promising way to catalyze a wide range of reduction processes in BESs. Indeed, biocathodes have been tested for oxygen reduction, nitrate and fumarate reduction, as well as for bioremediation processes. The application of microbial biocathodes for biofuels production is a new and emerging field. In a pioneering study, it was shown that a pure culture of Desulfovibrio vulgaris immobilized on carbon electrodes could catalyze H₂ production with methyl viologen (E°' = −0.446 V vs SHE) serving as redox mediator. More recently, other researchers showed that hydrogenophilic dechlorinating bacteria could also catalyze H₂ production either in the presence or in the absence of the redox mediator. Specifically, in the presence of methyl viologen, both a Desulfotobacterium-enriched and a Dehalococcoides-enriched cultures could produce H₂ at −0.450 V vs SHE; hence, a cathode potential very close to the reversible H⁺/H₂ potential value. The obtained rates of H₂ production were however relatively low (0.01 m³/m³ day).

Interestingly, in the absence of methyl viologen, only the Desulfotobacterium-enriched culture was capable of catalyzing H₂ production (via direct extracellular electron transfer) at cathode potentials lower than −0.700 V vs SHE. Even though the obtained rates of H₂ production were relatively low and not comparable with those reported for noble metal-catalyzed cathode, it was pointed out that they could greatly be enhanced by increasing the biomass density at the electrode surface. The observed ability of dechlorinating bacteria to catalyze H₂ production was attributed to the abundance in these microorganisms of hydrogenases, the enzymes catalyzing the interconversion of H₂ and water in the microbial H₂ cycle. Bioelectrocatalytic H₂ production was also reported for Geobacter sulfurreducens and for a naturally selected mixed culture fed with bicarbonate. These latter microbial biocathodes were also successfully applied in fully assembled MECs.

In a recent study, the microbial composition of a H₂-producing biocathode of an MEC was analyzed. The predominant microorganism in the mixed culture was D. vulgaris, pointing to a key role of this microorganism in the bioelectrocatalytic H₂ production process. The biocathode operated at −0.700 V vs SHE produced a current of 1.1 A/m² and 0.63 m³ H₂/m³ cathode liquid volume per day. The enhanced H₂-producing capability of Desulfovibrio species has been also reported in a very recent research paper in which the biocathode set at −0.900 V vs SHE produced up to 3 A/m² current density and 0.2 m³ H₂/m³ cathode liquid volume per day.

So far, the main perceived limitations of H₂-producing biocathodes are the slow start-up before a stable current density is reached and the low H₂ production rates. An attempt to address both issues was made by some researchers, who were able to show that by feeding a mixed culture biocathode with acetate (instead of bicarbonate) as the carbon source, the start-up time could be substantially reduced. According to the authors, the faster start-up was likely caused by a higher biomass yield for acetate compared with bicarbonate. To increase the H₂ production rates, the authors used a flow-through system; the maximum obtained production rate was 2.2 m³ H₂/m³ reactor per day at a cathode potential of −0.700 V vs SHE.

Microbial biocathodes can also be employed to produce methane from carbon dioxide reduction (E°' = 0.244 V vs SHE). For these biocathodes, two distinct mechanisms of methane production have been identified: (1) direct reduction of CO₂ with methanogens accepting electrons from a polarized cathode and (2) indirect (H₂-mediated) reduction of CO₂ with methanogens using abiotically (or biotically) produced H₂ gas as electron donor in their metabolism. In principle, the production of methane at the cathode of an MEC could exploit both the electrons and (part of) the carbon dioxide released at the anode from the microbial oxidation of the organic matter contained in a waste stream.

A methane-producing MEC combines features that are typical of conventional aerobic biofilms processes, such as the possibility to oxidize diluted streams, with those of conventional anaerobic biofilms processes, such as the possibility of energy recovery in the form of methane (Fig. 6).

Such a process potentially holds some specific advantages compared with a traditional AD process. These include the physical separation of the organic matter oxidation from methane generation that allows producing a biogas that is richer in methane and protecting methanogens against inhibitory compounds possibly contained in the waste stream. Moreover, less thermal energy (if any) is needed to control the temperature of the cathode because the waste stream does not need be warmed up, being processed only at the anode side and, analogously to other BESs; low strength wastewater can also be treated.

Moreover, the previously discussed compatibility between the influent of BES and the effluent of conventional anaerobic digester makes methane-producing MEC a suitable and promising technology to extract additional energy (in the form of methane) from the residual organic matter contained in the effluent of the AD systems (Fig. 7).

So far, very few studies have investigated the application of MEC technology for methane production; therefore, fundamental information regarding the optimal operating conditions and achievable performances
are rather limited. In a recent study, the start-up and performance of a fully biological MEC operated at ambient temperature, consisting of a *Geobacter sulfurreducens*-enriched bioanode and methane-producing biocathode were described. The MEC was successfully started up by sequentially controlling the cathode and the anode potentials at values that are favorable to the establishment of an active methanogenic biocathode (i.e. $-0.850 \text{ V vs SHE}$) and acetate-oxidizing bioanode (+0.500 V vs SHE), respectively. The highest methane production rate (approximately $0.05 \text{ m}^3/\text{m}^3 \text{ day}$) was obtained by controlling the cathode at $-0.850 \text{ V vs SHE}$, whereas a three times lower value was achieved when the MEC was operated at a controlled anode potential of +0.500 V vs SHE. However, similar to other biocathode studies, the performance of the system was found to be primarily limited by the concentration of biomass both at the anode and at the cathode, thereby suggesting that a possible strategy to optimize the process involves saturating the electroic surfaces with microorganisms.

**OUTLOOKS AND PERSPECTIVES**

Bioelectrochemical systems represent a novel and highly versatile technology for simultaneous wastewater treatment and bioenergy generation. Although initially main attention was placed at the possibility of producing electric power with MFCs, the attention of the scientific community is now shifting on the more attractive possibility of producing renewable biofuels or even other chemicals with MECs. In this regard, the discovery that microorganisms can be employed at the cathode of these systems to catalyze the production of a variety of reduced products represents a major scientific breakthrough. Not only microbial biocathodes have been studied (in place of more expensive metal catalysts) to produce gaseous biofuels such as...
hydrogen or methane gas, but also ethanol\(^{[95]}\) and acetate\(^{[96]}\) have been obtained from the bioelectrocatalytic reduction of carbon dioxide. However, in spite of the increasing interest in their application in reactions of industrial or environmental relevance, the biochemical mechanisms involved in microbial electron uptake from a cathode still remain largely unknown. This is expected to be a main research area for future investigations.

In the perspective of gaseous biofuels production, a number of biocathodes mainly consisting of hydrogenase-possessing microorganisms have been described to produce \(\text{H}_2\). For some of them, the electron transfer from the polarized cathode to the hydrogenase was mediated by externally supplied redox mediators such as methyl viologen. It is worth noting that the application at the MEC anode of soluble redox mediators is commonly regarded as an economically unfeasible strategy due to the continuous flow of wastewater\(^{[97]}\) whereas it could be successfully employed to enhance hydrogen (or other products) formation at the cathode, which can be operated in closed loop mode. Moreover, because redox mediators such as methyl viologen were found to drastically inhibit the activity of hydrogenophilic methanogens\(^{[93]}\) their use could also possibly result in higher \(\text{H}_2\) yields compared with mediatorless systems in which \(\text{H}_2\) loss through methanogenesis is a main limiting factor.\(^{[98]}\) Clearly, the long-term stability of redox mediators in BESs is another main issue that requires further investigations.\(^{[93]}\)

Overall, the MEC process, either employing mediator or mediatorless biocathodes, allows, at least in principle, producing \(\text{H}_2\) from a variety of organic substrates including wastewater. This is made possible by the physical separation of the electron-releasing reaction (i.e. wastewater oxidation) from the electron-consuming reaction (i.e. \(\text{H}_2\) production) and the external addition of small amounts of electrical energy to overcome thermodynamic barriers, commonly limiting the hydrogen yield in dark fermentative processes. However, the \(\text{H}_2\) production rates achieved thus far with this approach are at least one order of magnitude lower compared with those obtained through dark fermentation, which are typically between 0.6 and 29 m\(^3\) \(\text{H}_2\)/m\(^3\) reactor volume per day.\(^{[18]}\)

In general, \(\text{H}_2\) production is preferred over methane production because from an economic point of view, the value of \(\text{H}_2\) is higher than that of methane. However, as already mentioned, there are several reasons for which the significance of methane in BESs should be considered, especially in consideration of the recently reported possibility of using microbial biocathodes as effective electrocatalytic agents of the methane-producing reaction.

In brief, the possibility to control both oxidation and reduction reactions at separate electrodes makes BESs a promising technology for a wide range of applications. However, a scale-up from the laboratory-scale to industrial-scale is still underway. A pilot scale MFC to treat brewery wastewater has been installed and operated in Australia; recently, a pilot scale MEC has been started at Napa Valley (California) to treat winery wastewater.\(^{[1,99]}\) To boost full scale implementation of the technology, several limitations and challenges need to be further faced. First of all, more experience with real wastewaters is required so that strategies can be developed for improving the degradation of complex materials and controlling the desired reactions occurring both at the anode and the cathode of the systems. Moreover, new reactor designs are needed to minimize electrochemical losses and optimize performance. Additionally, the capital costs should be reduced to make the technology more economically feasible and applicable. As an example, on the basis of the materials currently used in laboratory-scale systems, the capital costs of large-scale BESs would be orders of magnitude higher than those of conventional wastewater treatment technologies.\(^{[100]}\)

On the basis of these premises, it has been suggested that bioelectrochemical wastewater treatment can become economically competitive only if its higher capital costs are compensated for increased revenue deriving from the final products, as occurs in MECs. However, the electrical energy input required in MECs still accounts for a significant portion of the operating costs, whose reduction could be achieved by powering them with photochemical cells such as dye sensitized solar cell (DSSC). The latter is a low-cost solar cell that uses a photoelectrode (dye sensitized TiO\(_2\)-coated conducting glass) and a counter electrode (platinum-coated conducting glass) to convert light energy into electrical energy. A combined MEC-DSSC process for hydrogen production, in which the electrons that originate from bacterial oxidation of organic substrates at the anode of the MEC are energized by light energy when flowing through the DSSC to the MEC cathode, has recently been proposed.\(^{[101,102]}\) Similarly, sunlight could also be used as input energy in methane-producing MEC.

Overall, gaseous biofuels seem to be excellent products to push the BES technology from the laboratory to the market.

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REFERENCES


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