Chapter 7
Valorization of Liquid End-Residues of H_2 Production by Microbial Fuel Cell

Microbial fuel cell (MFC) technology can be employed in order to add value to the metabolic products of acetogenesis fermentation after H_2 production. Considering the total conversion of glucose into acetic acid as the only bioproduct, only 1/3 of the energy available is converted in H_2 from a thermodynamic point of view (Chap. 5). Furthermore, in practical terms, the energy necessary to carry out the pretreatments must be considered as energy used to perform the energy balance, in order to render the organic contents accessible to microorganisms (Chap. 9). Nevertheless, the aim of this book is to focus on anaerobic technology and its energy sustainability; the conversion of H_2 production residues, such as VFA, into additional H_2 or CH_4 or electricity needs to be investigated. Even if the MFC technology is in its infancy, it deserves a very close attention for the possibility of generating electrical energy or additional H_2. The present chapter is devoted to explore the potential of MFC technology.

7.1 Overview of Bioroutes for Recovery of Additional Energy

The way to increase the overall energy production of an H_2 process is to extract the residual bioenergy embedded in the liquid metabolites at the end of acidogenesis. This bioenergy can be recovered as methane, or directly as electrical energy, or as additional H_2. Table 7.1 gives a list of biotechnologies that are under research studies for extracting the remaining potential bioenergy from liquid end-products, while in Fig. 7.1 the same information is reported in a more synthetic way.

7.1.1 Photofermentation

The combination of *photosynthetic bacteria* with hydrogen-producing bacteria (HPB) can provide a system for additional hydrogen production from residual

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Table 7.1 Alternative strategies for additional recovery of energy, after a first stage of H_2 anaerobic production

Second stage after H_2 production by AD	Principle and kind of produced energy	Advantages	Disadvantages
Photofermentation	N_2ase and captured solar energy used to convert organic acids to **H_2**	Solar light exploitation	• Little knowledge about potential pathways • Light-dependent
Biogas production by AD	Standard anaerobic digestion used to convert organic acids to **biogas** (**methane**)	Well-known technology widely used	• Low production rate • Large reactor volume required due to HRT greater with respect to H_2 production
Microbial fuel cells (MFCs)	Electrons realised from organic acid oxidation are used to produce **electricity**	• Directly conversion of VFA into electricity • Production of a gas without energy value (only CO_2) which does not require treatment • Long lifetime (up to 3–5 years)	• Large surface area electrodes required • Ohmic losses • Low power density (0.001–0.1 mW/cm^2)
Enzymatic fuel cells (EFCs)	Employ enzymes to catalyze the oxidation of fuel at the electrode surface, releasing **electrons**	Higher power density (1.6–4 mW/cm^2)	• Incomplete oxidation of fuel • Low lifetimes High costs
Organelle-based biofuel cells	Immobilized mitochondria on conductive electrode that convert chemical energy into **electricity**	• Power density between MFCs and enzymatic FCs • Complete oxidation of the fuel at high rate	• High costs • Necessity to immobilize mitochondria on electrode; difficult at large scale
Microbial electrolysis cells (MECs)	Electrical energy added to drive conversion of organic acids to **H_2**	Theoretical production of pure H_2	• Added energy required • Low current density • Large surface area electrodes required
Metabolic engineering	Genetic modification, gene addition to create novel **hydrogen-producing pathways**		• Unproven • Little knowledge about potential pathways

chemicals such as VFA at the end of anaerobic digestion (AD). In such a coupled system, the low-molecular-weight organic acids are converted in a second step to hydrogen by photosynthetic bacteria at the expense of light energy. Non-sulfur purple photosynthetic bacteria are able to capture light and to convert organic acids

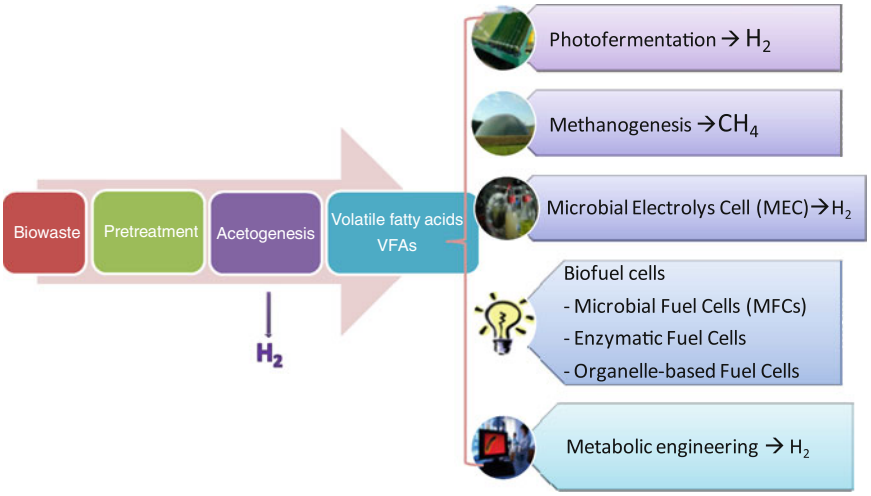

Fig. 7.1 Schematic view of alternative biological routes to harvest additional bioenergy from organic waste refuses

to hydrogen by a photofermentation reaction, where H_2 is driven by nitrogenase; the required ATP is formed via the photosynthetic capture of light energy [1]. Even though the overall yield in such a two-stage fermentation process was found to be higher compared to a single one [2], many factors restrict the practical application of this process. In fact, nitrogenase-driven hydrogen production is potentially sensitive to the nitrogen content of the medium/substrate since it represses nitrogenase synthesis and moreover inhibits the enzyme activity [1]. The most severe constraint is the fact that photosynthetic efficiencies, with either solar radiation or tungsten lamps, are very low: the majority of captured light is dissipated as heat (>80 %), even at moderate light intensities [3]. The combinations of many technical parameters present very significant hurdles for the development of a full-scale process, with high costs, e.g. for transparent impermeable photobioreactors with enhanced surface area.

7.1.2 Biogas Production

Biogas production by AD in a second stage, here called two-step anaerobic digestion (TSAD), is the process that appears to be the most suitable for reaching the maturity of a full plant application in a short time. It consists of the separation into two stages of the natural metabolism of an anaerobic bacteria consortium

present in a digester. This approach will be treated in detail in Chap. 8, along with the results obtained by a pilot plant at laboratory scale using fruit and vegetable refuses as feed.

7.1.3 Microbial Fuel Cells

MFC technology represents the newest approach to bioelectricity generation from biomass using bacteria. In an MFC, microorganisms mediate direct conversion of chemical energy stored in organic matter into electrical energy: the oxidation of various fermentable substrates (e.g. glucose and sucrose), complex substrates (e.g. domestic wastewater and paper wastewater) and organic matter present in aquatic sediments produces electrons under microbiological activities at the anode; these electrons are recovered on the electrode surface and they travel through an external circuit to reach the cathode, where a reduction reaction occurs.

7.1.4 Microbial Electrolysis Cells

A *bio-electrochemically assisted microbial reactor* (BEAMR), or more simply a *microbial electrolysis cell* (MEC), permits the generation of hydrogen gas and other reduced products at the cathode using organic electron donors. The terms "electrochemically" and "assisted" are used in this process because additional voltage is required by the circuit compared with MFC [4]. An external power supply can support the process and therefore circumvent thermodynamic constraints that, in its absence, render the generation of hydrogen impossible [5]. Examples of electron donors might be acetate and wastewater. The anodic oxidation of acetate is the same reaction occurring in the anode of a MFC that harvests electrical energy through an external resistance. In the case of MECs, hydrogen is produced at the cathode with platinum as a catalyst. The separation between the anode and cathode chambers of a MEC is accomplished with an ion-selective membrane (cation exchange membrane, CEM). CEMs are used to obtain cathodic hydrogen gas that is as pure as possible. However, CEMs give rise to higher ohmic voltage loss in the cell and a pH gradient over the membrane, resulting in a lower current production for a given applied voltage. Removing a part of the membrane helped to minimize the pH gradient between anode and cathode: Clauwaert and Verstraete [6] conducted operations of MECs without an ion exchange membrane and found that this could help to lower the construction costs, to lower the ohmic cell resistance and to improve MEC conversion efficiency by minimizing the pH gradient between anode and cathode. They demonstrated that membrane-less MECs with plain graphite can be operated for methane production without pH adjustments and that the ohmic cell resistance

was reduced by approximately 50 %; as a result, the current production increased from 66 to 156 A m^{-3} MEC with an applied voltage of –0.8 V. In this condition, methane was the main product.

7.1.5 Metabolic Engineering

Another possible path towards increasing hydrogen yields is to alter the metabolism of microorganism species through *metabolic engineering* by genetic manipulation in order to overcome the thermodynamic/metabolic barriers. These tools are not yet well developed and there is a lack of studies. Several avenues can be pursued in the future in attempts to introduce NAD(P)H-dependent hydrogenase/pathways. In a recent study [7], a ferredoxin-dependent NAD(P)H:H_2 pathway was introduced into an *E. coli* strain expressing a [Fe–Fe] hydrogenase, the maturation enzymes and ferredoxin. Some increase over basal activity was noted, and variations in hydrogen production led the authors to conclude that the system was thermodynamically severely limited, and thus impractical. However, levels of expressed hydrogenase activity are quite low in this strain, making it difficult to draw definitive conclusions. Another suggested approach is to use reverse electron flow to generate reduced ferredoxin with enough reducing power to drive hydrogen evolution by hydrogenase [1]. Of course, this requires energy input and it was suggested that a small amount of electrons from the respiratory chain could be used to generate an electrochemical gradient that would drive ferredoxin reduction. This approach has not yet been tested and it is not known how to construct the appropriate pathways. Thus, metabolic engineering pathway deserves some criticism, but with further investigations the metabolic system could be adapted to drive higher biohydrogen production by AD.

7.1.6 Mitochondria-Based Fuel Cells

Mitochondria are present in the cytoplasm of living cells of animals, plants and fungi, and are responsible for energy conversion. This organelle, called “the powerhouse of the cell” contains the enzyme and coenzymes of the Krebs’ cycle and the electron transfer chain, but unlike a cell it has no need to transport fuel across the cell wall and therefore mitochondria can completely oxidize fuel at a faster rate, ensuring that no toxic byproducts are produced as waste. This organelle-based fuel cell theoretically can provide power densities between those of enzyme-based fuel cells (EFCs) and MFCs, with a lifetime and efficiency similar to that of MFC. A proof of the concept was made for the first time by Arechederra and Minteer [8], who immobilized mitochondria within tetrabutylammonium bromide-modified Nafion membrane, obtaining a power density of 0.2 mW/cm^2, lower than

those obtainable in both MFCs and EFCs. Moreover, they obtained a complete oxidation of pyruvate into carbon dioxide without an external mediator with stability of up to 60 days. However, this pathway seems to be difficult to apply to full-scale plants that valorize waste, at least at present.

7.1.7 Enzyme-Based Fuel Cells

EFCs utilize isolated enzymes as catalysts for fuel oxidation at the anode and reduction at the cathode. Appropriate choice of enzymes permits such reactions to occur under relatively mild conditions: neutral pH and ambient temperature. In addition, immobilization of specific enzymes for reaction on electrodes, such as carbon, can eliminate the separation into compartments of components required in conventional MFCs [9]. EFCs have the advantage of higher power density (1.65–4.1 mW/cm^2) [10] but they are limited by incomplete oxidation of fuel at the electrode surface. Due to the exclusion of such components, EFC have the capacity to be miniaturized and, consequently, micrometer-dimension membrane-less EFCs have been developed [9, 11]. However, due to the short lifetime of enzymes and their specificity, they are not indicated as a second step for H_2 production.

7.2 MFCs: Principles and Applications

The MFC is a promising green technology that permits the conversion of biodegradable materials present in wastewater directly into electricity. In the anode chamber, the decomposition of organic substrates by microorganisms, via the respiratory chain, generates electrons (e^-), which are transferred to the cathode through an external electric circuit, and protons (H^+), which travel via CEM to the cathode [12]. A schematic view of a MFC is shown in Fig. 7.2.

The anode and cathode chambers are separated by a CEM. On the anode side a bio-electrochemical reaction occurs between microorganisms and a fuel/substrate which is oxidized, releasing CO_2, electrons and protons to the solution. The CO_2 produced moves toward the top of the anode, protons are transported to the cathode chamber through the membrane and electrons are harvested on the electrode surface and go through an external electric wire; the bacteria grow on the anode, colonizing it. The cathode chamber is sparged with air to provide dissolved oxygen at the electrode surface for the reduction reaction; a resistor completes the circuit, utilizing the electrical energy.

The following equations illustrate the redox reactions occurring in a MFC in the case of glucose as fuel:

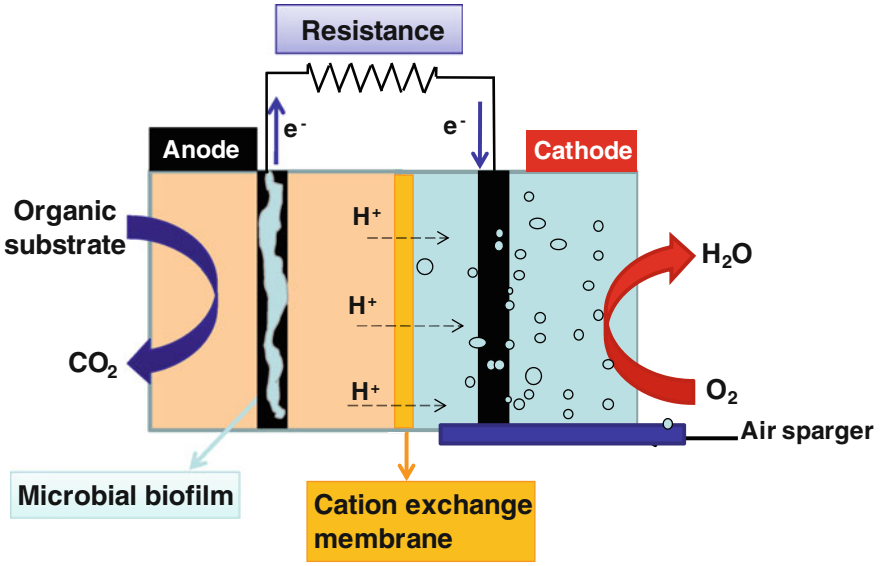

Fig. 7.2 Schematic views of components of a microbial fuel cell

$$\text{Anode: } C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (7.1)$$

$$(\text{Theoretically } \Delta G_0 = -2{,}840\,\text{kJ/mol})$$

$$\text{Cathode: } 24H^+ + 24e^- + 6O_2 \rightarrow 12H_2O \quad (7.2)$$

In the case of the use of VFA formed in the first acidogenic step for H_2 production, the following theoretical reactions should be taken into account in the case of the use of acetate, where $8e^-$ per mol are produced:

$$\text{Anode: } C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^- \quad (7.3)$$

$$\text{Cathode: } 8H^+ + 8e^- + 2O_2 \rightarrow 4H_2O \quad (7.4)$$

similarly, butyrate produces $4e^-$ per mol:

$$\text{Anode: } C_4H_8O_2 + 2H_2O \rightarrow 2C_2H_4O_2 + 4H^+ + 4e^- \quad (7.5)$$

Among various energy technologies (e.g. incineration, gasification, bioethanol-based fermentation, AD for bioH_2 and biogas production), the MFC presents a number of attractive features, such as direct electricity generation, high conversion efficiency and a reduced amount of sludge production [13]. Although the functionality of a MFC is similar to that of a chemical fuel cell, as they both include

reactants, two electrodes (anode and cathode), the MFC has the following substantial advantages:

- working at ambient temperature and pressure;
- using neutral electrolytes and avoiding expensive catalysts (e.g. platinum);
- not using a traditional fuel supply but organic wastes;
- not needing to be recharged/replaced after exhaustion, as happens for lithium ion batteries.

Conversely, there are still several problems associated with a low yield of power generation, such as biofouling, adequate and lower cost of the electrode material, cation transport rather than protons, and high costs of the system causing a decrease in the global energy efficiency.

However, the use of MFCs has great potential for a broad range of applications, e.g. electronic power sources for space shuttles, self-feeding robots and also as an additional sustainable step in wastewater treatment plants, especially in remote situations, where difficulties in electrical energy supply can arise. In fact, MFCs can potentially be installed in environments that are not easily reachable, where bacteria flow in the MFC compartments can be self-sustained by the surrounding environment. Even if not suitable for high energy demands, the MFCs can generate enough power to permit microelectronics and sensor systems to run at acceptable specifications. The main challenge for MFC technology is to replace the energy-expensive aerobic wastewater treatment, alone or in association with AD plant. Virtually any biodegradable organic material can be used in a MFC, including VFA, carbohydrates, proteins, alcohols and even recalcitrant materials like cellulose [4]. Furthermore, large amounts of excess sludge are produced, requiring an appropriate treatment and disposal. MFCs are therefore a valid option for extracting the energy still embedded in liquid residues of acidogenic fermentation thanks to VFA-oxidizing microorganisms. The bacterial reaction can be carried out over several different temperatures ranges, depending on the tolerance of the bacteria, ranging from mesophilic (15–35 °C) to thermophilic (50–60 °C) and psychrophilic temperatures (<15 °C). The current that can be generated by an anodic biofilm typically increases with the amount of active biomass attached to the electrode. MFCs exhibit low coulombic efficiencies (the ratio of the electrical charges harvested to the input charges produced by the oxidation reaction) determined by the internal resistance of the device, mainly due to inefficient electron transfer between the microbial cells and the anode. This inefficiency results in incomplete oxidation of the fuel, the production of liquid metabolites and the adsorption of organic substrates used as fuel on the biomass; all these problems could be addressed by future research programs.

7.2.1 Anode Microbiology

In a bioanode, electrochemically active microorganisms, generally bacteria, oxidize a substrate (electron donors) and transfer these electrons to the electrode (Fig. 7.3).

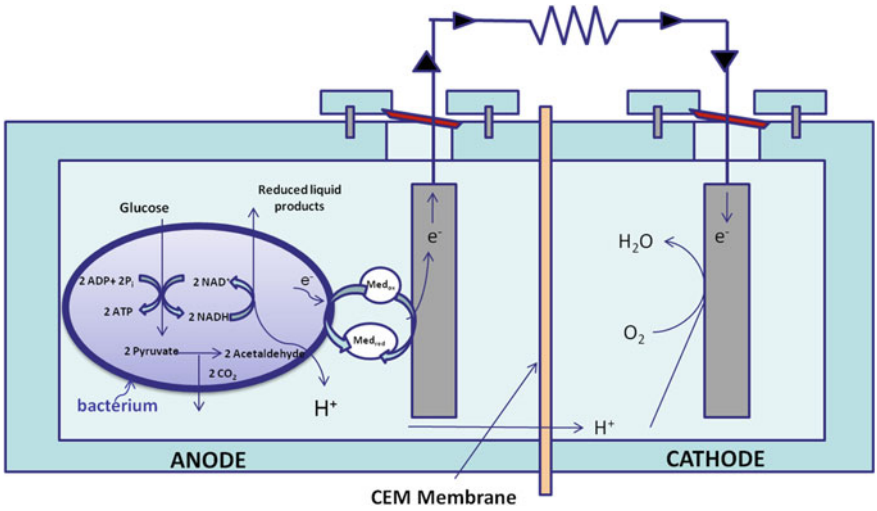

Fig. 7.3 Working principle of an MFC

In anaerobic conditions, reducing equivalents generated during fermentation move through a series of redox components towards an available terminal electron acceptor. In this way, a proton motive force is generated, helping to produce energy-rich phosphate bonds in ATP, which is useful for microbial growth and subsequent metabolic activities. MFCs are mainly based on harvesting available electrons using non-biological materials (electrodes) as intermediate electron acceptors [14].

Electron transfer to the electrode can occur by different mechanisms grouped into two different modes, *direct electron transfer* (DET) and *mediated electron transfer* (MET), based on the carrier involved (Fig. 7.4). DET occurs due to a physical contact between bacterial cells (e.g. cytochromes and nanowires) and anode, without the involvement of any redox species or mediator. *Geobacter*, *Rhodoferax* and *Shewanella* are the most-studied bacterial genera that use DET through membrane bounds for electron transfer. MET indicates the involvement of mediators for extracellular electron transfer from the biocatalyst to the anode. The mediators may be added artificially or may be shuttles naturally excreted by bacterial activity as primary and/or secondary metabolites [15].

In *bio-electrochemical systems* (BES), depending on the configuration as well as the intended application, the microbial catalysts can be an axenic culture or a mixed culture. MFCs that use mixed bacterial cultures have some important advantages: a lower sensitivity to process disturbances, larger substrate versatility and higher power generation [16].

While in axenic culture the bio-electrocatalysis is attributed to the activity of only one bacterial culture, in a BES operating with a mixed culture the interactions within members of the entire microbial community determine the so-called electrochemically active consortium. These electrochemically active consortia could be

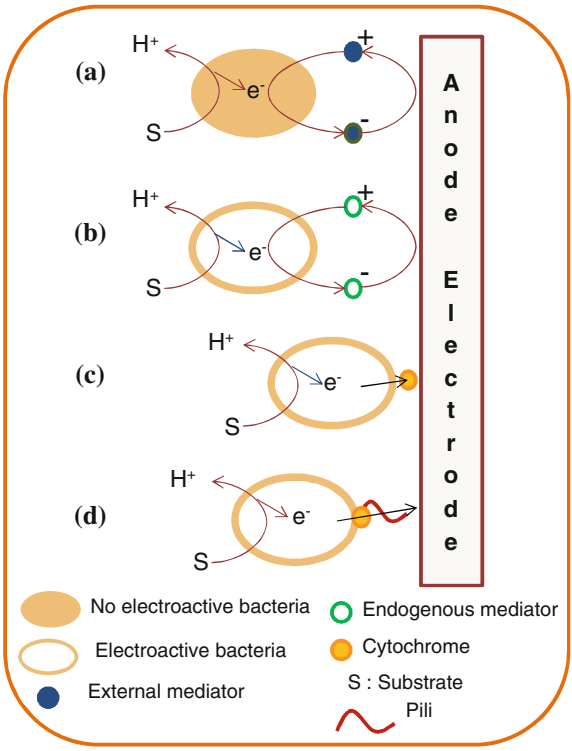

Fig. 7.4 Schematic illustration of electron transfer: **a** addition of artificial mediator; **b** "natural" redox mediator; **c** direct surface contact; **d** direct electron transfer

obtained by either sediment (both marine and lake sediment) or activated sludge from wastewater treatment plants by subsequent enrichment techniques. By means of molecular analysis, electrochemically active species of *Geobacter* spp., *Desulfuromonas* spp., *Alcaligenes faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Clostridium* spp., *Bacteroides* spp., *Aeromonas* spp. and *Brevibacillus* spp. have been detected in mixed consortia. The microbial community established on the bioanode may vary in a mixed culture depending on the inoculum, type of substrate, operational conditions and reactor design. It is evident that a change in the community composition will influence the efficiency of the corresponding anode performance [17] but, to date, no typical composition for the anode community has been found [18]. However bacteria belonging to the taxonomic class of *Proteobacteria* usually comprise the majority of the bioanode community [19, 20] on different electrode materials. In addition many bacteria which have been isolated from the mixed culture communities can be characterized as metal reducers or as other types of anaerobic respirers [21, 22].

The study of the electron transfer mechanisms within microbial biofilms is one of the most challenging aspects of MFC research. Many microorganisms possess the ability to transfer the electrons derived from the metabolism of organic matters to the anode. Microbes transport electrons to the electrode through an electron transfer system which consists of many components in the bacterial extracellular matrix,

together with electron shuttles dissolved in the bulk solution. *Geobacter* belongs to the metal-reducing microorganisms which produce biologically useful energy in the form of ATP during the reduction of metal oxides under anaerobic conditions in soils and sediments. The electrons are transferred to the final electron acceptor, such as Fe_2O_3, mainly by direct contact of mineral oxides and metal-reducing microorganisms. The anodic reaction in MFCs without external mediators belongs primarily to the families of *Shewanella*, *Rhodoferax* and *Geobacter*, where the anode acts as the final electron acceptor just like the solid mineral oxides. *S. putrefaciens*, *G. sulferreducens*, *G. metallireducens* and *R. ferrireducens* transfer electrons to the solid electrode (anode) using this system. Mediators play an important role in electron transport for those microbes that are unable to transfer the electrons to the anode; they take electrons from microbes and discharge them at the anode surface. *Actinobacillus succinogenes*, *Desulfovibrio desulfuricans*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, and *Pseudomonas fluorescens* need external mediators, while some microbes can provide their own. For example, some dominant Gram-negative bacteria such as *Pseudomonas* spp. can produce metabolites, such as pyocyanin and/or phenazine-1-carboxamide, which can act as electron shuttles (mediators), not only for *Pseudomonas* but also for other species. When an MFC is inoculated with anaerobic sludge, mixed-culture microbes are in the anode chamber and interact with themselves, enhancing the overall performance and allowing different substrate utilization.

A diversity of mechanisms by which microorganisms may transfer electrons to the anode of a MFC has been proposed, as shown schematically in Fig. 7.4. Currently, artificial mediators are employed only in specific cases, for example using *Saccaromyces cerevisiae*, which is not able to produce endogenous redox mediators. Today, much attention is directed to the study of natural shuttling of electrons via soluble compounds (Fig. 7.4b) and direct contact (Fig. 7.4c, d).

7.2.2 Electrical Parameters

The difference between positive cathodic and negative anodic potentials is considered to be the cell voltage which drives the electron flow from anode to cathode, called the electromotive force (*emf*). According to standard electrical principles, due to the positive potential difference (ΔV) between cathode and anode of a MFC, the flow of electrons (I) generates a useful power (P):

$$P = I \cdot \Delta V \tag{7.6}$$

The ratio between the voltage and the current is determined by the external resistance (R_{ext}) according to Ohm's law:

$$\Delta V = I \cdot R_{ext} \tag{7.7}$$

When the external resistance is infinite (open circuit conditions), no current flows and the open circuit voltage (*OCV*) is obtained. Conversely, when the R_{ext} is

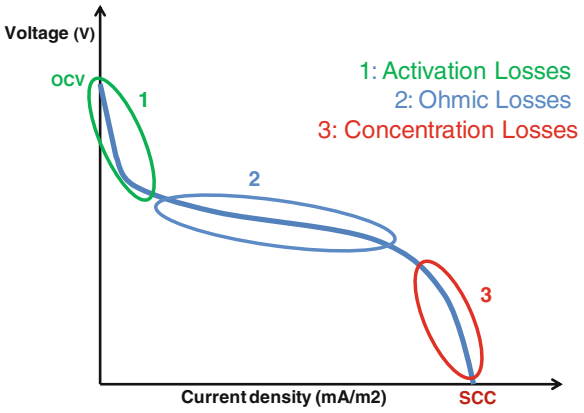

Fig. 7.5 Voltage–current polarization curve; the *circles 1*, *2*, *3* represent losses occurring

zero (short circuit conditions, $\Delta V = 0$), the short circuit current (I_{scc}) is generated. Alternatively, the relationship between the cell voltage and the current (density) can be calculated by a polarization curve (Fig. 7.5). Despite the microbial nature of the process, it is affected by electrochemical laws and principles which generally result in lowering the attainable voltage [23] and, consequently, in limiting the power generation of MFCs. In order to improve performance, a wide range of techniques have been utilized, to understand either scientific fundamentals or the role of materials, as well as to investigate MFC performance bottlenecks [24].

The power performance curve can be calculated by considering the experimental evaluation of the polarization curve (Fig. 7.6). The power delivered by a fuel cell is maximized when the external load matches the internal resistance of the fuel cell

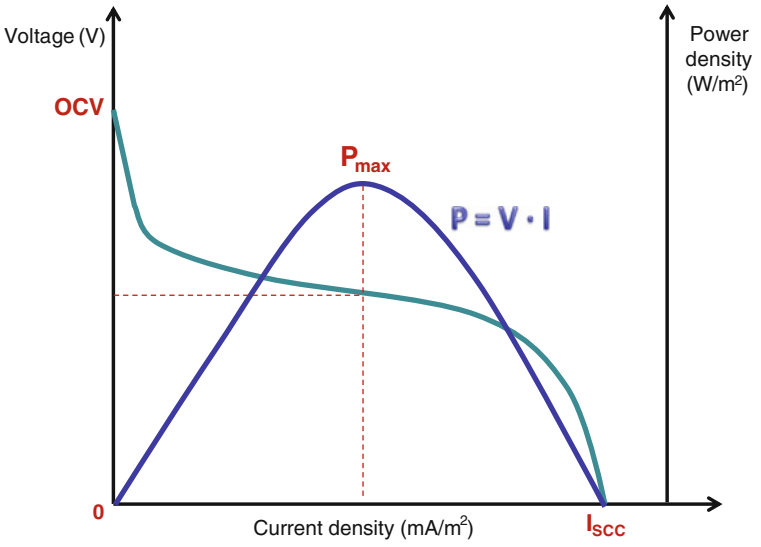

Fig. 7.6 Cell voltage and power density as a function of the current density

system [25]. The microbial system that leads to electrical production is dependent on the total electron transfer chain, i.e. from bacteria to anode and from cathode to final electron acceptor. While the overall thermodynamics are quite favorable for anaerobes, there are numerous internal resistance mechanisms that diminish the potential use of the electrons at the cathode via the anode [26]. The bacteria involved in electricity production are able to overcome the resistance posed by the system. One way to minimize losses is to operate the MFC under optimal conditions for power generation using an optimal external resistance [27], which is usually correlated with the internal resistance of the MFC [28], following electrochemical theory supported by biological investigations.

The following equation is used for monitoring the energy production under an external resistance as electrical load during MFC usage.

$$\text{Energy (J)} = \int_{t_0}^{t_{final}} V \cdot I \cdot \mathrm{d}t \tag{7.8}$$

7.3 Application of MFCs

The MFC is able to deliver power outputs at the desired voltage or current by connecting the electrical polarities of several MFC devices in series or in parallel. Therefore, the system could permit linkage of the advantage of wastewater treatment to the recovery of electricity.

MFC principles have been demonstrated at laboratory-scale devices even though real full-scale applications are currently limited by power yield and long-term performance. One of the first applications could be the development of pilot-scale reactors using the digester effluents of a bioreactor producing H_2 using food processing wastewater as feed. In the long term, more dilute substrates, such as domestic sewage, could be treated with MFCs, decreasing the need for substantial amounts of electrical energy for the treatment. Furthermore, the conductive materials used to manufacture electrodes play an important role in MFC performance (e.g. power generation), costs and energy sustainability.

Many alternative applications could also emerge, ranging from biosensor development and energy generation from the seafloor, to MFCs operating on various biodegradable fuels. A simple monitoring system has been reported [29], comprising an ultra-lowpower impulse-radio ultra-wide-band transmitter (TX) and a nanostructured piezoresistive pressure sensor connected to a digital read-out circuit requiring 40 μW powered by two MFCs connected in series. Tests conducted with a laboratory prototype with a total volume of 0.34 L, continuously fed with sea water and a synthetic substrate, has given 0.7 ± 0.1 V as open circuit voltage and 3 ± 1 A m^{-2} as short circuit current; the maximal power density was 0.6 ± 0.2 W m^{-2} (Fig. 7.7).

Fig. 7.7 MFC design used to conduct tests for continuous generation of electricity

The success of specific MFC applications in wastewater treatment will depend on the concentration and degree of biodegradability of the organic matter in the influent, the temperature, and the absence of toxic chemicals. The growing pressure on the environment and the call for renewable energy sources will further stimulate development of this technology, soon leading to its successful implementations. MFCs undoubtedly have potential in terms of energy recovery during wastewater treatment: they may occupy a niche market in terms of a stand-alone power source and also in the direct treatment of wastewater.

7.4 Integrated Bioenergy Production System

An environmental and sustainable alternative to the traditional wastewater treatment plant is represented by an integrated biological system which produces hydrogen using organic waste; it comprises two promising and innovative technologies, TSAD (H_2 + CH_4) and MFC (Fig. 7.8).

The incoming biomass is first hydrolyzed (mechanical/chemical or enzymatic treatment), then fermented by TSAD and finally used in a MFC. These biological processes permit purification and transformation of waste by microorganisms into energetic products: either H_2 or CH_4, and electricity. Biogases rich in H_2 and CH_4, after an upgrade for CO_2 removal, are subsequently converted in a *combined heat and power* module (CHP). The heat obtained from the CHP is usually returned to the digester to ensure the working temperature, which could be in the mesophilic or thermophilic range. The electrical power (direct from MFC and indirect from AD) can be used for running reactors, and the overflow is used to furnish an energy

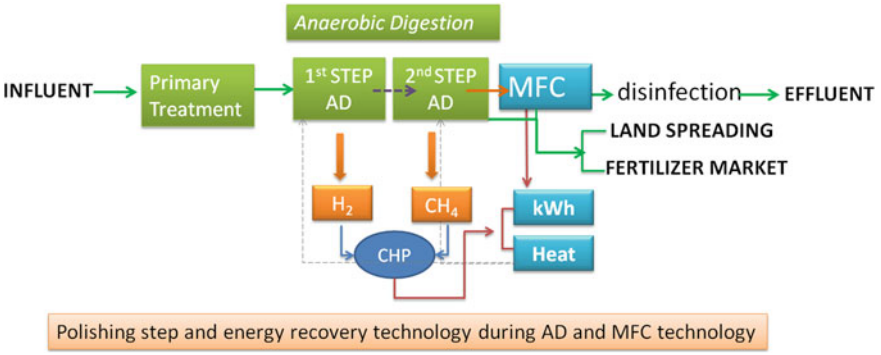

Fig. 7.8 Conceptual suggestions for alternative processes for wastewater treatment and energy recovery

service to society. The MFC step, unlike AD, needs to be fuelled by more hydrolyzed biomass, such as VFA. In fact, suspended and particulate organic matter is difficult to process because it can cause clogging of the system, which poses a serious threat to technical operations. In contrast, the AD system is able of dealing with either suspended or particulate waste streams.

Direct benefits of using the suggested integrated system as an alternative to the usual aerobic treatment could be the lack of need for aeration and the recovery of electrical energy useful for the wastewater plant, saving in this manner the energy cost. In fact, aerobic treatments cost about 0.4 € kg^{-1} COD removed. In contrast, AD permits a slight positive balance thanks to the energy recovery, saving 0.06 € kg^{-1} COD removed. MFC technology is in the middle at the moment, costing about 0.29 € kg^{-1} COD removed [23].

The production of hydrogen and biogas at mesophilic temperatures, combined with no need for external electrical energy input, permits the energy sustainability of the production process. An MFC completes the $H_2 + CH_4$ gas production system, contributing to lowering the energy costs of AD technology, even if the power density generation is low ($\sim$10 mW/L anode). This integration could be a promising challenge for organic waste residues because its implementation can give a positive energy contribution, after, however, ensuring an adequate disposal system. The TSAD process integrated with MFC technology could increase the efficiency of harvesting the energy embedded in the substrate as chemical bonds into H_2 and CH_4, compared with the standard anaerobic process for biogas production.

7.5 Experimental Study

Here, two case studies are reported: H_2 and electricity production by MFC using sodium acetate as fuel; this choice is aimed, in the first instance, to simulate the treatment of metabolite residues obtained at the end of H_2 production by dark fermentation.

7.5.1 Production of H_2 from Acetate by MEC

A MEC prototype made of two Plexiglas frames (8 cm × 8 cm × 2 cm per frame) was used; the total reactor volume of 0.256 L is the sum of the total anodic and cathodic compartments both connected with a recirculation vessel, as showed in Fig. 7.9. The anode and cathode frames were completely filled with granular graphite and connected to the external electric circuit with a graphite rod (5 mm diameter, Morgan, Belgium). A CEM (Ultrex CMI7000, Membranes International Inc.) was used to separate the anode and the cathode chambers. The medium contained 6 g L^{-1} $Na_2HPO_4 \cdot 2H_2O$, 3 g L^{-1} KH_2PO_4, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.1 g NH_4Cl, 0.0146 g L^{-1} $CaCl_2$ and trace elements; 1 g of sodium acetate ($C_2H_3O_2Na$) was fed in discontinuous mode to the anodic frame upon depletion. Peristaltic pumps (Watson Marlow) were used to circulate the anodic and cathodic liquid at 6 L day^{-1}. The external vessel of the anodic compartment was inoculated with 10 mL anaerobic sludge, taken from the anode of running MFCs in order to have a conversion of acetate into H_2; tests were performed at room temperature (22 ± 2 °C). A power supply was used to obtain an applied voltage of 800 ± 2 mV. The current was measured by placing a 1.07 Ω resistor in the electrical circuit. A data acquisition unit (HP 34970A, Agilent, USA) was used to record voltage and current every minute. The coulombic H_2 recovery was calculated as the ratio of the actual current produced and the theoretical one obtainable from the substrate. The gas volume was measured with a water replacement method, while the composition of the gas produced, methane and carbon dioxide, was analyzed with an Intersmat IGC 120MB gas chromatograph; the qualitative hydrogen presence was determined by a H_2 sensor (OPUS, Zellweger Analytics, UK). Polarization curves were obtained with a potentiostat at a scan rate of 0.2 mV s^{-1} after an open circuit stabilization of 15 min.

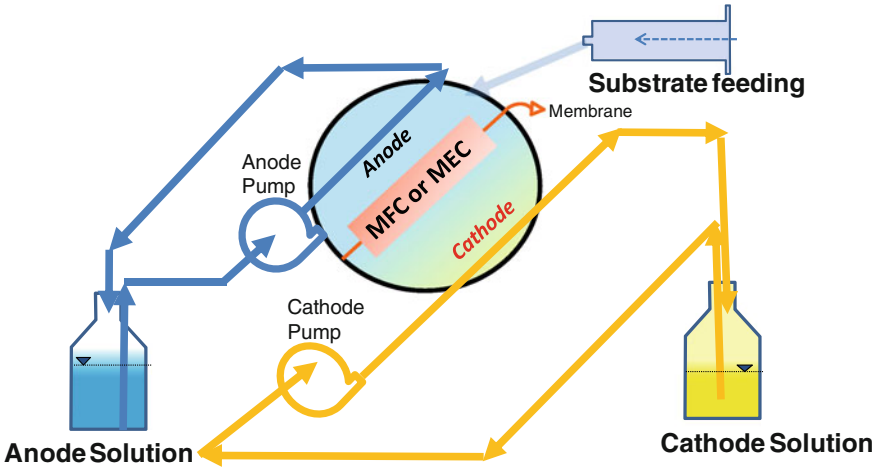

Fig. 7.9 Schematic overview of an MFC or MEC with anode and cathode chamber connected with a recirculation pump and separated by CEM membrane

7.5.1.1 Experimental Results

Figure 7.10 shows that after 2 days of lag phase the number of moles of electrons (Coulombs) sharply increases, and the cumulative H_2 production follows the same curve. This is due to the direct proportionality between Coulombs (mol e^-) and hydrogen. The coulombic H_2 recovery expresses the recovery of electrons as current generated over the amount of electrons dosed as sodium acetate, which is 84.4 %. The generated current recorded $I(t)$ was used to calculate the amount of electron recovery versus time. Sodium acetat was oxidized in the bioanode by microorganisms while H_2 was electrochemically produced in the cathode. The following half reactions describe the reactions occurring:

$$-\text{Anode: } CH_3COONa \rightarrow Na^+ + CH_3COO^- \tag{7.9}$$

$$CH_3COO^- + H_2O \rightarrow CH_3COOH + OH^- \tag{7.10}$$

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+ \tag{7.11}$$

$$-\text{Cathode: } 8H^+ + 8e^- \rightarrow 4H_2 \tag{7.12}$$

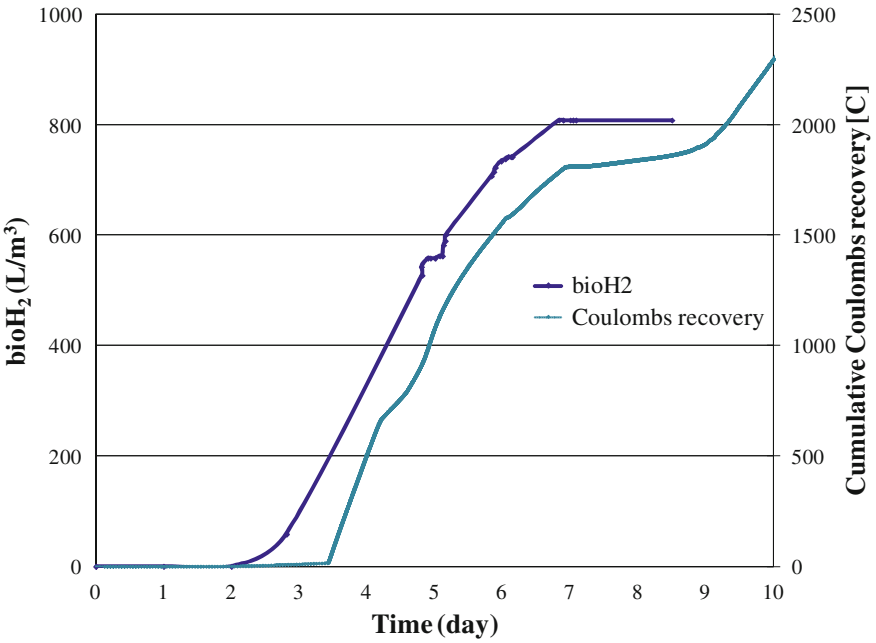

Fig. 7.10 Cumulative chemical H_2 and Coulomb production in MEC test batch-fed with sodium acetate

Table 7.2 Experimental yield and efficiency of MEC tested

Efficiencies of the MEC	
Y_{H2}	2.4 $mol_{H2}/mol_{Sodium\ acetate}$ or 5.31 $g_{H2}/g_{Sodium\ acetate}$
$\eta_{H2} = Y_{H2}/Y_{theoretical}$	60 %
Theoretical V_{H2}	0.29 L
V_{H2} obtained	0.21 L
$\eta_{vol\ H2} = V_{H2,obtained}/V_{H2,theoretical}$	70.69 %

Other process efficiencies are indicated in Table 7.2: the high coulombic H_2 recovery, based on the amount of substrate consumed, demonstrates that sodium acetate is a good substrate as electron donor at ambient temperature also.

The CEM proved to be very useful into achieve a high flux of H^+ protons from anode to cathode. The CEM used in a MEC produces relative pure hydrogen gas at the cathode [30]. The CEM, however, causes a higher ohmic cell resistance and a build-up of a pH gradient across the membrane, causing a lower current production for a given applied cell voltage [6, 31] with such an alkalinization effect, more dominant in poorly buffered solution. When the CEM is omitted in the MEC, methanogenesis can easily become dominant and gas composed of carbon dioxide, hydrogen and methane is produced. Other tests conducted with the same configuration of Fig. 7.9 but without the separation with an ion-selective membrane (CEM) confirm the production of a high value H_2 + CH_4 gas; methane production in a MEC may also be more robust than hydrogen production.

Hence, an MEC (either with or without CEM membrane) installed after a conventional anaerobic digester to remove the residual organics present in the effluent of the digester could be a practical finishing step at ambient temperatures.

7.5.2 Production of Electricity from Acetate by MFCs

The MFC device used (Fig. 7.7) consists of two circular frames, anode and cathode; both compartments were made in polymethyl methacrylate (PMMA) with an internal diameter of 12 cm and thickness of 1.5 cm (internal volume for each chamber ~170 ml) separated by a robust cation exchange membrane (CEM, CMI 7000, Membranes International Inc., Glen Rock, NJ, USA). Carbon felts (soft felt SIGRATHERM GFA5, SGL Carbon, Germany) were used in both chambers as a planar anode electrode and assembled with a graphite rod (diameter 5 mm, SGL Carbon, Germany) to ensure an effective electron recovery capability. The experiments were conducted at room temperature (24 ± 2 °C). MFCs were inoculated in the anode chamber by sea water, previously enriched with subsequent culture inoculum in anaerobic conditions. The first 5 days of tests were conducted in batch mode, in order to permit the adaptation of bacteria to the new conditions inside the MFC. After the start-up period, MFCs were operated in semi-continuous mode (fed-batch)

using a syringe pump (NE-1600 Programmable Syringe Pump, USA) with HRT of 3 days and organic loading rate of 1 g L^{-1} day^{-1}. The medium contained other nutrients such as Na_2HPO_4, NaH_2PO_4, CH_3CO_2Na and peptone, dissolved in a solvent comprising 30 % of distilled water and 70 % of filtered fresh sea water, in order to guarantee a good support of oligoelements and vitamins. The cathode compartment was filled with potassium ferricyanide (6.58 g L^{-1}), used as an oxidant compound, and a buffer solution of mineral salts Na_2HPO_4 (8.2 g L^{-1}) and NaH_2PO_4 (5.2 g L^{-1}). The mixing of the solutions at both anode and cathode chambers was obtained by the recirculation of anolyte and catholyte with a 500 mL reservoir, at a high flow rate (30 mL min^{-1}) by multichannel peristaltic pumps at both anode and cathode chambers (Peri-Star Pro 4 and 8 channel, USA, respectively).

Electrochemical experiments were run on a multi-channel potentiostat (BioLogic) in a two-electrode set-up configuration: a working and counter electrodes, inserted in anode and cathode positions, respectively. Measurements were recorded using EC-Lab® software version 10.1x (BioLogic) for data acquisition. Experiments were performed to measure cell open circuit voltage (OCV), current density and linear sweep voltammetry behavior, in order to gain information on the dynamics of electron transfer and, hence, on power generation. Polarization curves were measured at a scan rate of 1 mV s^{-1}. The power density was calculated from the *I–V* curves by $P = IV/v$, where I, V and v represent current, voltage output and medium anode volume, respectively. Furthermore, both anode potential (referred to Ag/AgCl electrode) and total MFC potential were continuously monitored for 1 month in open circuit voltage conditions and under resistances (1000, 820, 330, 220 Ω), by using a Data Acquisition Unit (Agilent, 34972A). The current interrupt (CI) technique was used in order to obtain information on the internal resistance of the MFC and its dependence on electrode materials and operating conditions. The CI method was carried out using a perturbation to the system with a very short duration (50 ms); when the MFC produced a stable current output (I) at fixed potential (0.30 V), the circuit was opened, and thus an initial steep potential (V_R) was followed by a further slow increase in the potential (V_A). The steep increase is related to the ohmic losses caused by the internal resistance (R_{int}), which can be calculated as $R_{int} = V_R/I$. Carbon felt was used as the anode electrode because it exhibits high mechanical strength and good conductivity; furthermore, its plane structure reduces the distance between the two electrodes, improving MFC performance.

7.5.2.1 Experimental Results

The evolution of open circuit voltage and maximal power density (P_{max}) over time was observed through polarization tests (Fig. 7.11). At the start, the uncolonized anodes were initially inactive and hence, during the first 5 days of operation, only the cell potential at OCV conditions was monitored, in order to avoid perturbation of the system, considering that in the first period only the acclimatization of bacteria to the new conditions inside MFCs occurs. After 5 days of operation, the operating voltage was increased to values greater than 0.3 V, indicating a successful start-up,

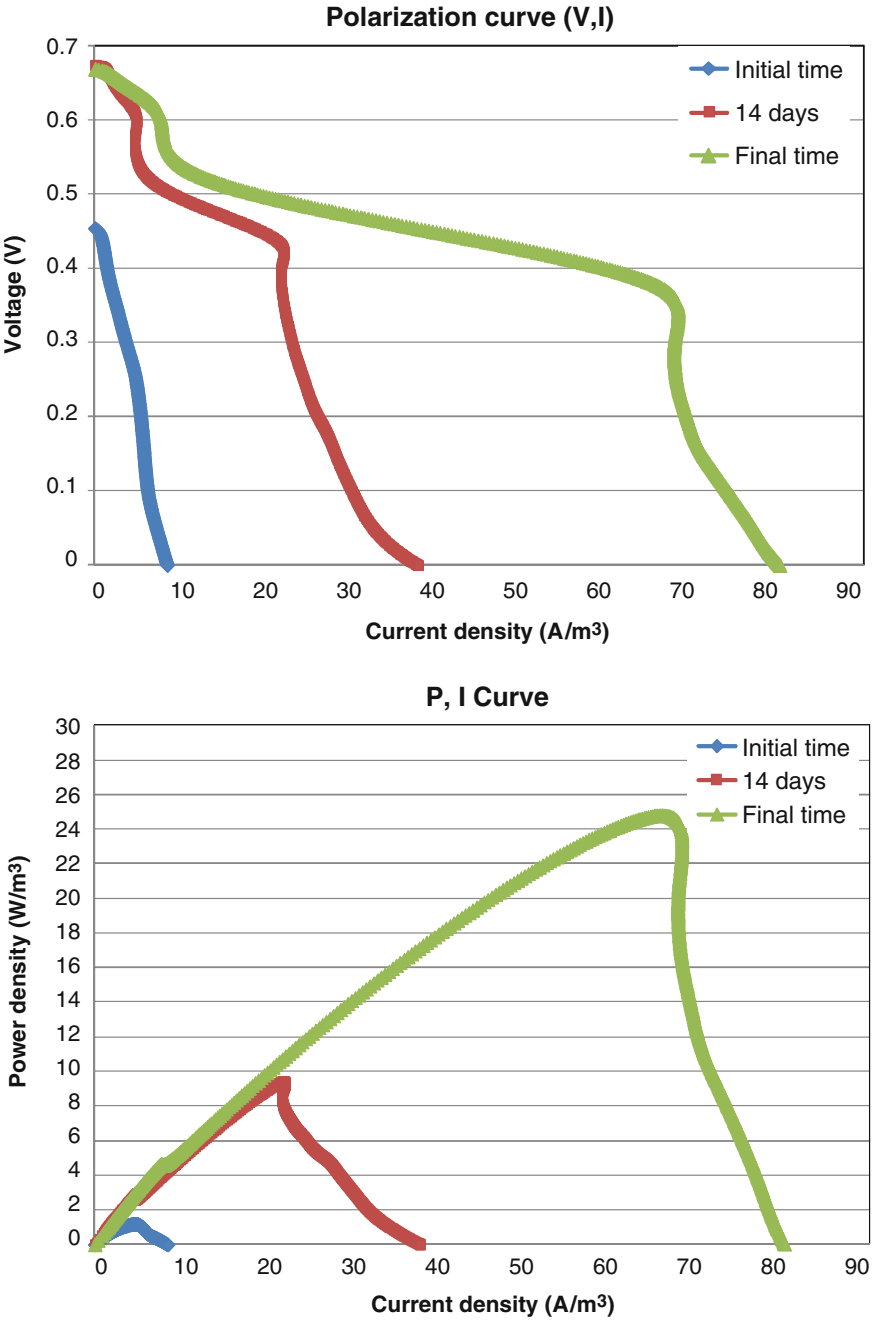

Fig. 7.11 *V–I* and *P–I curves* for the MFC with carbon felt as anode at the start time, after 14 days and final time of test running

Table 7.3 Electrical parameters during time course of MFC test

Time (days)	OCV	P_{max} (mW)	P_{max} (mW m^{-2})	P_{max} (W m^{-3})	I_{SCC} (mA m^{-2})	R int (Ω)
0	0.45	0.2	18	1.2	127.0	37.4
3	0.68	1.4	134.6	8.3	603.7	7.4
14	0.67	4.2	373.0	24.8	1204.3	7.4
27	0.71	3.3	290.0	19.3	920.5	6.5

confirmed also by the turbidity of the anode compartments. The internal resistance was determined using the CI method; accordingly to the *I–V* curves, the internal resistances decreased by about 14 % between the start time and the 10th day. This reduction can be explained by the growth of reducing microorganisms at the anode, which colonize the electrode material creating a biofilm on the carbon felt surface.

Colonization improves the electron transport mechanisms between cell membrane and anode and hence reduces the internal resistance, which limits the current output. Carbon felt gives the best performance in terms of maximal power output (P_{max}), reaching a value of about 25 W m^{-3} after 14 days (Table 7.3). The results are in accordance with other results obtained using mixed populations [32]; moreover, stable operating conditions were successfully achieved after 10 days and for more than 3 months of test running. As a consequence, the internal resistances decreased about fivefold (∼83 %) compared with the initial values, with a positive effect on the current and power densities (Table 7.3). This is a well-known issue with the CI method, since MFCs are bio-electrochemical systems in which the polarization resistance associated with the microbial activity is not negligible compared to the ohmic resistance.

Linear sweep voltammetry measurements were carried out 1 h after the replacement of fresh acetate medium at the anode and reducing cathode solution, when the power generation returned to a steady value. Moreover, good maintenance of fresh reagents in both chambers proved to be extremely important; some effects were revealed even if anolyte and catholyte recirculation was continuously applied to guarantee reduction of the influence of environmental factors, maintaining constant operating conditions (such as substrate concentration and pH) and facilitating mass transfer. For example, short circuit current (I_{SCC}) increased about sixfold after refilling, reaching 173 A m^{-3}_{anode} (2.6 A m^{-2}), and *OCV* changed from 0.54 to 0.61 V. The huge increase of current is due to the reactivation of bacteria metabolisms after famine conditions due to depletion of the fuel, which is mainly glucose, with the consequent release of electrons to the anode. Consequently, the power output also tripled, reaching 31 W m^{-3}_{anode} (∼0.5 W m^{-2}).

One of the most important parameter in evaluating MFC performance is the energy. Energy is expressed either in joules (J) or kilowatt-hours (kWh), and it can be calculated by multiplying power by time. Over a period of 1 month, changing the resistances step by step from 1,000 to 220 Ω, an energy value of 600 J (3.5 kJ/L_{anode} or 3.5 MJ/m^3_{anode}) was recovered in the present test.

Bearing in mind that 1 kWh = 3.6 MJ, a MFC in 1 month can produce 0.17 Wh with an energy density of ~ 1 Wh/L_{anode} and 1 kWh/m^3_{anode}.

This analysis shows the distance towards achieving positive energy performances using MFC technology.

7.6 Conclusion

Different biological routes to further increase the overall balance of bioenergy production are briefly presented in this chapter; routes that vary from conventional AD to novel biofuel cells, underlining the role of MFCs and microbial electrolysis cells in an integrated system.

Considering that the net energy balance of biological H_2 production via dark fermentation cannot be positive, an integrated system producing H_2, CH_4 and electrical energy via MFCs could be a promising challenger in the use of organic waste as an energy-positive waste treatment system. The two-step AD process, integrated with MFC technology, can increase the global energy efficiency of using organic wastes relative to the standard anaerobic process for biogas production.

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