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Analytical applications of microbial fuel cells. Part I: Biochemical oxygen demand

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ABSTRACT

Microbial fuel cells (MFCs) are bio-electrochemical devices, where usually the anode (but sometimes the cathode, or both) contains microorganisms able to generate and sustain an electrochemical gradient which is used typically to generate electrical power. In the more studied set-up, the anode contains heterotrophic bacteria in anaerobic conditions, capable to oxidize organic molecules releasing protons and electrons, as well as other by-products. Released protons could reach the cathode (through a membrane or not) whereas electrons travel across an external circuit originating an easily measurable direct current flow. MFCs have been proposed fundamentally as electric power producing devices or more recently as hydrogen producing devices. Here we will review the still incipient development of analytical uses of MFCs or related devices or set-ups, in the light of a non-restrictive MFC definition, as promising tools to asset water quality or other measurable parameters. An introduction to biological based analytical methods, including bioassays and biosensors, as well as MFCs design and operating principles, will also be included. Besides, the use of MFCs as biochemical oxygen demand sensors (perhaps the main analytical application of MFCs) is discussed. In a companion review (Part 2), other new analytical applications are reviewed used for toxicity sensors, metabolic sensors, life detectors, and other proposed applications.

Contents

1. Introduction

Fuel cells are electrochemical systems designed to convert energy released in a chemical reaction directly to electrical power. Unlike a battery, a fuel cell continuously supplies current as long as

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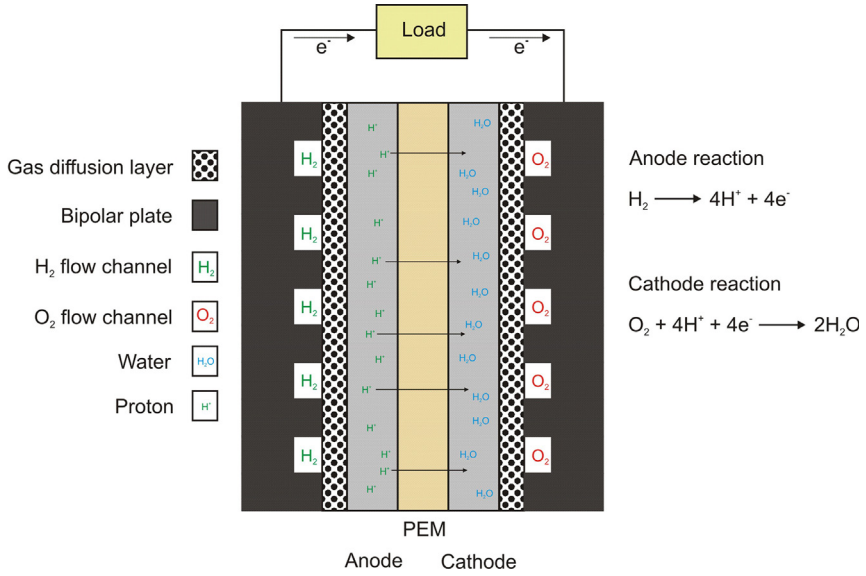

Fig. 1. PEM hydrogen fuel cell unit; microbial fuel cells are based on the same principle.

chemical reactants are available or replenished. Hydrogen fuel cells, where this gas is used as fuel and oxygen is the oxidant, are the more developed and mature systems. When oxygen and hydrogen combine to form water, energy is released because the electrons in the water molecule are in a lower energy state than those in the gas molecules. In a combustion reaction, as in a rocket engine, the energy appears as heat. In a fuel cell the majority of the released energy (typically about 50–60%) is converted directly to electrical energy (Wieckowski and Norskov, 2010). At least five types of H_2 fuel cells, frequently classified by the electrolyte they use, have been proposed up to now.

The obvious and more important application of the aforementioned electrochemical systems is the production of electricity. A main advantage of this technology is that the production of greenhouse gases can be avoided, if they are running on hydrogen derived from a renewable energy source. Although hydrogen fuel cells principles are well known in the scientific community since a long time, only when the space race to the Moon began they showed their real potential as a competitive way to produce electric energy. Hydrogen fuel cells were selected to replace batteries given the increased energy needs for more ambitious missions, as the Gemini during the early 1960s, that was powered by two 1 kW power plants designed by General Electric (NASA webpage). Later the Apollo spacecrafts launched by the NASA used similar technology, where a solid polymer membrane (Nafion® or similar membranes are used actually) was used as electrolyte. Because of that they were usually denominated solid polymer fuel cells (SPFCs), or more usually nowadays, proton exchange membrane fuel cells (PEMFCs). In Fig. 1 the general outline of this system is shown. The Gemini cell was composed of stacks of this individual cell shown in Fig. 1, in order to reach the necessary potential and power. For space exploration uses PEMFCs have several advantages over conventional batteries: they produce several times more energy per equivalent unit of weight, and not less important, as fuel cells operate, oxygen and hydrogen are combined to produce water as well as electrical power. Apollo crews used that water for drinking.

The current produced by such electrochemical systems is related to the concentration of the limiting reactant at a given concentration range. Some researchers have taken advantage of this phenomenon to develop a still secondary area of research and applications of fuel cells, as sensors or transducers. In the early 1970s this technology was incorporated into hand held instrumentation to give the first fuel cell based "breathalyzer" (Jones et al., 1977), in order to check the alcohol level in drivers. In this system the fuel is methanol. This fuel and other simple and easily oxidable substances can also be measured by fuel cells. These devices have been proposed as gas chromatography detectors (GCD) capable to detect methanol, ethanol, ethanal, diethyl ether, and others (Criddle et al., 1995). Although the use of fuel cells as GCD is not significant when compared with other widespread used detectors (as flame ionization detectors), given its limited sensibility and relatively higher complexity, this detector could be valuable, still, in some applications where simplified chromatographs are required or for other specific uses.

A particular type of fuel cells, where biological material (mainly enzymes or microorganisms) is used as bio-catalysts, became important as a very active research area in the last 20 years. Microbial fuel cells (MFCs) have been defined as devices that can convert chemical energy from organic matter to electricity in one step (Chaudhuri and Lovley, 2003; Min and Logan, 2004). Although the demonstration of electric current production by active microbial cultures and some of their relevant features ("…, the rise of the voltage being determined by the concentration of the glucose solution, the temperature, and the quantity of yeast added.") in an appropriate electrochemical cell have been shown previously in the early twentieth century (Potter, 1911), Bennetto et al. (1985) visionary work, beginning in the earlier 80s started a new, very exciting and active period in MFCs research. Also, the discovery of particular genera of bacteria, as *Geobacter* and *Shewanella*, described as capable of direct electron transfer (DET) from their metabolic pathways to a solid electrode, has awakened the interest in this very interdisciplinary area, involving at least microbiology, electrochemistry and engineering experts.

The reactions at the MFC electrodes generate a potential difference, that is usually studied in terms of the overall cell electromotive force (*emf*), E_{emf} (*V*), defined as the potential difference between the cathode and the anode. Theoretical E_{emf} using as cathodic reaction the oxygen reduction and acetate oxidation at the anode is in the order of 1.1 V. A detailed analysis of this and other electrochemical MFC characteristics were reviewed previously (Logan et al., 2006). However, several potential losses occur on the electrode surface and cell, and because of that, the maximum potentials obtained are usually lower than 1 V. The MFCs can be measured with or without an external resistor intercalated between the anode and the cathode or with a resistor (load resistor, R_L) chosen in such a way that the total discharge of

the cell is avoided and still affects the E_{emf} obtained at open circuit. Typically, larger electrodes can be measured with a lower R_L. The E_{emf} obtained without an R_L is called open circuit potential (OCV). Also, some MFCs operate with external polarization, mainly when they are used as H_2 producing devices or during an initial time period when the biofilm is expected to grow after inoculation.

To date, a myriad of different MFCs designs have been proposed by a number of groups working in this active and technologically oriented research area. Nevertheless, any fuel cell consists basically of two electrodes sandwiched around an electrolyte. In a typical two compartment fuel cell, a cation-exchange membrane (for example Nafion) separates two compartments (anodic and cathodic), where the electrodes (anode and cathode) are introduced. The anodic compartment is kept anoxic, generating a reduced environment, where heterotrophic bacteria are inoculated, whereas the cathodic compartment is kept oxidant, whether oxygen ("oxygen cathode") or another oxidant is used.

In this review we will introduce first the current uses of microbial analytical biosystems including bioassays and biosensors, which will lead us to establish valid criteria for the design of useful assays and devices. Later, MFC working principles, configurations and their possibilities in order to become valuable analytical sensors are discussed. After that, comprehensive details of the proposed analytical uses of MFCs as BOD detectors are presented, including earlier works presented from 1977 to date. Other analytical uses are also presented in the second part of this review.

2. Microbial systems proposed for analytical applications

Life in general and any microbial organism in particular have a limited set of chemical and physical conditions where their survival is possible. Indeed, when a life form is exposed to conditions that affect negatively or positively its survival then growing, metabolic rate, or any other life-related phenomena are also affected. A long time ago this observation gave birth to the use of organisms for different bioassays in order to measure insignificant amounts of highly active substances (as hormones) and later a variety of single substances or substances mixtures, including toxicological, pharmacological, clinical and environmental applications, among others.

The biological assays or bioassays were defined as "a procedure for determining the concentration or biological activity of a substance (e.g., vitamin, hormone, plant growth factor, antibiotic) by measuring its effect on an organism or tissue compared to a standard preparation" (IUPAC "Gold Book", 1997). In microbial bioassays, the biological material is not in close contact with an electric transducer (as in biosensors), the organisms are typically free in the liquid media (ISO, 2007), or growing over jellified substrates (ISO, 1995). Bioassays can be developed and used as quantitative or qualitative methods. Even though the use of bioassays was more important (the only possibility to measure a number of substances o mixtures) before the development of modern instrumental analytical equipment, they still remain useful in a number of areas. This is because of the unique ability of life (in vivo, using animals, plants or microorganisms) or of living material (in vitro, as tissue or mammalian cells in culture) to differentiate biologically active from inactive isomeric molecules. As previously noted, they are also able to detect very small amounts of active compounds (like hormones). This is useful for the rapid screening for new biologically active molecules, or to calculate the effective dose or concentration of a given compound or compound mixtures, among other uses. Mediated MFCs, where the cells and mediators can move/diffuse freely (see Section 3) are comparable to bioassays.

More recently, a new type of bioanalytical devices has been proposed and called biosensors. One of the main differences with bioassays (as previously stated) is that the biological component is always immobilized on the surface of an adequate chemical or physical transducer, originating an electrical signal, usually allowing the quantitative determination of the analyte targeted. A biosensor has been defined as "a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" (IUPAC "Gold Book", 1997). Following the aforementioned biosensor definition, the systems based upon electrogenic biofilms self-immobilized over electrodes, where the electron transfer process (ET) is related to non-mediated mechanisms are positively a type of biosensor. When mediated ET is considered, depending of the mobility of microorganisms and mediators, the classification could be more or less ambiguous. Some authors described MFCs based on the use of immobilized (polymerized) artificial redox mediators: if they are in close contact with immobilized microorganisms, which are in turn in close contact with the anode, this system can also be considered as a true biosensor. Fig. 2 shows the possibilities and diversity of microbially-based analytical systems.

The history, applications, biological material and transducers used to design biosensors have been reviewed recently, including a short mention of MFCs (Su et al., 2011). In that work three papers describing analytical uses of MFC were also reviewed, including biosensors for biochemical oxygen demand (BOD), lactate and acetate. In particular, environmental applications are a growing area for bioassays and biosensors uses. In this matter, the lower specificity of most microbial based analysis (considered a negative characteristic in most instrumental analytical technique), has become a strong and positive aspect when global water quality parameters such as BOD and toxicity need to be measured.

Despite the still small participation on the worldwide biosensor market, where clinical glucose sensors are close to 90% of the total sales, a growing market for environmental related biosensors is expected. Several bioanalytical parameters, such as BOD (biochemical oxygen demand) and toxicity, among others, are related or can be related to the measurement of metabolic respiratory activity or changes in the culture media associated with it (Catterall et al., 2001). MFCs have huge possibilities as convenient biosensors and bioassays, given the mechanical and electronic simplicity the system is based on.

3. Working principle of microbial fuel cells

One fundamental aspect to comprehend MFC basics and operation is the understanding of the possible electronic pathways between electrodes and bacteria, assuming that these prokaryotic life forms are basically non-conductive structures (given the lipidic nature of the bacterial cellular membrane, and other isolating external structures, as the microbial cell wall). How the intracellular electron transport (ET) system is capable of diverting the generated electrons from their natural intracellular electron acceptors outside the cell to the anode, is a fundamental question. The ET can be achieved either through the use of natural (produced by the bacterium) or artificial redox mediators (incorporated as a reagent), systems or set-ups that have been named *mediated* MFCs. Some particular types of bacteria have the amazing ability to transfer electrons by direct contact with the electrode *via* membrane associated cytochromes, conductive pili or other proposed mechanisms discussed later in this section. In this situation where any soluble mediators are absent, the systems are denominated *non-mediated MFCs*.

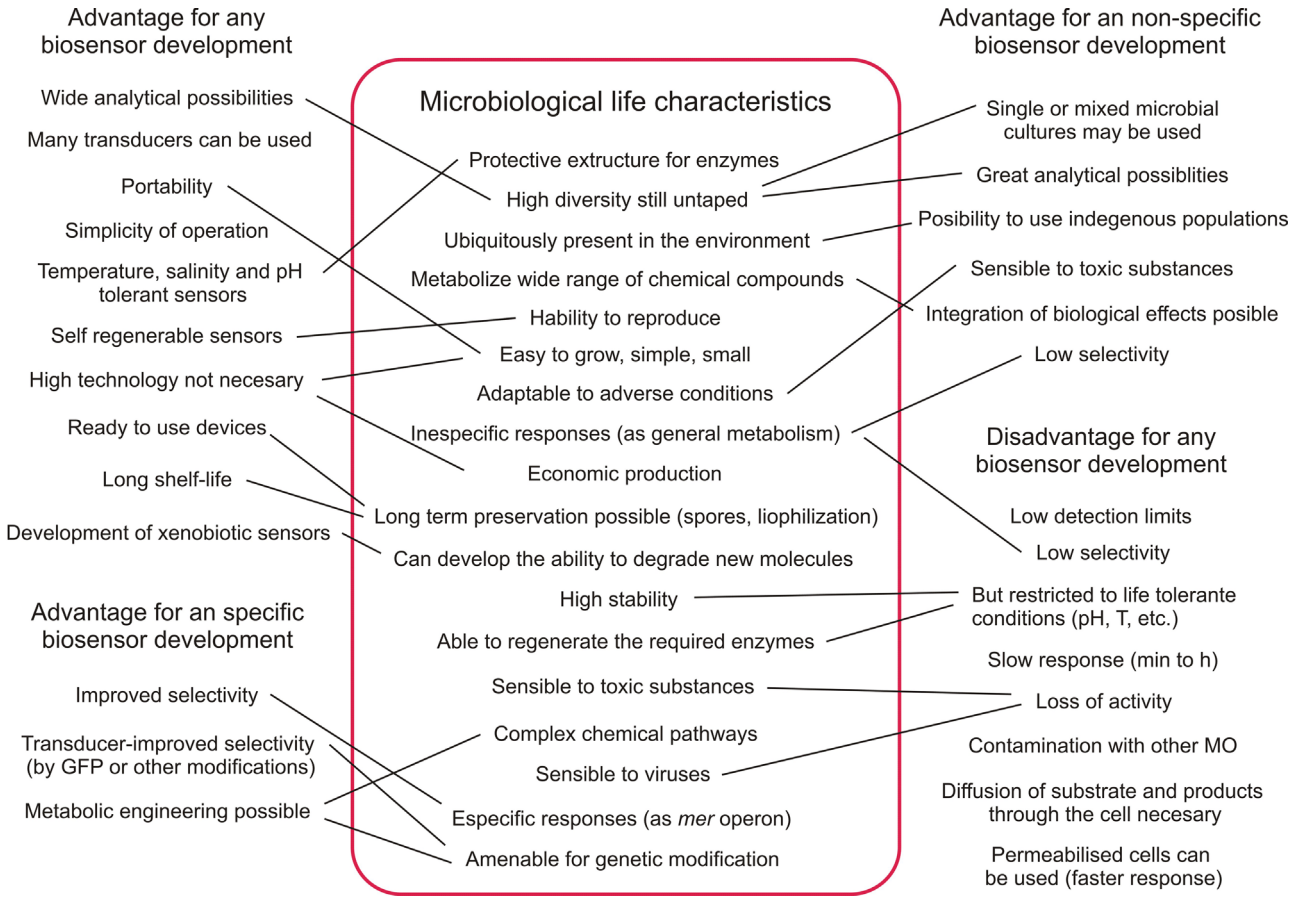

Fig. 2. Relevant characteristics of microbial life and microbially-based analytical systems, as biosensors and bioassays.

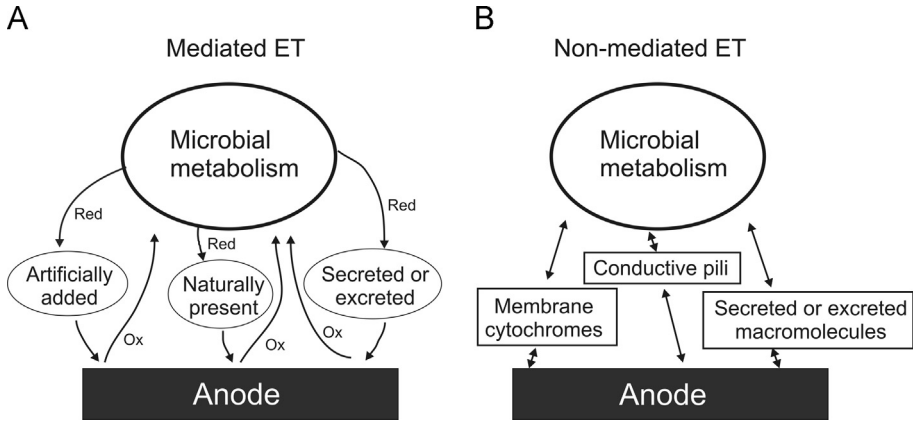

Fig. 3. Charge transfer mechanisms in MFCs. A, Mediated; B, Direct transfer. In A, the mediator (ellipses) diffuses freely to and from the microbial cell, transporting metabolically released electrons to the anode surface. In B, the transporters (squares) are relatively non-mobile. Red, reduced mediator; Ox, oxidized mediator.

A simple scheme in Fig. 3 shows the two basic charge transfer mechanisms proposed for MFCs. The level of participation of any of the mechanisms shown for a given MFC system is still an area of scientific controversy. For example, when the mediator is naturally produced by the bacteria, the system is sometimes named as *non-added mediator MFC*, although probably the ET mechanism could be similar, regardless of the origin (artificially added or naturally synthesized) of the mediator. The ET proposed mechanisms have been recently reviewed in a very interesting work (Osman et al., 2010).

In mediated MFCs (Fig. 3A), a soluble redox mediator is used as a way to transport electrons between the oxidative microbial metabolism and the anode surface. A proton conductive membrane (as Nafion®, a sulfonated tetrafluoroethylene based fluoropolymer-copolymer) or other membranes should be able to selectively transport cations, allowing protons to be transferred to the cathode, whereas electrons must travel across an external circuit, originating a current. Depending on the ionic concentrations and pH at a given experimental set-up, other change transporters can travel across the Nafion® membrane. Mediator based systems are usually called first generation or mediated MFC, since in the absence of a suitable electron shutter, the production of current is minimal or absent. Oxygen is reduced to water at the cathode with the help of protons and electrons provided by the anode (Allen and Bennetto, 1993; Bennetto, 1984). In order to facilitate the cathodic oxygen reaction, metallic catalysts as Pt or others are often used. Additionally, in

some set-ups oxygen is replaced by other oxidants as ferricyanide, avoiding in this case the use of metallic catalysts at the cathode. Mostly carbon based electrodes have been used as cathode and anode in MFCs.

Mediators usually employed are relatively small molecules, as Methylene Blue, Neutral Red, or others, as a mean to enhance power output (Davis and Yarbrough, 1962). Also some naturally complex media as soil, submerged soil or sediments and waste-water, among others, can contain a considerable amount of organic or inorganic compounds that can perform as electron mediators. Humic and fulvic acids and sulfur compounds have been postulated to be responsible for charge transfer between bacteria and electrodes as examples of organic and inorganic mediators (Stams et al., 2006). Most authors agree that to add mediators is not possible or practical to any large scale operation, as waste-water treatment plants,. That is because of the cost of the mediators and possible problems (as toxicity) caused by the release or treatment of these compounds. However, the use of minimal quantities of soluble mediators at analytical laboratory scale could be a useful approach in the design of valuable bioassays or biosensors.

In addition, it is known that some bacteria can actively secrete pigments, as redox-active flavins, that undergo reversible redox processes, as in several *Shewanella* strains (Brutinel and Gralnick, 2012; Marsili et al., 2008). The mediator can be actively secreted or excreted by the microbial cells, as shown in Fig. 3A.

Since adding a redox mediator to the anodic compartment is unpractical for the reasons previously stated, other type of MFC has been described based on a special type of microorganism, usually called electrogenic bacteria, where the addition of any mediator is not necessary. In this type of system, the transfer of electrons from the bacteria to the anode is proposed to occur directly (DET). These systems are called non-mediated MFCs (Fig. 3B), and it was proposed that the charge transfer from a microbial cell to the electrode occurs by means of external membrane associated cythocromes or by specialized structures described as conductive pili (Lovley, 2011; Richter et al., 2008). The contribution in non-mediated systems of endogenous secreted or excreted mediators is actually in discussion, at least for some microbial strains (Jiang et al., 2010). Therefore some authors prefer to avoid the denomination “mediator-less MFC” and simply state if a redox mediator was or not added to the system. Besides, there are systems utilizing non-diffusive mediators (that can be used to attach bacteria on electrodes), or other electron facilitating molecules or ions, where the mediated or no-mediated classification was be not adequate or was confusing.

4. Design and construction aspects of MFCs

Considering the engineering and design aspects, the most frequently used MFC set-up is based on the dual-chamber strategy, typically named “H-cell”, that consists of two compartments joined by a horizontal pipe that connects electrically the anodic and cathodic compartments. In the middle of this pipe, an ion-exchange membrane, usually a perfluoro-sulfonated cation exchange membrane (as Nafion®) is fixed, in order to separate the solutions at both compartments while allowing ionic conductivity. The membrane ionic conductivity is particularly important: it should be as high as possible to keep small the resistance losses in the MFC and to maintain high output power density in the cell (Okada et al., 1998). This is especially important when the production of energy is the rationale. But in order to use a MFC as an analytical device the possibility to obtain constant and reproducible current or power values is more a concern. Nowadays, μA–nA currents, for example, can be easily measured with standard and economic electronics, levels of current that are typically produced by simple MFCs systems.

Some other membrane types have also been assayed, and sometimes the two MFC compartments are separated just by a saline bridge. Also the operation mode (batch, flow systems) is important when applicability is considered. The main types of architecture and operation modes are presented in Fig. 4.

In the more studied set-up, the anode is maintained anaerobic, while the cathode is kept in aerobic conditions. The anodic compartment contains the biocatalizers, usually anaerobic or facultative bacteria; whereas the anode, usually aerobic and abiotic, contains the oxidant (the two more used are oxygen and ferricyanide, but also permanganate). The electrodes are normally some form of carbon, including glassy carbon, graphite, and different highly porous materials as Toray paper, woven or non-woven carbon fabrics, etc., with or without a catalyst as Pt or others. Catalysts are typically used to accelerate oxygen reduction at the cathode. The different architecture, electrode materials and other construction aspects have been compressively reviewed elsewhere (Du et al., 2007; Logan, 2008; Logan et al., 2006).

The design denominated “one compartment MFCs” or air-cathode MFC has been developed more recently, when compared with the “two compartment” ones. These cells are usually constructed applying at one part of the anode chamber a membrane-electrode assembly (MEA), where a carbon/Pt cathode is directly exposed to the oxygen available in the air (Liu and Logan, 2004). More recently, and in order to avoid the use of expensive Nafion membranes, simplifying the architecture and, eventually, lowering the internal resistance of the cell, membrane-less systems have been proposed. This is important since membrane resistance was described as a factor that strongly limits the maximum obtainable power. In a typical set-up a fluid moves through a slow flow column, where the anaerobic catolite is injected in one extreme. After passing the anode and a separator (glass wool, glass beads) the fluid is oxygenated and allowed to pass through a porous cathode (Jang et al., 2004). In this system the mixing of anodic and cathodic solutions or media is prevented or limited by directional flow and separators that prevent turbulent flow and mixing.

Other systems that avoid membranes are the denominated sedimentary or benthic microbial fuel cells (Reimers et al., 2001), where anode and cathode are separated by several cm of sediment or mud. This layer avoids mixing and allows the establishment and persistence of a redox gradient between the anode (in a reduced environment) and the cathode, usually in the overlying water (in an oxidant environment).

5. Basic fuel cell electrochemistry and reaction kinetics

MFCs are studied in the same way as hydrogen/oxygen chemical fuel cells; both are electrochemical energy converters, where chemical energy is transformed directly to current. But the reactions that limit the maximum potential E produced at biological fuel cells are way more complex, because they depend on a naturally complex microbial metabolism, enzymatic kinetics, and sluggish electron and mass transport processes. Moreover, different anodic and cathodic reactions, that can occur simultaneously, complicate the theoretical studies of this biologically-based energy producing system. Different reactions considered to be relevant to MFCs technology have been revised elsewhere (Logan, 2008), including E^o and E'^o values (this last adjusted to a microbiology relevant pH of 7). As they are basically DC current sources, they are typically operated using an adequate R_L (see Introduction). By using a number of R_L to challenge the current production of a MFC, it is possible to obtaining the corresponding E for each load

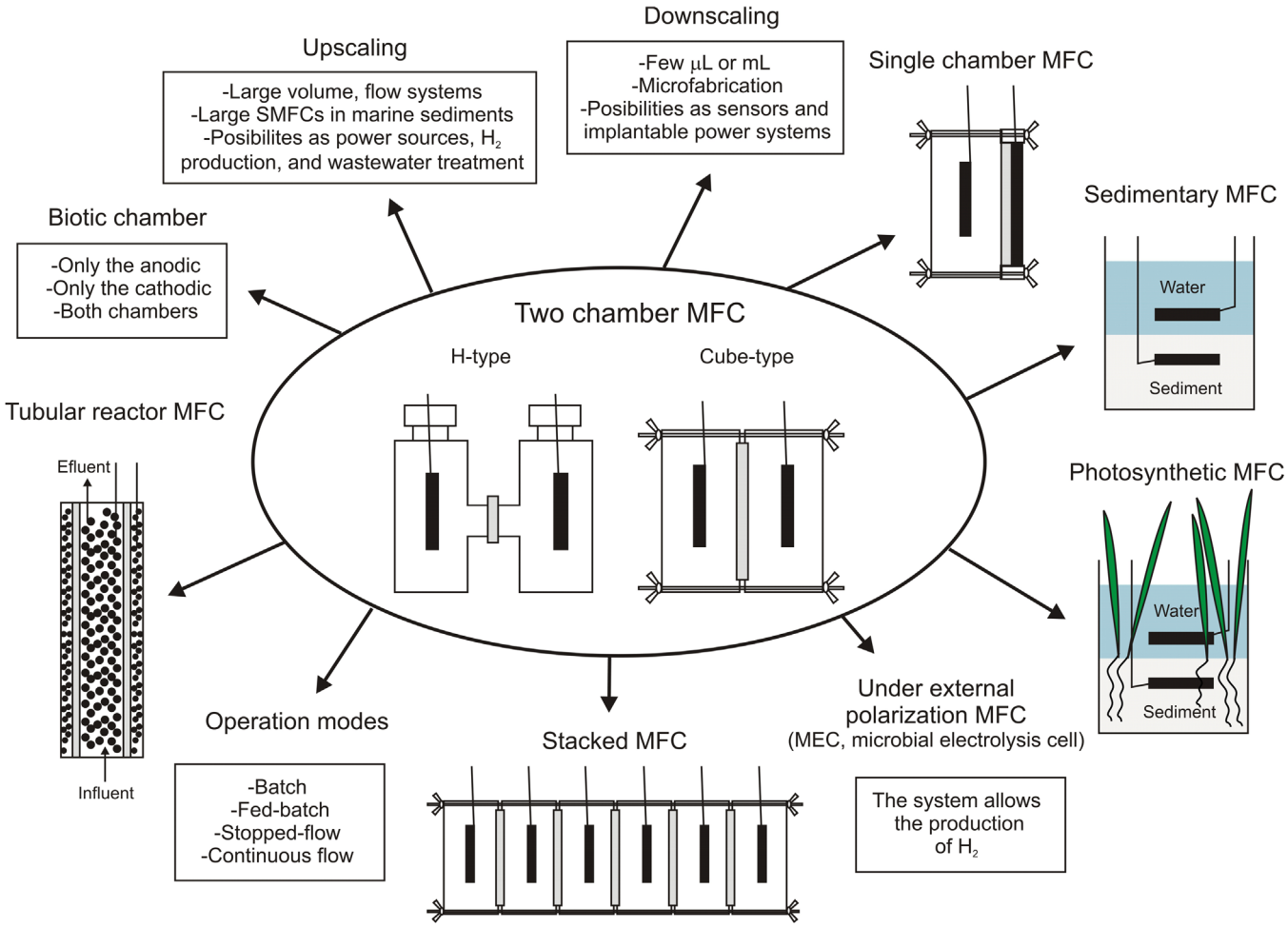

Fig. 4. MFC architecture, construction aspects and operation modes. From the two more used systems (center), new devices, operation modes, and applications have been proposed. Electrodes are in black and separation membranes in gray.

condition. By using Ohm's law (Eq. 1), it is easy to calculate the current i circulating through the system.

$$E = iR_L \tag{1}$$

The electricity (i) produced in fuel cells is proportional to the electrode area (A). Because of that it is customary (allowing the comparison between different set-ups) to use current density (j) values to present the obtained data, calculated following Eq. 2.

$$j = i/\mathrm{A} \tag{2}$$

Both E and j values allow the construction of polarization curves, as shown in Fig. 5A. Polarization curves are of great value, allowing to study the performance and to calculate some important MFC characteristics, as the internal resistance (R_{int}) that limits strongly the maximum power produced by a cell. R_{int} could be calculated as the slope of the curve, in the ohmic polarization losses section, where it remains relatively constant (Fig. 5A). This curve shows three zones, where different phenomena are the main causes of the observed voltage drop. The activation losses are caused by the slowness of the reactions taking place on the surface of the electrodes. Also, a proportion of the voltage generated is lost in driving the chemical reaction that transfers the electrons to or from the electrode. In the ohmic losses region the voltage drop is explained mainly by the resistance to the flow of electrons through the material of the electrodes, bacterial biofilm (if present) and DET; but perhaps the more important factors at MFCs are the electrolyte and PEM membrane capacity to transfer charge. The last part of the curve, denominated mass transport or concentration losses region, result from the change in concentration of the reactants at the surface of the electrodes (or at the surface of a biofilm) as the fuel is used. The losses or polarization losses explain why the OCV potential never reaches the theoretical or calculated potential predicted considering the reduction potentials of cathodic and anodic reactions. Moreover compatible conditions with life limit strongly the temperature and chemical environment where microbial life is able to thrive. The chemical and electrochemical phenomena behind power generation at microbial MFCs have been reviewed (Rabaey and Verstraete, 2005).

By using Eq. 3 the power (P) produced at different load conditions can be obtained. After being normalized by surface area, a power density curve can be drawn, as shown in Fig. 5B. This curve have a bell shape, and shows the current where the power production would be the maximum, usually denominated P_{max} (about 4.20 mW cm^{-2}), which corresponds to the denominated j_{max} (about 9.6 μA cm^{-2}) in the given example.

$$P = i^2 R_L \tag{3}$$

As the main proposed practical use of MFCs is as an energy source, much of the work is also concentrated in scaling-up possibilities and economic materials to make MFCs a viable commercial option. Moreover, the efficiency as power producing systems, including the coulombic efficiency, is expressed by how much of the chemical energy present in the substrate used by the microorganisms is finally converted into electricity. Furthermore, for practical energy-related application, the power density that a given system can provide is a very important factor. However, in order to use MFCs systems as sensors, the main concerns are

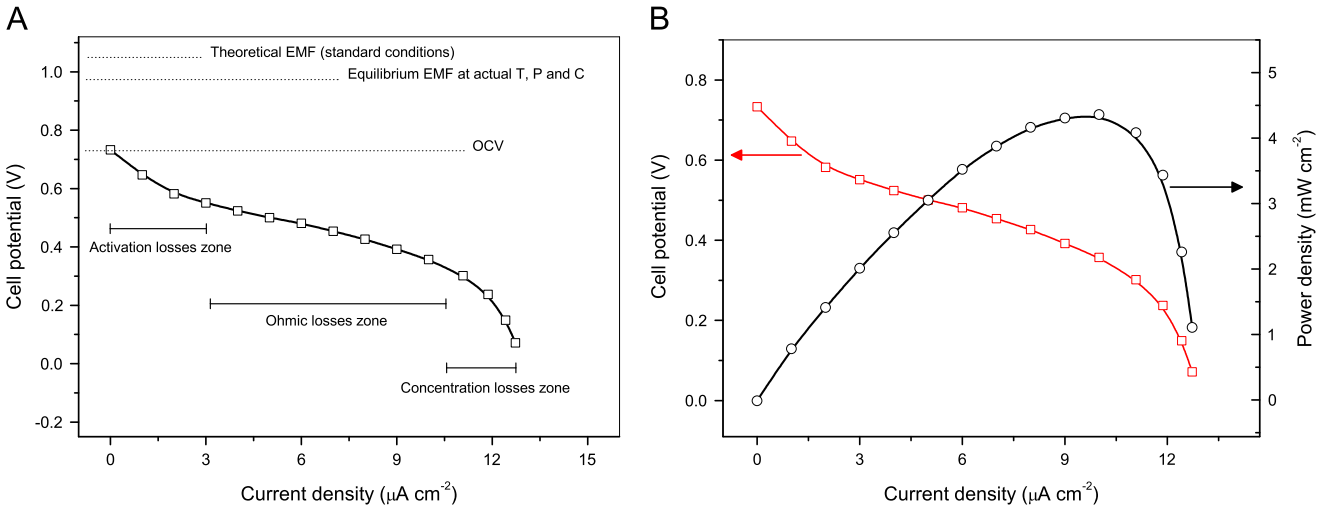

Fig. 5. Characterization of a MFC. A typical polarization curve (A) is shown; the theoretical maximum *emf* never is reached because the potential losses and the actual conditions, mainly related to reagents concentrations. The curve is divided in three regions, where different types of losses limit the current production. Usually, a polarization and power density curve are shown in a single plot (B).

stability, reproducibility and detection limit, among other analytical performance parameters, given that just very low currents are necessary and efficiency is not an issue.

6. BOD, concepts and limitations

The concentration of easily biodegradable biomass is an important characteristic of any natural or artificial water body, strongly related to the biological (especially microbial) metabolic activity, which is used to quantify the degree of organic contamination in natural waters as well in wastewater monitoring process, among other industrial processes.

The concept of BOD has been defined by a recent IUPAC publication (Duffus et al., 2007) as "The amount of oxygen taken up through the respiratory activity of microorganisms growing on organic compounds present when incubated at a specified temperature (usually 20 °C) for a fixed period (usually 5 days). It is regarded as a measure of that organic pollution of water which can be degraded biologically but includes the oxidation of inorganic material such as sulfide and iron (II). The empirical test used in the laboratory to determine BOD also measures the oxygen used to oxidize reduced forms of nitrogen unless their oxidation is prevented by an inhibitor such as allyl-thiourea".

From the definition is clear that any form of nitrogen oxidation in the water can be considered as interference. Depending on the amount of nitrifying bacteria and reduced forms of nitrogen present in the sample, the consumed oxygen could be important. Also, even though is not directly stated in the aforementioned definition, other compounds as reduced metals are also habitually considered interfering substances. Because of that a denominated carbonaceous BOD (CBOD for short) has been defined, where the oxygen to be considered is exclusively the oxygen needed to oxidize organic materials, a parameter that is habitually accepted for regulatory water quality agencies.

Given the way MFCs typically work (with the electrogenic bacteria or community at the anaerobic anodic MFCs compartment, and the predominant metabolisms of the used bacteria), the reduced nitrogen compounds and metals would probably not generate an appreciable current or signal. But instead of considering this fact a drawback we considered it as an advantage, given that the BOD calculated using MFCs based biosensors or bioassay may be equivalent to CBOD, with the bonus that nitrification inhibitors would be unnecessary.

This fact is also true when BOD biosensors based on transducers different from the typical oxygen Clark electrode or other oxygen sensors are used. For example, a system for BOD determination based on a potentiometric carbon dioxide electron that claims to be insensitive to reduced nitrogen or metal compounds (at non-toxic concentrations) has been proposed. Oxygen is not measured but, instead, estimated by means of carbon dioxide evolution (Chiappini et al., 2010).

7. Analytical applications of MFCs

Microbial fuel cells were proposed and studied mainly in the same way as H_2 fuel cells, as energy-producing devices. However, the use of a MFC as a detector or transducer is very interesting given that they are relatively simple, not only for its construction but also when signal acquisition and electronic requirements are taken into consideration. Any electrical output of a MFC could be *a priori* useful as an analytical signal, but electrical current at a given external charge (R_L) has been shown useful and reliable. Usually R_L is chosen in the way that current flow through the electric circuit will modify the cell equilibrium potential (affecting as well the biological reactions occurring in the cell), avoiding large resistors that would not produce any appreciable diminution of open circuit voltage (OCV, a non-current flow condition) or small R_L that would short-circuit the cell. In most works, currents at mA–nA level are obtained that are easily amplifiable to required levels with low cost, off-the-shelf electronics. The electrical current at a MFC external circuit (i) is related among others to many construction, design and concentration factors. It increases with the electrode area, reactants concentration, and catalysts activity, and decreases by aspects related to the architecture such as the distance between electrodes, and membrane and media resistivity, that affects the maximum voltage a MFC can sustain. The difference between the theoretical maximum potential and currents and the obtained in a real MFC is explained by activation, resistance or concentration losses, which have been classified and described elsewhere (Logan, 2008).

But for a given design and operational conditions, much of the described factors could be maintained at a constant level. Also if the biological reactions at the anode are the limiting reactions, the electrical current produced by the MFC will be related only to it avoiding any consideration about cathodic reactions. Cathode size is usually larger than the anode, and/or the cathodic reactant concentration is maintained at high levels, among other approaches to

maintain the anode as the electrode where the limiting reactions occur. The microorganisms at the anode of the MFC oxidizes the substrate as fuel and resulting electrons are directly transferred to the electrode, suggesting that the MFC system can monitor substrate concentration because electric signal is proportional to fuel (substrate) strength (Chang et al., 2006).

Perhaps one of the more studied MFC application is related to the ability of these systems to produce electricity from domestic wastewater, while at the same time accomplishing biological wastewater treatment (removal of chemical oxygen demand; COD) as was shown by Liu et al., 2004. This type of MFC, where a very complex mixture is used as fuel, including not only domestic wastewater and other types of relatively non-toxic wastewater but also marine or continental sediments, is usually operated without any added mediator. Still, given the very complex nature of the materials, where humic and fulvic acids, sulfur compounds and others redox active substances are commonly present. These substances, naturally present in the sampled material, can perform as soluble mediators; because of this, they can be considered as mediated systems or mixed mechanism systems (where mediated and not mediated process would probably occur).

Some advantages of MFCs, as the direct utilization of most organic compounds as microbial carbon/energy source are, indeed, counterbalanced by its very low-density power, usually in the mW–μW cm^{-2} range, limiting its real-world applications as an energy source. Besides, most of the organic molecules present in wastewater, but not some xenobiotics or recalcitrant molecules, can be converted into energy by the different microbial metabolic pathways.

The goal of the authors reviewed here was to design and assay microbial electrodes (usually in a MFC set-up) as useful analytical systems, able to provide practical and relevant information. Depending on the intended use and other considerations, some of the devices proposed here and in the second part of this review, can be classified as bioassays, where the biological material is not immobilized in close contact with the electrode. Still, the majority of the devices proposed are more related to biosensors since electrogenic bacteria grow as biofilms over the electrodes; therefore the aforementioned condition (immobilization) is accomplished by biofilm formation or other natural or artificial methodology. In the biosensor configuration the microbial cells, entrapped or immobilized at the MFC anode (or the cathode, but this configuration is still very rare) allow a more integrated detection system that has more chances to be used as detector at analytical FIA (flow injection analysis) systems, or in other potential analytical set-ups.

The possible applications of MFCs as biosensors were very briefly noted in several reviews, as presented by Logan et al., 2006, that state “A varied array of alternative applications could also emerge, ranging from biosensor development and sustained energy generation from the seafloor, to biobatteries operating on various biodegradable fuels”. The same year (Bullen et al., 2006), the possibility of using MFCs as biosensors was also mentioned; they stated very briefly that MFC “…can act as a specific biosensor (if enzyme based) or a non-specific one if microbe based”. Later, another review had made focus in the advances and application of biofuel cells (Davis and Higson, 2007) where some interesting applications of MFCs have been described. However analytical MFC uses were not mentioned in this work.

Of all the reviews considered here and probably, to our best knowledge, published the only one that dedicates a complete subsection to MFC biosensor applications is the work published by Du et al., 2007. In this work, they mentioned and commented without great depth the more frequently studied application, i.e. biochemical oxygen demand (BOD) biosensors.

The objective of this paper is to describe all the analytical applications of MFCs in any configuration, to compare them for their performances and limitations as well as their advantages and disadvantages and to present various factors affecting the experimental results. The reviewed papers are critically interpreted in order to facilitate future work and technological development.

7.1. As BOD sensors

The need for a rapid and simplified method to replace or complement the cumbersome five days assay used to evaluate the standard biochemical oxygen demand (BOD_5) had been recognized some time ago. Rapid biosensors and bioassays, mainly based on the measurement of consumed oxygen through Clark amperometric electrodes or other oxygen measurement methods have been designed, and in some opportunities, commercialized (Liu and Mattiasson, 2002).

Still, some other electrochemical principles and devices have been proposed using redox mediators that replace the oxygen, as ferricyanide. This compound can be reduced to ferrocyanide by the enzymatic cellular machinery and in turn re-oxidized at a conveniently polarized amperometric electrode (Bonetto et al., 2011; Catterall et al., 2001; Trosok et al., 2001; Yoshida et al., 2000). Details of relevant BOD sensors reviewed in this paper are summarized in Table 1; also a relatively recent work summarizes some mediated BOD MFC-based systems (Kim et al., 2006).

Karube et al. (1977) suggested, probably for the first time, the use of MFCs as a BOD sensor. The designed MFC included two chambers separated by an anion exchange membrane; in the anodic chamber, *Clostridium butyricum* bacteria was immobilized, by means of a collagen membrane, over a Pt electrode. The bacteria were maintained in anoxic conditions, the cathode being a simple carbon electrode in an aerated solution. As the BOD (glucose-glutamic solution standard) strength increased, the production of hydrogen and formate by the immobilized bacteria also increased, and reacted over the electrode. The measured current was proportional to the BOD solution, reaching saturation at about 400 mg L^{-1} BOD (110 μA).

After that early work, the utilization of MFCs as a convenient BOD biosensor or bioassay was recognized and studied by Kim et al. (2003). Using wastewater as a source of electrogenic bacteria is able to colonize the anode (apparently form a starch processing plant) and after a non-determined colonizing time, the system was able to function up to 5 years in a stable manner without any servicing. The biosensor gave a good correlation between the BOD value and the current measured or the coulomb produced. Employing the former, a wider calibration range was obtained (but with an excessive detection time for concentrated BOD samples); charge was integrated between the addition of the sample to the time where current decreased to 5% of the maximum current. Reproducibility (at the 10% level) and a comparison with the standard BOD_5 method showed interesting and positive results. Later, a very similar system for on-site, on-line and real-time monitoring of real wastewater was proposed. In this case, samples were filtered after measured (to avoid clogging) given that a flow system was used. Synthetic wastewater was used to verify the system. They found that the electrical behavior of the flow MFC correlates well with the standard 5 days BOD (Kim et al., 2003).

Oligotrophic enriched anodes were proposed as a way to measure low BOD concentration, as naturally found in non-contaminated fresh-water rivers and lakes (Kang et al., 2003). Mediator-less microbial fuel cells (MFC) were enriched using river sediments as inoculum, and operated during 8 weeks of continuous flowing, at low BOD concentration (6 mg L^{-1} BOD). Low oxygen concentration in the cathode (2 mg L^{-1}) and a reduced cation exchange membrane area

Table 1
Summary of the analytical performance,architecture and functional characteristics of MFCs used for the determination of BOD.

Microbial/s assayed (origin)	Mediator added?	Anode	Cathode	Membrane?	Detection range (BOD_5, mg L^{-1})	Saturation signal	Measurement time	Reference
Clostridium butyricum	No	Pt	Carbon	Yes, anion exchange	10–300[a]	120 μA	70 min	Karube et al. (1977)
Enriched consortium (wastewater)	No	Graphite felt	Graphite felt	Yes, cation exchange	2.6–25 (current) 2.6–206 (charge)	1.1 mA 58°C	30 min–10 h	Kim et al. (2006)
Consortium (river sediments)	No	Graphite felt	Graphite felt/Pt	Yes, cation exchange	ND	ND	ND	Kang et al. (2003)
Consortium (activated sludge)	No	Graphite felt	Graphite felt	Yes, cation exchange	20–100	7 mA[a]	1 h	Chang et al. (2004)
Consortium (river sediments)	No	Graphite felt	Graphite felt/Pt	Yes, cation exchange	2–10	6 mA[a]	1 h	Moon et al. (2005)
Consortium (anaerobic sludge)	No	Carbon cloth	Toray paper/Pt	Yes, cation exchange	50–400[b]	0.4 mA	40 min–2 h	Di Lorenzo et al. (2009)
Consortium (primary clarifier)	No	Toray paper	Toray paper/Pt	Yes, cation exchange	10–250	233 mA m^{-2}	40 min	Zhang and Angelidaki (2011)
Escherichia coli	Poly-neutral red[c]	Glassy carbon	Pt	No	50–1000	1 μA	ND	Liu et al. (2012)[d]
Consortium (anaerobic and aerobic sludge)	No	Graphite rod	Carbon paper with carbon nanoparticles	No	32–1280	70°C	5–20 h	Modin and Wilen, (2012)

ND. No data available in original work.
[a] Estimated using data presented by the authors.
[b] COD (mg L^{-1}).
[c] As immobilized mediator at the anode.
[d] Not really a MFC set-up, detection range not fully assayed.

was used as a way to limit oxygen diffusion to the anode, optimizing this system to low microbial activity and therefore, able to measure low BOD concentration. This oligotrophic-type MFC was described to have high operational stability, good repeatability and reproducibility, but calibration curves were not presented. Currents of about 3 μA for 6 mg L^{-1} BOD, relative to the baseline noise allow us to speculate a detection limit of about 1 mg L^{-1} BOD. Later, some of the same authors proposed a similar system for real-time wastewater monitoring (Chang et al., 2004). Graphite felt ($20 \times 120 \times 5$ mm^3) was used as electrodes in a two 20 mL compartment system. An oxygen saturated solution was employed as oxidant at the cathode (flow rate equal to 5 mL min^{-1}). This MFC was assayed as a continuous BOD sensor, using a flow rate at the anode of 0.35 mL min^{-1}. The presented results indicate that BOD values from 20 to 100 mg L^{-1} BOD could be measured based on a linear relationship. Higher BOD values (up to 200 mg mL^{-1}, with a saturating current of ca. 6 mA, $R_L = 10\ \Omega$), can also be measured using either a non-lineal fitting method or a lower anolite flow rate. Perhaps, the main drawback of the proposed method is the long time necessary for stabilization once the MFC is inoculated by activated sludge (one month, stable current of 5 mA). On the other hand, only almost 60 min was required to reach a new steady-state current after the MFCs had been fed with different strength artificial wastewaters or samples, with a repeatability in the order of 10%, which are relatively good performance parameters for BOD analysis. In the same year and group, the dynamic response of a similar MFC was studied, optimizing the hydraulic retention time of the designed flow cell and reducing the anode volume to 5 mL, reaching a response time of only 5 min (Moon et al., 2004).

Min and Logan (2004) proposed a MFC that, even though not intended for analytical uses, gives relevant new information. A calibration curve was given, where a relation between chemical oxygen demand (COD) and power density was found and studied. They designed and assayed the ability of a flat plate microbial fuel cell (FPMFC) containing a single electrode/PEM assembly. The PEM (Nafion) was hot pressed to the cathode. Power density showed a Monod-type trend as a function of the wastewater strength over a range of 38–324 mg COD L^{-1}. They also showed that several single organic substrates (at 1 g L^{-1} COD concentration) can produce an electric signal (in this case power, in mW m^{-2}) including glucose (212), acetate (286), butyrate (220), dextran (150), and starch (242). These results demonstrated that complex polymeric hydrocarbons can be measured using MFC transducer principle.

Using a similar MFC set-up as previously described (Kang et al., 2003), Moon et al. (2005) assayed the denominated "oligotrophic MFC", inoculated with river sediments, for the continuous monitoring of low BOD concentration water; calibration was made by using artificial wastewater, containing glucose and glutamate (GGA). Ten times diluted trace mineral solution was used to minimize the background current level, which was proposed to be generated from the oxidation of nitrilotriacetate used as a chelating agent. The response time related to a concentration change of 2 mg BOD L^{-1} was about 60 min. They also showed that current signal increased with the increase in the salts concentration (probably by lowering the MFC internal resistance). With a similar MFC set-up, the same group further presented interesting results (Chang et al., 2005). They shown that the signal from MFCs decreased in the presence of electron acceptors of higher redox potential such as nitrate and oxygen in the assay medium that will behave as interferences for this analytical system. First, it is shown that the addition of azide and cyanide did not change the signal in the absence of the electron acceptors and later it was probed that the respiratory inhibitors (azide and cyanide) eliminated the inhibitory effects of the electron acceptors (oxygen and nitrate) on the current generation from MFCs. Similar results were obtained using an oligotrophic MFC fed with an environmental sample that contained nitrate. For these reasons, the use of respiratory inhibitors is recommended by these authors, for the accurate BOD measurement of environmental samples containing nitrate and/or oxygen with this type of sensor.

A single chamber MFC (SCMFC) was also proposed (Di Lorenzo et al., 2009) allowing a simplification in the architecture since the cathodic compartment is absent, and replaced by a humidified cathode in the presence of air. Because of that, this MFC type is also called "air cathode MFC". Stability over 7 months of operation and high reproducibility (better than 1%, CV) were reported. The authors also showed that by reducing the anode chamber

volume from 50 to 12.6 cm^3, the biosensor response was faster (40 min); the smaller reactor also gave coulombic efficiencies nine times higher than the larger one.

A sensor based on a submersible microbial fuel cell (SUMFC) was developed for in situ monitoring of microbial activity and BOD in groundwater (Zhang and Angelidaki, 2011). The set-up proposed was interesting, made of a polycarbonate cathodic chamber completely independent of the anode, having on one side a membrane electrode assembly (MEA), prepared of a Nafion membrane hot pressed to a Toray paper electrode, containing 0.5 mg cm^{-2} of Pt catalysts. The produced current was measured between this and a Toray anode, after 2 months bacterial colonization period. Possible problems with the proposed set up and their intended use in real situations are the fragility of Toray electrodes and the necessity to bubble air at the cathode. Later, a similar set up was used with domestic wastewater. In this paper the authors showed that the MFC had an optimum (higher current density) pH of about 7.0, and that the current increased with temperature and conductivity, at the maximum values they reached, of 33 °C and 13.5 mS cm^{-1} (Peixoto et al., 2011).

A recent work (Liu et al., 2012) presented a modified electrode that, even though not really a MFC, could eventually behave as an MFC anode. We decided to discuss this paper here because neutral red (NR) is a well know redox shuttle used frequently in mediated MFCs. The authors presented an interesting method by using co-immobilized *Escherichia coli* as a biocatalyst and poly-NR, obtained by electrochemical polymerization (cyclic voltammetry), over a glassy carbon electrode (GCE). Two different modification approaches of GCE were utilized and compared. In one approach, NR was electro-polymerized on the surface of GCE, and later *E. coli* cells were mixed with a self-gelatinizable graft copolymer of poly(vinyl alcohol) with 4-vinylpyridine (PVA-PVP) as an immobilization matrix. In the second approach, both NR and *E. coli* cells were mixed with the copolymer and used to modify the GCE. In this case it was electro-chemically treated, similarly as above for obtaining poly-NR over the electrode. Based on the electrochemical evaluation, they found that the performance of the latter was better, which may be caused by the fact that the NR and poly-NR deposited on the surface of *E. coli* resulting in a good electron transport and permeability of the cells membrane. Also, the authors showed that when complex wastewater is used for calibration (as OECD BOD standard is) a pretreatment with TiO_2 nanotubes and photocatalysis increases the obtained signals, probably by breaking down polymeric organic substances present in the wastewater OECD standard.

Finally, a bioelectrochemical cell where the anode was polarized to 0.2 V vs. a SHE (standard hydrogen electrode) using a reference electrode, has been described (Modin and Wilen, 2012). The authors presented this approach to avoid the use of an external resistor, boost current production and, eventually, microbial activity. Feed-batch and coulometry was used as sensor operation and measuring principle; perhaps as result of this selection the analytical system shows very slow response time, as the normal measurement involve the total consumption of the BOD content, that seems impractical. The one compartment, membrane-less cell is possible by using a gas diffusion carbon paper cathode, coated at the air-facing side with a 40% polytetrafluoroethylene (PTFE) solution containing 200 mesh graphite powder, to prevent liquid leakage from the chamber. Absence of any ion exchange membrane was proposed as a way to avoid anolyte acidification, which could be detrimental for microbial metabolism.

8. Summary and conclusions

High reproducibility in the current output and the capacity to use numerous organic molecules as fuel substances, including proteins, polysaccharides and others of macromolecular nature, are prerequisites for a successful implementation of a MFC as a BOD sensor. The biosensor set-up, based in immobilized microbial cells or communities is usually preferable to design on-line or automatic systems, while for some other applications the bioassay format, where the microbial cells are free, could be a better analytical approach. The reproducibility of the systems presented here are limited by the intrinsic variability of biological processes, but this relatively low reproducibility is considered acceptable for biologically based methods used to analyze highly complex mixtures, as wastewater.

The main problem to be addressed by any rapid method to measure BOD, is to avoid or control the possible lack of agreement between the standard BOD_5 method and rapid BOD methods, which depends mainly on the water composition. Long chain polymers as cellulose or starch, that could be present in the sample, are not easily biodegradable by the microbial machinery; because of that when the BOD present in a given sample is related mainly to these long chain organic molecules a poor agreement between the rapid method and standard BOD can be expected.

Besides, MFCs based BOD sensors face other more specific challenges, given that much of the published work is based on electrogenic bacteria. Those are mainly bacterial groups usually limited in the types of organic substances they are able to use as carbon source and with metabolic rates much lower than other typically biosensor and bioassay-used microorganisms, as *E. coli*, *Pseudomonas putida* or *Saccharomyces cerevisiae*, among others. Both characteristics will negatively affect the performance of MFC-based BOD analytical systems.

Table 1 shows more of the relevant work done in the last decade, where the analytical uses of MFCs were rediscovered, after some preliminary efforts in 1977. The work done in this area follows the trend in other MFC research areas. There is a tendency to simplify the MFC architecture avoiding the cathodic chamber (single chamber MFCs, see Fig. 4) and to modify the electrode materials to improve electron transfer using nanoparticles or other materials (Modin and Wilen, 2012). Moreover, later work tends to concentrate on systems able to determine BOD in industrial and municipal wastewater without dilution, looking for wide calibration range and relatively insensitive to BOD concentrations expected for natural or slightly contaminated waters.

Soluble mediators were avoided in the work reviewed in Table 1 following the general tendency in MFC studies, where their uses have been considered unpractical. Mediators are cumbersome when the MFC system is aimed towards electricity production or wastewater treatment systems, the main applications described in the literature, where very large volumes and flow rates are considered. Electrogenic bacteria as *Geobacter* or *Shewanella* that do not need any added mediator and industrial systems could be therefore simpler and economically viable. But Liu et al. (2012) have used an anode modified with electropolymerized mediator with very interesting results.

We hypothesize that the use of small quantities of mediators, immobilized or free, in conjunction with weakly, or not, electrogenic bacteria or other microorganism could open new analytical possibilities of MFCs. The use of mediated systems, based in relatively low toxicity mediators could be economic and practical for low volume (few μL), disposable mediated systems. Mediated non-biofilm based systems have as advantage their easier industrial fabrication (no biofilm growing facilities), possibly higher reproducibility (biofilms are very dynamic and inhomogeneous systems) and ability to fast measurements after simple lyophilized culture rehydration.

9. Future perspectives

A way to improve the applicability of this MFC-based BOD rapid systems is the screening of new microorganisms, or microorganisms groups or consortia, with electrogenic capabilities, able to metabolize

relatively fast a wide range of organic substances, combined with sample pretreatment designed to split larger biomolecules. Construction of recombinant bacteria could also be possible, but the knowledge about the genetics related to extracellular electron transfer to electrodes is perhaps not yet mature enough to pursue knowledge based methods (as directed mutations, or gene manipulation). Still, other approaches, like directed evolution of relevant molecules involved in direct ET form bacteria to electrodes, could probably be applied. Besides, the MFC-based BOD biosensors are not limited to electrogenic bacteria. By using other heterotrophic bacteria (free or immobilized) and soluble or immobilized redox mediators, small, cost-effective and practical devices might also be envisioned.

Perhaps, the more important problem of microbial-based biosensors to become a fully and stand-alone competitive analytical system (when compared with traditional analytical instrumentation and methods) is to improve strongly their stability, reproducibility, sensitivity and selectivity. With better analytical characteristics, MFCs based biosensors and bioassays could gain acceptance and become eventually, approved standard methods. This path will lead probably in the medium-term to very convenient and economic equipment able to be used as stand-alone technology to accomplish several analytical tasks, as BOD determination, a relevant water quality analytical parameter, also useful for wastewater management.

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