PERSPECTIVES ON RESEARCH AND DEVEOPMENT OF MICROBIAL FUEL CELLS

A. Ortega-Martínez[1], A.L.Vázquez Larios[1], O. Solorza-Feria[2], H.M. Poggi Varaldo[1]

[1]Depto. Biotecnología y Bioingeniería, Centro de Investigación y de Estudios Avanzados del IPN, Apdo. Postal 14-740, 07360 México D.F., México. Correo electrónico: hectorpoggi2001@gmail.com y rareli@hotmail.com.
[2]Depto. Química, CINVESTAV-IPN, A. Postal 14-740, 07360 México D.F., México.

ABSTRACT

Microbial fuel cells (MFC), is an anoxic electrochemical bioreactor where bacteria grow in the absence of oxygen in a chamber containing an anode which it may be covered by a biofilm. Microorganisms anoxically oxidize the organic substrate and electrons generated are released to the anode. Released protons are transferred to the cathode. Natural or forced aeration of the cathode supplies the oxygen for the final reaction $2H^+ + 2e^- + (1/2)\ O_2 = H_2O$. In this work, we present a critical review on MFC focused on subjects that are receiving a growing interest from the research and technological communities: (i) types of MFC, their relative advantages and disadvantages and ranges of application; (ii) development of biocathodes; (iii) enrichment procedures of microbial communities in MFC.

Recent research shows that one-chamber fitted with cathode aerated by natural aeration, and other special types of high performance MFC, have displaced the historical two-chamber MFC. Recent studies showed that electrochemically active bacteria (EAB) can be successfully enriched in MFC. The cost and eventual poisoning of the platinum catalyst used at the cathode is a major limitation to MFC application and economic viability. Researchers have started working on the concept of biocathodes that would use bacteria instead of platinum as a biocatalyst. Microbial enrichment of inocula seeded to MFC may provide a way to enrich the consortium with EAB, thus substantially increasing the transfer of electrons to the anode. Bioaugmentation of consortia in MFC with strains EAB, could contribute to the same goal.

Keywords: Microbial fuel cell, biocathodes, enrichment.

1.- Introduction

The current energy crisis has launched a renewed interest on alternative energy sources and non-fossil fuels. One promising technology is the direct production of electricity from organic matter or wastes in MFC. A MFC can be envisioned as a bio-electrochemical reactor that converts the chemical energy stored in chemical bonds into electrical energy via the catalytic activity of microorganisms under anoxic conditions.[1, 2, 3] In addition to electricity generation, a MFC may significantly decrease the organic load of the effluent, acting as a wastewater treatment unit. MFC have also been tested as biochemical oxygen demand (BOD) sensors for monitoring treatment of wastewaters.[4] Although the MFC research has increased in the last years, several issues remain to be solved.

Microbial fuel cells (MFC) that utilize wastewater or degradable biomass as fuel is a promising technology to carry out energy recovery and pollution control. A MFC is fundamentally an anaerobic process where bacteria grow in the absence of oxygen in a chamber containing an anode and form a biofilm that covers the anode. These bacteria act as a biocatalyst at the anode and replace the use of costly platinum catalyst at the anode.[5] Of major concern is the optimization of MFC design in order to maximize power output and reduce the installation and operation costs. The cost of the platinum catalyst used at the cathode is a major limitation to MFC application and economic viability. For this reason some researchers have recently started working on the concept of biocathodes that would use bacteria instead of platinum as a biocatalyst at the cathode.[6]

The organic contaminants in wastewater are oxidized by electrochemically active bacteria or microbial consortium, and the resulting electrons are transferred directly or through natural mediators to the electrode.[7, 8, 9, 10, 11, 12, 13, 14, 15] Mediatorless microbial fuel cells (ML-MFC) have been reported that do not require mediators to facilitate electron transfer to MFC electrodes.[16] ML-MFC utilize electrochemically active bacteria or microbial consortium as biocatalysts to convert chemical energy into electrical energy. Several studies showed that electrochemically active bacteria can be successfully enriched in a microbial fuel cell format.[7, 17, 18, 19, 20, 21] It has been proposed that the nature of both the inoculation source and the fuel used in ML-MFC is crucial to the types of EAB that eventually cover at the anode.[22]

2.- Types of MFC and relative advantages and disadvantages

2.1. Two-compartment systems

A typical MFC consists of an anodic chamber (anaerobic) and a cathodic chamber (aerobic) separated by proton exchange membrane (PEM).[23, 1] Microorganisms in the anodic chamber act as biocatalysts for the oxidation of the biodegradable organic matter. During this oxidation process, protons and electrons are produced. The latter are transferred to the anode and afterwards are transported through an external circuit to

the cathode, where they reunite with protons and oxygen to produce water. In this way, the flow of electrons produces an electrical current and an associated power. On the other hand, protons in the anodic chamber migrate through the solution reaching the proton exchange membrane. They pass through this membrane to the cathodic chamber and eventually they reach the cathode where the above mentioned formation of water occurs.[3, 23, 24, 25]

This conventional two-compartment system has shown limitations in terms of power output due to the internal resistance of the system. Moreover, generally forced aeration of the cathodic chamber is required with a negative effect on the overall, net energy gain of the MFC which in turn it translates in higher operating costs of the cell.[26]

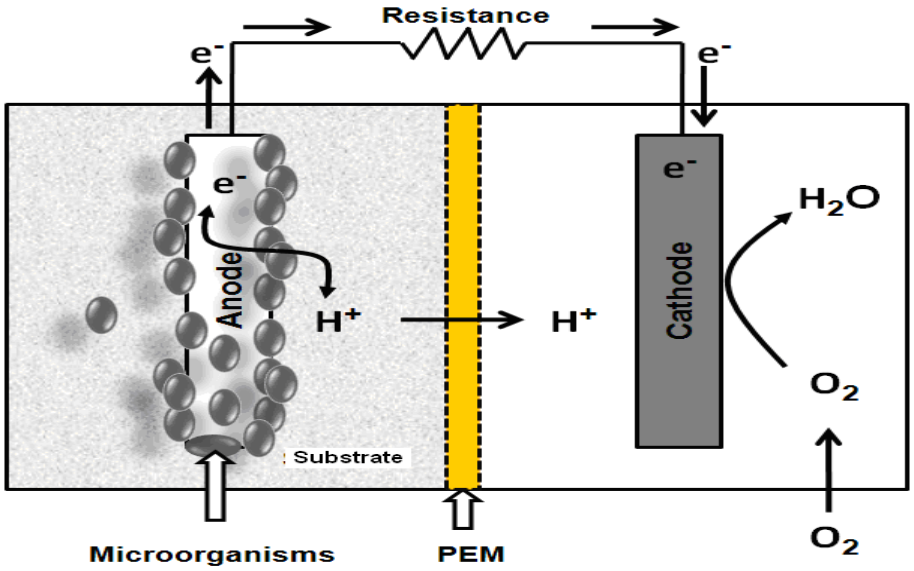

Figure 1. Two-compartments system

2.2. One-compartment systems

A simple and effective MFC design is that with one chamber (anodic chamber). In this design, the cathodic chamber is eliminated. In fact, the PEM is generally bound to the cathode, which is exposed to atmospheric air in one extreme side of the cell. Contact between cathode and air allows for aeration by natural convection, *i.e.*, the need for forced aeration of the cathode is circumvented, at least for lab scale MFC.[1, 25] Some special one-chamber MFCs have been built without PEM, although their long term efficient operation remains to be demonstrated.[26]

Liu and Logan[26] have designed an MFC consisting of an anode placed inside a plastic cylindrical chamber and a cathode placed outside, Figure 2a shows the schematic of laboratory prototype of the bioreactor. The anode was made of carbon paper without wet proofing. The cathode was either carbon electrode/ PEM assembly fabricated by bonding the PEM directly onto a flexible carbon-cloth electrode.

Rabaey *et al.*[13] built an MFC that consisted of a robust cation exchange membrane, which was folded and sealed through soldering to provide a cylindrical structure (Figure 2b). The reactor was filled with graphite granules that performed as the anodic electrode material. The cathode comprised of a woven graphite mat with a high surface area tightly matched around the membrane. The cathodic electrolyte was a $K_3Fe(CN)_6$ solution in a phosphate buffer.

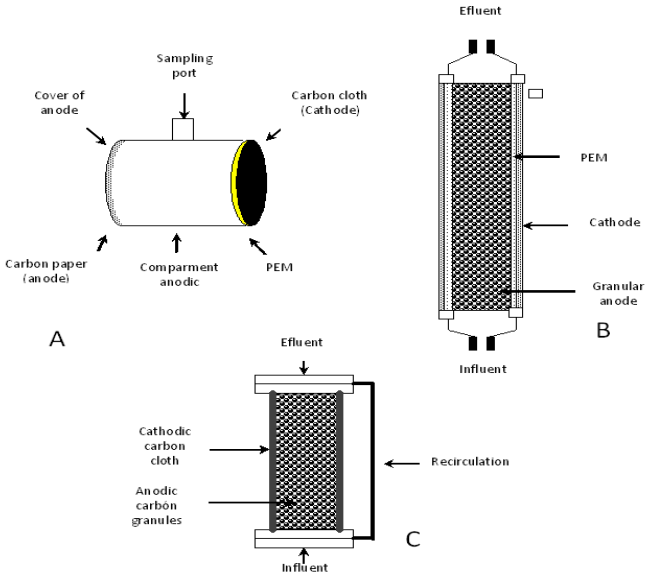

Figure 2. *Squematics of one-comparment sytems. A.[26] B.[13] and C.[27]*

Zhang et al.[27] worked with an innovative MFC coined UAMMFC (for upflow air-cathode membrane-free microbial fuel cell) that consisted of a 4 cm-diameter and 13.5 cm-height cylindrical Plexiglas columns shown in Figure 2c. This MFC lacked a PEM. A conical shaped cover was placed at the top of the MFC to collect the treated effluent. Homogeneous holes were drilled on the wall of the anodic zone to form a total holes area of 62 cm^2 that were available for proton transport. Anodic carbon granules filled the anodic zone External transport of electrons was facilitated with a graphite rod connected into the anode. The cathode was made of a flexible carbon cloth tightly bound to the wall in the anodic zone outside, resulting in an electrode distance as

close as possible. The cathode was prepared with fine C/Pt powders. The two electrodes were connected by copper wires to close the circuit.

There are several advantages of using a single-chamber MFC versus a two-chambered system: increased mass transfer to the cathode; decreased operating costs, because it is not necessary to sparge the water; an overall decrease in reactor volume; and a simplified design.[28]

3.- Biocathodes

Biocathodes are feasible in potentiostat-poised half cells, but only very few studies have investigated them in complete MFCs (Table I). Biocathodes may be advantageous over abiotic cathodes for several reasons. First, the cost of construction and operation of MFCs may be lowered. Metal catalysts or artificial electron mediators could be made superfluous in MFCs with biocathodes, because microorganisms can function as catalysts to assist the electron transfer. In addition, under some special conditions, microorganisms, such as algae, can produce oxygen through photosynthetic reactions, omitting the cost for an external oxygen supply. Second, biocathodes may improve MFC sustainability, because problems with sulfur poisoning of platinum or consumption and replenishment of electron mediator will be eliminated. Third, the microbial metabolism in biocathodes may be utilized to produce useful products or remove unwanted compounds. Biocathodes can also be involved in the nitrogen removal process during wastewater treatment by reducing nitrate compounds (i.e., denitrification). By using denitrification in MFCs, electrons from organic waste oxidation can be used, rather than external electron donors, such as ethanol, methanol, or hydrogen gas, which are commonly added to denitrification bioreactors.[6]

3.1 Aerobic Biocathodes

In general, biocathodes can be classified as aerobic and anaerobic biocathodes, depending on the terminal electron acceptors adopted in the cathode. Oxygen is the most popular terminal electron acceptor for the cathode reaction in MFCs, because of its high redox potential and abundance in the air, as well as relatively low cost to supply. Several studies utilized microorganisms to assist the oxidation of transition metal compounds, such as Mn(II) or Fe(II), for electron delivery to oxygen. In addition, bacteria in the cathode benefited the reaction by supplying oxygen.[6]

3.2. Anaerobic Biocathodes

In the absence of oxygen, other compounds, such as nitrate, sulphate, iron, manganese, selenate, arsenate, urinate, fumarate and carbon dioxide can function as terminal electron acceptors (note here that iron and manganese do not function as electron mediators under anaerobic conditions).

Among commonly found compounds, nitrate, iron, and manganese have a relative metabolic activity close to oxygen, while sulfate has a much lower relative activity. Regarding their redox potentials, the cathodic potential with nitrate, manganese, and iron as terminal electron acceptors is comparable to oxygen. On the other hand, sulphate has a negative potential, eliminating a favorable energy generation. Thus, based on metabolic activity and their electrochemical property, nitrate, iron, and manganese are promising as terminal electron acceptors in an anaerobic cathode. An advantage of using an anaerobic biocathode instead of an aerobic biocathode is the elimination of oxygen diffusion into the anode via the PEM, preventing the loss of electrons to oxygen rather than the electrode.[6]

Table I. Application of biocathodes in microbial fuel cells

Type Cell	Electrodes	Operation Mode	Inoculum	Substrate	Power density (W/m^3)	Membrane	Terminal electrons acceptor	Reference
Two-chamber	Plain granular graphite	Batch	Sludge	Start-up stage: domestic wastewater	0.13±0.04	Ultrex	Oxygen	85
				Steady stage: Synthetic wastewater	6.46±1.93		Nitrate	
Two-chamber	Non wet-proof carbon paper	Batch	Swage	Start-up stage: domestic wastewater	0.19	Nafion	Nitrate	5
				Steady stage: Synthetic wastewater				
Tubular	Granular graphite	Batch	Effluent from MFC, sludge and sediment	Sodium Acetate	83±11	Ultrex CM17000	Manganese	86
		Continuous			65±5			
Tubular	Granular graphite	Continuous	Effluent from MFC, sludge and sediment	Sodium Acetate	8	Ultrex CM17000	Nitrate	87
Two-chamber	Graphite roads	Batch	Aerobic sludge	Sodium Acetate	0.11	Ultrex CM17000	Oxygen	88

4.- Enrichment

4.1 Microbial fuel cells make it possible to generate electricity using bacteria

It has been known for almost one hundred years that bacteria could generate electricity,[29] but only in the past few years has this capability become more than a laboratory novelty. The reasons for this recent interest in using bacteria to generate electricity are a combination of the need for new sources of energy, discoveries about microbial physiology related to electron transport, and the advancement of fuel-cell technologies. In a microbial fuel cell (MFC), bacteria are separated from a terminal electron acceptor at the cathode so that the only means for respiration is to transfer electrons to the anode. The electrons flow to the cathode as a result of the electrochemical potential between the respiratory enzyme and the electron acceptor at the cathode. Electron transfer from the anode to the cathode must be matched by an equal number of protons moving between these electrodes so that electroneutrality is preserved.[30]

Microbial fuel cells (MFCs) are devices that convert chemical energy into the form of electricity through the catalytic activity of microorganisms.[15, 16, 17, 31, 32, 33, 34, 35, 36, 37, 38, 39] Many different bacteria can produce a modicum of electricity in an MFC if a mediator (electron shuttle) is used to facilitate the transfer of electrons between the bacterial cell and the working electrode (anode) in the system. [15, 35, 36] However, such mediated fuel cells (Figure 3A) tend to be inefficient, expensive, and produce low levels of power.

Recently, a number of bacteria (dubbed "electrochemically active bacteria, or EAB) have been found to possess the ability to transfer electrons from oxidized fuel (substrate) to a working it possible to establish mediator-less MFCs (Figure 3B).

4.2 Mediator-less MFC

A mediator-less MFC was first demonstrated by Kim *et al.*[16] in which anaerobically grown cell suspensions of the metal reducing bacterium, *S. putrefaciens* produced a quasi-reversible cyclic voltammogram (CV) with a reductive peak at -0.32 V and an oxidation peak at 0.03 V against a saturated calomel electrode (SCE). The apparent redox potential was -0.15 V against a SCE, which is about 0.05 V against a normal hydrogen electrode (NHE).[16] No redox peaks were observed in the CV test of the aerobically grown *S. putrefaciens* cells. These results suggested that, as long as anaerobic conditions were maintained, direct alectron exchange should be possible using *S. putrefaciens*.

Table II. Comparison of MFC performances

Biocatalyst	Operation condition	Operation Mode	Anode mediator	Cathode Mediator	Power density (mW/m^2)	Sources
Enriched microbial consortium	thermophilic	Continuous	none	None	1030 ± 340	63
Enriched microbial consortium	mesophilic	Continuous	none	None	560	21
Shewanella oneidensis	mesophilic	Continuous	yes	Yes	3000	62
Enriched microbial consortium	mesophilic	Batch	none	None	8.3	10
Shewanella putrefaciens	mesophilic	Batch	none	none	0.32	17
Geobacter sulfurreducens	mesophilic	Batch	none	none	16	58
Rhodoferax ferrireducens	mesophilic	Batch	none	yes	8.2	7
enriched microbial consortium	mesophilic	Batch	none	yes	480	66
enriched microbial consortium	mesophilic	Batch	none	none	1330	28
enriched microbial consortium	mesophilic	Batch	none	yes	3600	20
enriched microbial consortium	mesophilic	Batch	yes	yes	788	67

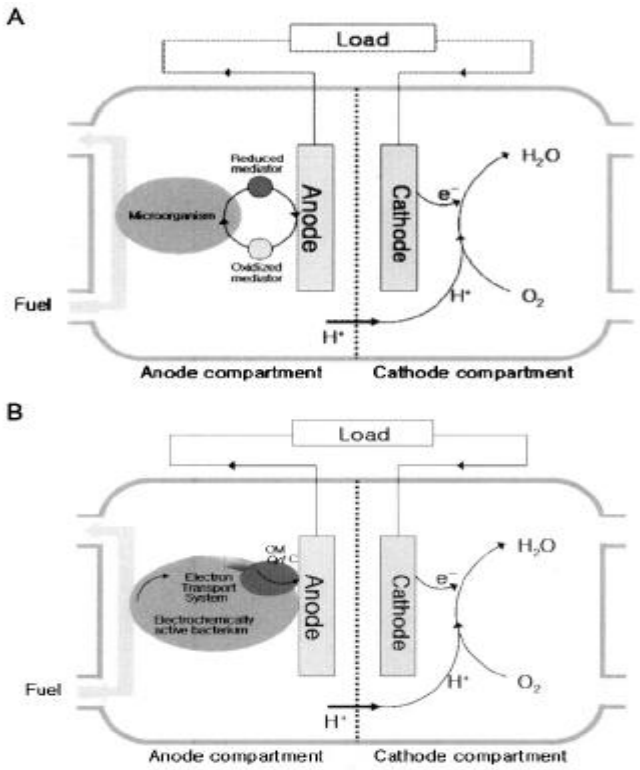

Figure 3. *Two microbial fuel cell systems. A. Mediated-MFC, B. Mediator-less MFC.*[40]

Direct electron transfer from cells of *S. putrefaciens* to an electrode was also tested using an MFC-type electrochemical device and lactate as the fuel.[16, 17]

When the circuit was not connected between anode and cathode electrodes (i.e., open circuit), the cells did not consume lactate,[41] while under the closed circuit condition, *S. putrefaciens* consumed lactate and generated electricity.[15, 16] To our knowledge, this was the first experimental verification of a mediator-less MFC operation. Based on both CV and MFC results, Kim *et al.*[16] also proposed that the electrochemical activity of bacterial cell suspensions was due to the presence of electrochemically active compounds on the cell surface such ascytochromes as depicted in (Figure 4). With regard to this, it is notable that, based on genome analyses, *S. oneidensis* strain MR-1 has 43 possible cytochrome *c* genes on the whole genome sequence,[42, 43] while another electrochemically active DMRB (dissimilatory metal reducing bacteria), *Geobacter sulfurreducens* has more than 100.[44, 45] It is also notable for both organisms, that a number of these c-type cytochromes are located on the outer membrane of the organism, well sited for their role in extracellular electron exchange.

To our best knowledge, there is no report on the thermophilic mediatorless microbial fuel cells using electrochemically active microbial consortium. However, there is a report that investigates the use of a single thermophilic microorganism to generate electricity in a MFC with a mediator at elevated temperatures (Table II).[45]

4.3Electrochemically Active Bacteria and Mediator-less MFCs

Recently, a number of microorganisms have been isolated based on their ability to use oxidized metal ions including Fe (III) and Mn (IV) as their electron acceptors.[46, 47] In anoxic environments, most microbial electron acceptors such as nitrate, sulphate and carbon dioxide are essentially water soluble both before and after reduction. However environmental Fe (III) and Mn (IV) minerals, which are used as electron acceptors by the DMRB, usually exist as insoluble (solid) oxyhydroxide minerals at neutral pH levels. Thus the DMRB face the problem of communicating electrochemically with solid substrates that are by definition unavailable to the membrane-bound enzymes usually involved in respiration. To overcome this obstacle, the DMRB employ several strategies including: 1) the utilization of naturally exiting electron shuttles (such as humic substances) as mediators;[48, 49, 50] 2) the production of their own mediators;[71] and, 3) the use external (outer membrane) components to effect directly electron transfer to the metals. Multiheme *c*-type cytochromes are thought to play a major role in this unique electron transport system (ETS) and outer membrane cytochromes are believed to be the contact point to externally located Fe (III) and Mn (IV) bearing minerals. Among the DMRB, *Shewanella oneidensis* (formerly *S. putrefaciens*),[42, 53, 54] and *Geobacter sulfurreducens*,[44, 45, 55, 56] are found to localize some of the *c*-type cytochromes on the outer membrane, rendering the cells electrochemically active in mediator-less MFC systems.[15, 16, 57, 58] Electrochemical activities have been observed in other DMRB such as *Aeromonas hydrophila*,[59] *Rhodoferax ferrireducens*,[7] *Desulfobulbus propionicus*[30] as well as some fermentative microorganisms such as *Clostridium butyricum*[61] and *Enteroccus gallinarum* (Table III).[19]

Dissimilatory metal reducing bacteria (DMRB), which are capable of the reduction of solid metal oxides, are known EAB species, including *Geobacter* and *Shewanella* spp (Table III).[16, 41, 45] It was shown that the anode electrode in MFCs served as the electron acceptor for growth and metabolism of EAB, which are capable of current production in the absence of a mediator.[41] Not only did single strains demonstrated this ability in MFC,[7, 58, 62] but some bacterial consortia also have been enriched on anodes of MFCs, using organic materials in wastewater as fuel and recovering electrons as the current.[10, 22, 63, 64] This enrichment technique was explored using sludge collected from a cornprocessing wastewater treatment plant as the inoculum. During the operation, repeated wastewater replacements were coupled with current increase.[10]

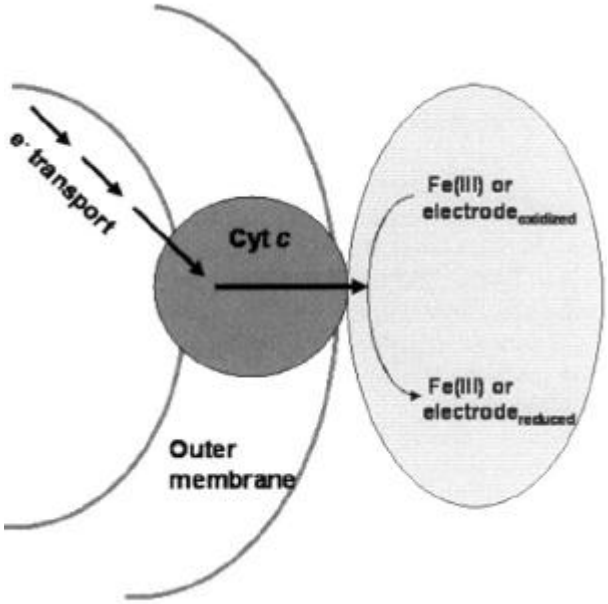

Figure 4. *Proposed electron transport by outer membrane cytochrome to oxidized metal or electrode.*[40]

Furthermore, enrichments of MFCs were made with continuous feeding of various different fuels, including artificial waewater containing acetate, propionate, or artificial wastewater containing glucose and glutamate.[22] In most cases, current from MFCs was stably generated within 3 weeks under given feeding rates and fuel concentrations. The results revealed that the bacterial population in the MFC was different from that in the original inoculum[10, 64] and microbial populations in MFCs were dependent on the fuel used. Among the previous research on MFCs, nonfermentative fatty acids such as acetate and butyrate were favorably used as the fuels [22, 63] to enrich EABs, which are capable of current production without co-metabolism of fermentation. The MFCs fed fermentable substrates such as glucose and glutamate[22] showed more diverse bacterial populations on anode electrodes than those fed nonfermentable substrates such as acetate.

However, many species known as non-EABs were also present in MFCs fed with acetate. Formate is also a nonfermentable fatty acid that can be metabolized directly by bacteria. It is also known both as an intermediate and as a precursor in the synthesis of acetate from a single carbon source by acetogenic bacteria. This synthesis process is known as the acetyl-CoA pathway. To our knowledge, there is no report on the use of formate as a fuel for MFCs.[65]

Tabla III. Mediator-less microbial fuel cell operation using metal reducing bacteria

Metal reducing Bacteria	Reference
Aeromonas hydrophila	59
Clostridium butyricum	52
Desulfobulbus propionicus	60
Enteroccus gallinarum	19
Geobactersulfurreduences	58
Rhodoferax ferrireducens	7
Shewanella putrefaciens (Shewanella oneidensis)	16, 17

4.4 Enrichment of electrochemically active bacteria

Electrochemical techniques are used in various fields of biology, for example in biochemistry to characterize redox proteins and in biotechnology to develop biosensors, bioelectrochemical synthetic processes, and biofuel cells.[68] Although a large number of redox proteins are electrochemically active, the peptide chain adjoining the redox center of the protein hinders direct electron transfer between the redox proteins and an electrode. Modifications of the protein or the electrode surface can increase the rate of the electrochemical reactions.[69] In most cases, intact microbial cells that contain active redox proteins are electrochemically inactive, as their cell walls and other surface structures are electrically non-conductive. Mediators can be used to facilitate the transfer of electrons between microbial cells and an electrode.[32, 70] Alternatively, the bacterial cells can be modified with hydrophobic conducting compounds to increase electrochemical activity.[71, 72]

Experiments with *Shewanella putrefaciens* showed that under conditions where electron were not present, its metabolism was stimulated by the presence of the MFC anode.[16] Based on these simple observations, it was posited that the anode itself should offer a pathway for the isolation of EAB via anaerobic anrichment. Thus, *"Enrichment"* using an MFC system was initially proposed[73] as a tool for selecting electrochemically active consortia (Figure 5). This technique was explored using sludge collected from a corn-processing wastewater treatment plant as the inoculum. The anode compartment of the MFC was inoculated with the sludge and fed with wastewater from the same source. The cathode compartment contained a buffer under continuous aeration and the two compartments were separated by a cation exchange membrane (i.e. it was an MFC!).[10]

Table IV. Comparison of bacterial communities in MFCs enriched with different fuels.

Fuel value as COD[a])	Class (%)						Ref
	α-Proteobacteria	*β-Proteobacteria*	*γ-Proteobacteria*	*δ-Proteobacteria*	*Firmicutes*	*Others*	
Glucose/ glutamate (copiotrophic, 200)	1.4	6.8	36.5	14.9	27.0	13.4	22
Glucose/ glutamate (copiotrophic, 200)	64.4	21.1	3.3	0	0	11.1	76
SPW[b] (400)	27.2	40.9	0	0	4.5	27.1	76
River water (≈ 5)	10.8	46.2	12.9	12.9	0	17.2	76
Acetate (300)	7.0	1.7	17.3	68.8	1.0	3.8	76
Propionate (100)	0	19.4	22.4	10.2	0	41.8	76

[a]COD unit measured in mg/L
[b]SPW: starch processing wastewater

An open circuit potential (OCP) of around 0.6 V was connected through a 10 Ω resistance the potential dropped to 20 mV, which corresponds to a current of 20 μA. When an aliquot of anode solution was replaced with new wastewater (fuel), the current increased. This current increase was concomitant with COD reduction. Repeated wastewater replacements were coupled with current increase up to 1.2 mA. Similar patterns were also observed in all MFCs which had been inoculated with activated sludge or anaerobic digester sludge.[10]

These results suggest that EAB propagated in the MFC and that wastewater and/or sludge of enrichment (Figure 5A top). The electricity production might be the result of electrons transferred to the electrode by EAB after they metabolized electron donor(s) in the wastewater in the absence of any other electron acceptors (Figure 5A middle). Because it is thought that the electrode reducing step is an energy conserving microbial respiration process,[10, 57] EAB could be enriched during MFC operation. If the enrichment step is stabilized, the electron donor(s) consumption in the wastewater could be metabolized faster than of previous step (Figure 5A bottom).

Furthermore, enrichment cultures were made with various nutritional characteristics, including copiotrophic cultures enriched with artificial wastewater containing acetate,[74] propionate or artificial wastewater containing glucose and glutamate,[22] and oligotrophic cultures with artificial wastewater,[75] or river water (Table V).[64, 75]

The small ribosomal RNA genes have been amplified and sequenced from the MFCs enriched with different fuels in the laboratory.[7, 48, 57] Comparison showed that the population of bacteria that dominates the electrode is dependent on both the type and concentration of the electron donor used as fuel (Table IV). When artificial

wastewater was used, *γ-Proteobacteria* and *Firmicutes* bacteria were most abundant under copiotrophic conditions; however, oligotrophic MFC was dominated by *α-Proteobacteria* . On the other hand, oligotrophic MFC anriched using river water contained a bacterial population dominated by *β-Proteobacteria*. Acetate and propionate, both non-fermentable electron donors, were occupied by different bacterial populations with around 70% of *δ-Proteobacteria* and 41% of other classes respectively. These differences were further substantiated by a denaturing gradient gel electrophoresis (DGGE) method to examine the small ribosomal RNA genes of the MFCs (Figure 6).

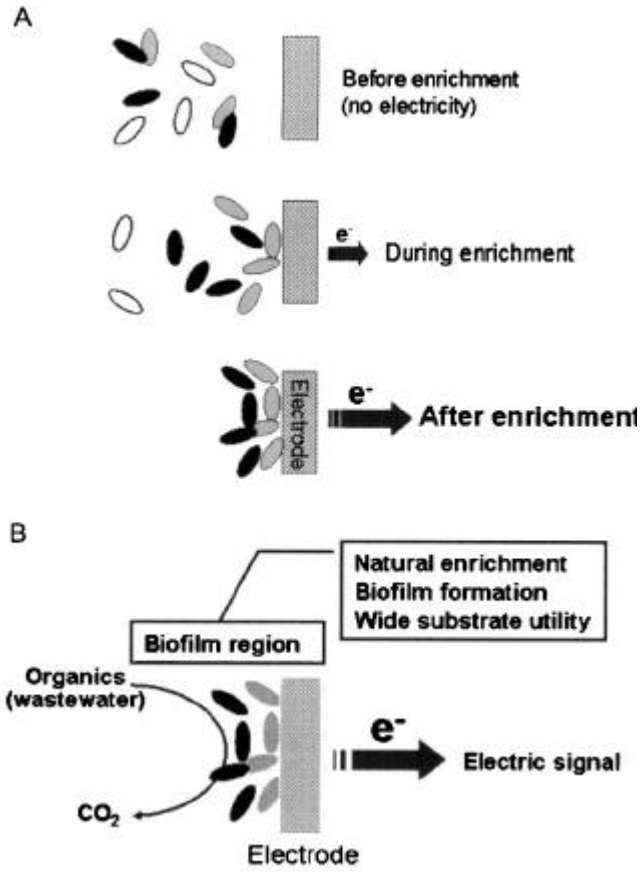

Figure 5. *Enrichment steps.* ***(A)*** *and enriched electrode* ***(B)*** *in microbial fuel cell.*[40]

Oligotrophs are microorganisms that grow in environments low in nutrient concentrations such as oceans, clear lakes and groundwater aquifers.[77] The microorganisms present in environments with low levels of nutrients grow under apparently optimal conditions. However, it is not easy to cultivate oligotrophs under laboratory conditions and most of them are therefore classified as ‘‘yet to be cultured bacteria’’. The ability of

bacteria to grow in this way has a number of important biotechnological, medical and environmental implications,[78, 79] especially regarding MFC application as a BOD sensor.[80] It has been found that the majority of the bacteria enriched in an MFC are not easily cultivated and no information about oligotrophic communities in MFCs has been available so far.[81] Molecular techniques are now widely applied to assess the diversity of microbial communities by analyzing the 16S rDNA sequence. Molecular ecological techniques include denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism (RFLP) and 16S rDNA sequencing. These methods are much less time consuming than traditional isolation and cultivation methods [82]-[84] and may be the methods of choice to analyse oligotrophic communities.

Fermentable substrates showed more diverse bacterial populations in the MFC than that of non-fermentable substrates (such as acetate) based on 16S rDNA analyses. This could be due to nutritional characteristics of electron donors.

Non-EAB may also be present in the electrochemically active MFC consortia. These microbes may pay a critical role in generating electron donor(s) for the EAB as a result of their metabolism.

Table V. The diversity comparison of oligotrophic communities in the mfcs enriched river water or artificial wastewater.

Classes	Oligotrophic MFCs fed with	
	River water (%)	Artificial wastewater (%)
Alphaproteobacteria	10.8	64.5
Betaproteobacteria	46.2	21.1
Gammaproteobacteria	12.9	3.3
Deltaproteobacteria	12.9	
Bacteroidetes	8.6	7.8
Actinobacteria		3.3
Acidobacteria		5.4
Verrucomicrobia		1.1
Chloroflexi		2.1

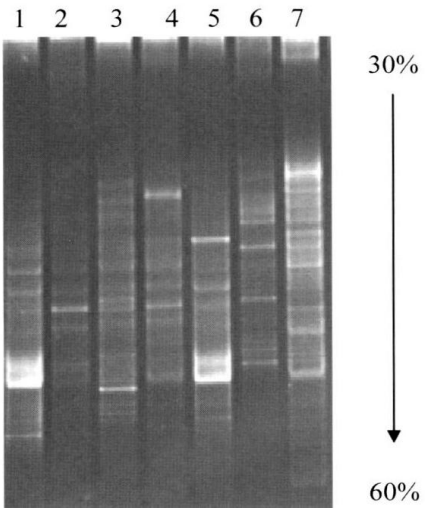

Figure 6. *Comparison of DGGE patterns of microbial communities of various MFCs. Denaturing gradients used from 30 to 60%. Lane 1, Sludge (inoculum); lane 2, Acetate enriched MFC; lane 3, Artificial wastewater (copiotrophic) enriched MFC; line 4, Starch wastewater enriched MFC; lane 5, Starch wastewater; lane 6, Acetate control MFC; lane 7, Artificial wastewater (oligotrophic) enriched MFC.*[64]

5.- Conclusion

Power output of MFC can be limited by high values of its internal resistance (Ri). So far, Ri has been minimized by the extensive use of Pt for connectors and cathode catalyst. Yet, Pt is very expensive and research on more economic new materials and catalysts is a dynamic area of development. Biocathodes are a welcome advancement in the quest to implement MFCs for practical applications, such as wastewater treatment and sediment MFCs, because of potential cost savings, waste removal, and operational sustainability.

Few studies have described biocathodes in complete MFCs. Results from potentiostat-poised half cells and electrolytic cells, on the other hand, showed that biocathodes are promising substitutions for abiotic cathodes in MFCs. Biocathodes are a welcome advancement in the quest to implement MFCs for practical applications, such as wastewater treatment and sediment MFCs, because of potential cost savings, waste removal, and operational sustainability.

The bacterial electron-transfer mechanisms in the cathode must be fully understood. This is required to ascertain the limitations of electron transfer from cathode to microorganism, and to subsequently reduce the biological overpotentials. Several successful implementations of biocathodes must be demonstrated without external power supplies. The current advances with biocathodes in potentiostat- poised half cells cannot

automatically guarantee the same results in complete MFCs, because an applied potential overcomes many limitations occurring in MFCs, such as anodic charge – and anolyte – resistances. A competitive advantage of electrochemically active bacteria over other bacteria in the cathode must be sustainable, especially in natural systems or when wastewater is used as the catholyte. The presence of other electron donors than the electrode could disturb the cathodic electron flow, and therefore the electric current in MFCs.

Mediator-less MFC systems can use biomass as a fuel source both directly and indirectly bye the catalytic activity of microorganisms. Additionally, MFC systems directly produce electricity suggesting that they don´t require advance processes to purify (separate) energy resources.

In this review, we have discussed physical and biochemical properties of electrochemically active bacteria as biocatalyst in mediator-less MFC systems and their unique electron transport chain. We also investigated several rate limiting steps. This was a very helpful approach to understand the working of MFC systems, and to optimize the systems for environmental process such as wastewater treatment systems. Based on the discussion here, we may propose that mediator-less MFC systems can be a novel energy production systems in terms of renewable, sustainable energy as well as pollutant control process. Indeed, mediator-less MFC systems have a great potential for future technology.

MFC systems can be a tool for selecting EAB consortia. The differences in bacterial populations between the enriched cultures may also be due to the types of fuel cells used for the enrichment studies. Diverse bacterial populations in MFCs enriched under different conditions show that electrochemical activity is not restricted to a few phyla of bacteria. With a deeper understanding of these EAB, we could manipulate them to play an important role in biogeochemical recycling in the future. MFC have the potential to be selective devices for culturing microorganisms, especially EAB, by determining the type and concentration of electron donor and acceptor the type of MFC to be used.

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