

Review

Microbial electrochemical technologies for wastewater treatment: principles and evolution from microbial fuel cells to bioelectrochemical-based constructed wetlands

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Abstract: Microbial electrochemical technologies (MET) rely on the presence of the metabolic activity of electroactive bacteria for the use of solid-state electrodes for oxidizing different kind of compound, that could lead to the synthesis of chemicals, bioremediation of polluted matrices, the treatment of contaminants of interest, as well as the recovery of energy. Keeping in mind those possibilities, since the beginning of the present century, there has been a growing interest in the use of electrochemical technologies for wastewater treatment, and if possible with simultaneous power generation. In the last years, there has been a growing interest to explore the possibility of merging MET with constructed wetlands, to offer a new option of intensified wetland system that could keep a high performance with a lower footprint. Based on that interest, this paper explains the general principles of MET, and the different known extracellular electron transfer mechanisms ruling the interaction between electroactive bacteria and potential solid-state electron acceptors. Also, the adoption of those principles for the development of MET set-ups for simultaneous wastewater treatment and power generation, and the challenges that the technology face. Ultimately, the most recent developments in set-ups that merges MET with constructed wetlands are presented and discussed.

Keywords: bioelectrochemical systems (BES); electroactive bacteria (EAB); extracellular electron transfer (EET); microbial fuel cells (MFC); treatment wetlands.

1. Introduction

Bioelectrochemistry is the merging field between microbiology and electrochemistry, which includes research interest of environmental engineering, electrochemistry, biochemistry and physics. Systems derived from this field are denominated bioelectrochemical systems (BES), and incorporate the study and knowledge applications of different subfields, such as microbial, enzyme, protein, DNA and neuro-electrochemistry (Figure 1) [1]. BES have being design to provide different products, such as electricity generation, synthesis of sub-products and environmental services including, soil bioremediation, desalination and wastewater treatment [2,3]. BES are based on the bacterial interactions with insoluble electron donors and acceptors, relying on the exchange of

metabolic electrons which are removed from an electron donor or supplied to an electrode acceptor (e.g. a solid state electrode) through an electroconductive material [4,5].

The first microbial electrochemistry experiences can be tracked back to the first half of XX century with the findings by Potter [6] regarding the ability of certain species of bacteria, *Escherichia Coli*, to generate electricity through substrate oxidation processes, and the first attempts made by Hooker to take advantage of the oxidation-reduction reactions aimed at creating an electrical cell [7]. The rapid evolution and interdisciplinary approach, has led to the development of different terms to describe their principles and applications; Schröder [1] remarked the distinction among the concepts microbial electrochemistry, microbial electrocatalysis and microbial electrosynthesis (Figure 1).

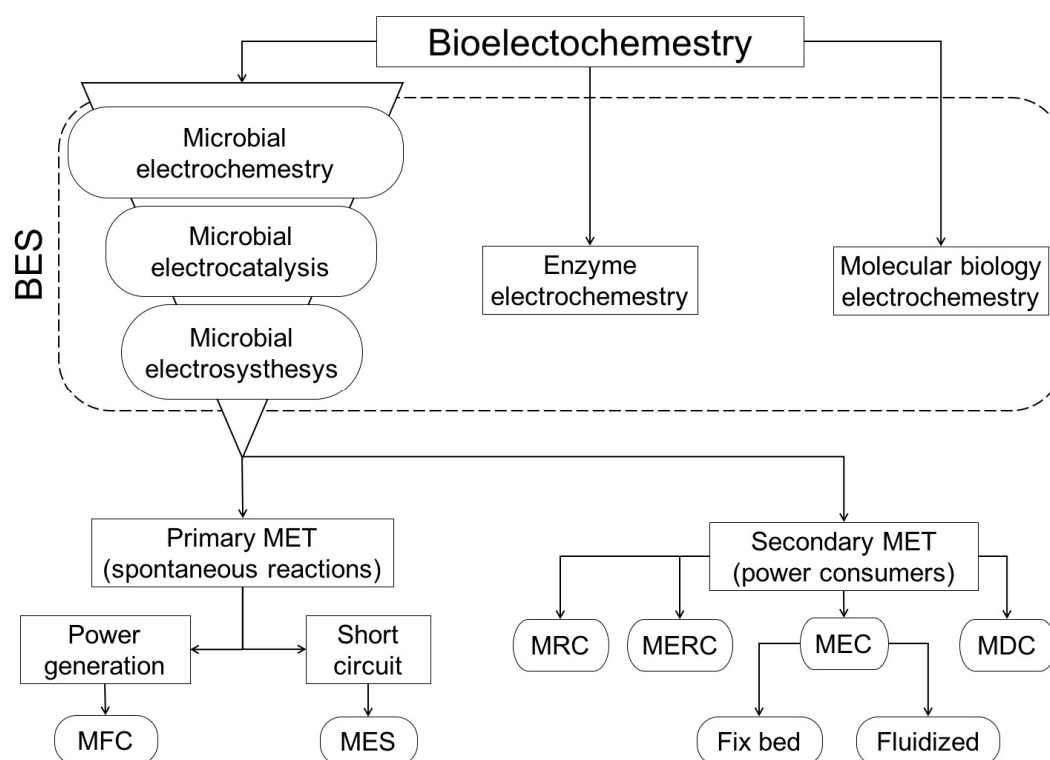


Figure 1. Diagram illustrating the disciplines of biochemistry field, the interrelations with bioelectrochemical systems, and their applications as microbial electrochemical technologies.

The Microbial electrochemistry concept refers to the study and application of interactions between living microbial cells and electrodes, through capacitive or Faraday interactions. In capacitive interactions, the changes occur in the double layer capacity of electrodes as result of cell attachment/detachment, when the lipid layer of microorganism enters in contact, displaces water molecules and ions from the electrode, resulting in a change of the electrochemical capacity, leading to the flow of electric current. Whereas in Faraday interactions, oxidation and reduction reactions occur mediated by microbial cells and molecular species involved in extracellular electron transfer, which could be done either by pseudo-capacitive processes (cells and biofilm get charge or uncharged, as a supercapacitor) or by microbial electrocatalysis process, where electrochemical reaction are accelerated by microorganisms based on extracellular electron transfer [1].

Microbial electrocatalysis increases the rate of the electrochemical reactions, mediated by microorganisms' extracellular electron transfer. The electron flow reduces the overpotential or increase the reaction rates, at a fixed potential, of an electrochemical reaction. Electrocatalysis can be used for substrates degradation, as in microbial fuel cells (MFC) allowing simultaneously wastewater treatment and energy recovery.

The Microbial electrosynthesis concept refers to the production of high-value chemicals through the reduction of carbon dioxide and other organic substrates, mediated microorganisms able to grow on cathode surfaces. The microorganisms act as catalysts and use the reducing

equivalents released from solid-state electrodes as energy source. Microbial electrosynthesis can be used for producing sodium hydroxide, methane, ethanol, hydrogen and hydrogen peroxide [8–10].

The applications of microbial-driven electrochemical processes for production of complex organic compounds, energy generation or environmental solutions are called microbial electrochemical technologies (MET). Depending on the operating conditions, they can be classified as primary or secondary MET. In Primary MET, the microbial electrochemical processes (exclusively Faraday processes) involve extracellular electron transfer (EET) mechanisms, either directly from cell to acceptor or mediated by electron shuttles. On the other hand, in secondary MET, the bioelectrochemical processes are controlled by the adjustment of the microbial environmental conditions (pH, oxygen pressure, metabolite concentrations, etc.), as in microbial electrolysis of hydrogen or soil remediation [1]. Roughly MET can be classified as power producers (electrons derived from oxidized organic matter are conducted from to cathode via external circuit), power consumers (consumption of external power to achieve bioelectrochemical cathodic reactions, due to small or negative potential differences that do not allow electron flow from anode to cathode) and as intermediate systems that does neither produce nor consume power, but require stable electrochemical conditions [5].

According to the application, METs are denominated by different terms (Figure 1), like microbial fuel cells (MFC), for electricity generation, microbial electrolysis cells (MEC), for synthesis of H₂ or other compounds through addition of external power to reduce cathode potential, microbial desalination cells (MDC), for water desalination, microbial remediation cells (MRC), for cathodic reduction of oxidized pollutants like uranium, perchlorate, chlorinated solvents in polluted environments [11,12]. Other MET systems are the microbial electroremediating cells (MERC), that aim at overcome electron acceptor limitation and maximize the biodegradation of pollutants, as herbicide isoproturon [12], or atrazine [13] in the environment.

Given the potential that MET have for wastewater treatment and simultaneous energy production, since year 2012 different studies have explore the technical possibilities and benefits of combining that technology with constructed wetlands (CW). A CW is a biological engineered wastewater treatment system, that rely on the presence of plants and microorganisms, and the interaction of physical, chemical and biological processes, as well as different removal mechanisms [14]. CWs can be classified according to the dominant macrophytes (free-floating, floating leaved, rooted emergent and submerged), hydrology (surface or subsurface flow) and flow direction (horizontal or vertical) [15].

CWs have been extensively investigated, demonstrating their capacity to treat wastewater of different origins like domestic, industrial, drainage mining, runoff and agriculture effluents [16–20]. CWs are characterized for being robust and cost-effective technology that require low operation and maintenance efforts [21,22], being nowadays used worldwide as a mature solution for decentralized wastewater treatment. However, the major drawback of CW implementation is its area footprint, much larger than other compact wastewater treatment technology. Therefore, to reduce the surface requirements, CW have been evolving from passive to intensified systems, including very recent designs that incorporate the combination of CW with MET [23,24]. Therefore, this paper reviews the principles and the state of the art of MET applied in the field of wastewater treatment, and focusses on the potential benefits, challenges and novel set-ups resulting of the merging with CW.

2. Microbial extracellular electron transfer (EET)

MET rely on anaerobic environments in aqueous solutions, to allow the establishment of synergic consortia between fermentative and bioelectrogenic microorganisms [25,26]. Fermentative microorganisms break-down complex organic compounds into simpler structures e.g., acetate, ethanol, glucose, hydrogen gas, aminoacids, polymers (polysaccharides, proteins, and cellulose) and other long-chain, fatty acids, which could be easily oxidized by bioelectrogenic microorganisms [27]. The metabolic characteristics of those electroactive microorganisms allow them to obtain energy from electron transfer to a terminal electron acceptor or from an electron donor characterized for

being a extracellular conductive and insoluble form, enabling their development in environments where the availability of electron sources or acceptors constitute a growth limitation [28].

The favorable conditions allow the establishment of microorganisms colonies that lead to the formation of electrochemically competent biofilms on solid-state electrodes [4]. It is known that development of electroactive biofilms enables the electron transfer, however a complete understanding of the dynamics related with the transfer between electroactive microorganisms and electron acceptors is still under study [29]. Extracellular electron transfer (EET) of bioelectrochemical microorganism is affected by the potential difference between the final electron carrier and the anode [11,30], and can be executed by two main mechanisms: direct extracellular electron transfer (DEET) and by mediated extracellular electron transfer (MEET).

2.1. Direct extracellular electron transfer (DEET)

DEET requires a physical contact between the microorganism and the electrode, usually attached forming a biofilm on the electrode surface. This implies that only bacteria in the first monolayer at the anode surface are electrochemically active [31], which causes a limitation of the catalysis by the maximum cell density in this bacterial monolayer (Figure 2a). However, it has been demonstrated that some species have developed nanowires or pili to reach and utilize distant insoluble electron acceptors or to interconnect inner layers in the biofilm (Figure 2b) [5]. The nanowires (or electroconductive pili) are vesicular extensions of the periplasm and outer membrane of cells (2 – 3 μm length) designed to facilitate the direct electron transfer between cells and electron acceptors [30–32]. The development is a direct response to a limited availability of electron acceptors, making possible the build-up of thick electroactive layers as well as the contact with distant electron acceptors [26,33]. This kind of strategy has been observed for *Geobacter* and *Shewanella* species [34,35].

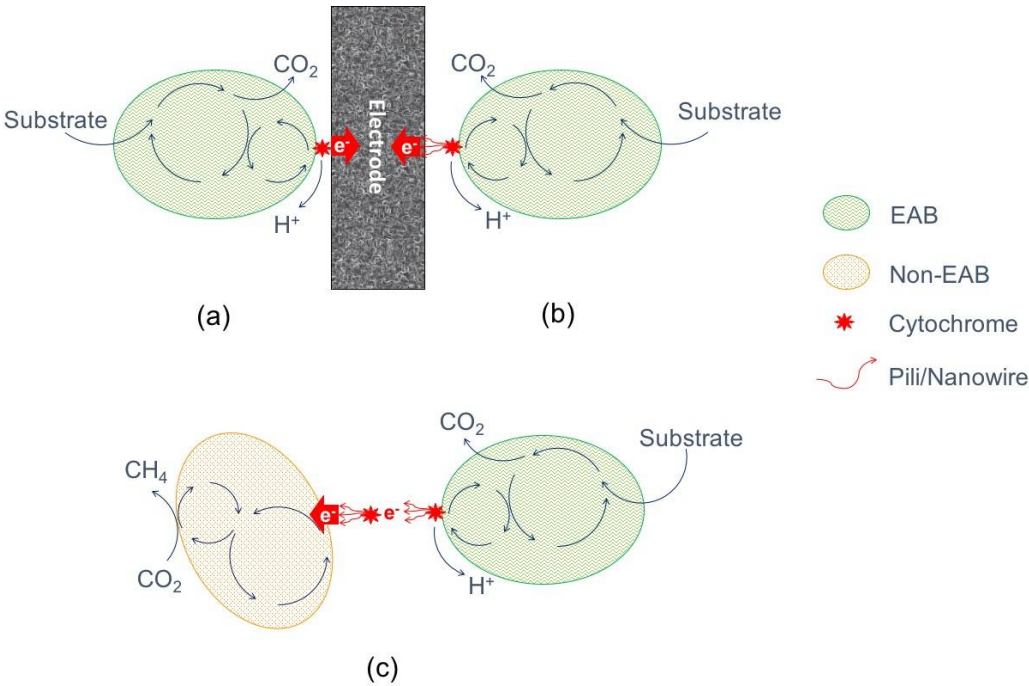


Figure 2. Simplified representation of extracellular electron transfer mechanisms. DEET: (a) by membrane-bound cytochromes, and (b) by electron-conductive pili/nanowires. Direct Interspecies Electron Transfer - DIET: (c).

The ability of microorganisms to directly use another cell as terminal electron acceptor, establishing an electrical contact between two microorganisms is called direct interspecies electron transfer (DIET) (Figure 2c) [36]. It is a interspecies electron transfer, which enables a diversity of microbial communities to gain energy from reactions that no one microbe can catalyze [37]. It is a

mechanism for exchanging electrons during syntrophic metabolism. In this way will be formed electrically conductive aggregates, like anode biofilms [38]. DIET can also take place with a mineral as mediator, a process in which different species use as conduits of electrons nano-mineral particles or conductive surfaces such as activated carbon granules, coke or biochar [28,39].

2.2. Mediated extracellular electron transfer (MEET)

As alternative for those bacteria that are neither able to use DEET or DIET, their metabolism have developed mechanisms mediated by electron shuttles to allow extracellular electron transfer [2]. That mechanism is known as mediated extracellular electron transfer (MEET). Some microorganisms, as *E. coli*, *Pseudomonas*, *Proteus* and *Bacillus*, can naturally synthesize and excrete endogenous redox-active molecules that function as electron mediators, such as flavins or phenazine compounds [31,40]. Also, these mediators can be external agents, artificially added to the media or present in natural environments, as humic substances [41,42]. In the oxidized form, the mediators can collect the electrons either from inside the bacteria cell or from their outer membrane, getting reduced and becoming oxidized after transferring electrons to a terminal electron acceptor [30]. Ideally, they should possess good electrochemical reversibility, a positive redox potential to ease the transfer, and without generating any interference in the cellular metabolism [33]. A disadvantage of this mechanism is that the stoichiometry involved in the conversion of electron donors to electricity is not clear. It is also possible that the fermentation processes can continue, leading to methanogenesis processes, hence reducing the electron transfer to the electrodes and consequently affecting the energy generation [26]. Additionally, their use at large-scale MET applications has some inherent limitations, such as the necessity of continuous addition, the unstable nature of the compounds, and their potential environmental toxicity [33].

MEET rely on the production of own secondary metabolites by bacteria (Figure 3) which are low-molecular shuttling compounds like phenazines, pyocyanine, ACNQ (2-amino-3-carboxy-1,4-naphtho-quinone) and flavins [33,43]. Despite of the reuse possibility as electron carrier, their synthesis are energy costly and will be suitable for closed environments with a minimal substrate replacement like in batch set-ups [26]. However, in the case of dynamic environments like wastewater treatment, those secondary compounds will be exhausted. Some examples of bacteria that are able to produce their own mediators to increase the EET rate are *Pseudomonas spp.*, *Shewanella putrefaciens* and *Geothrix fermentans* [44].

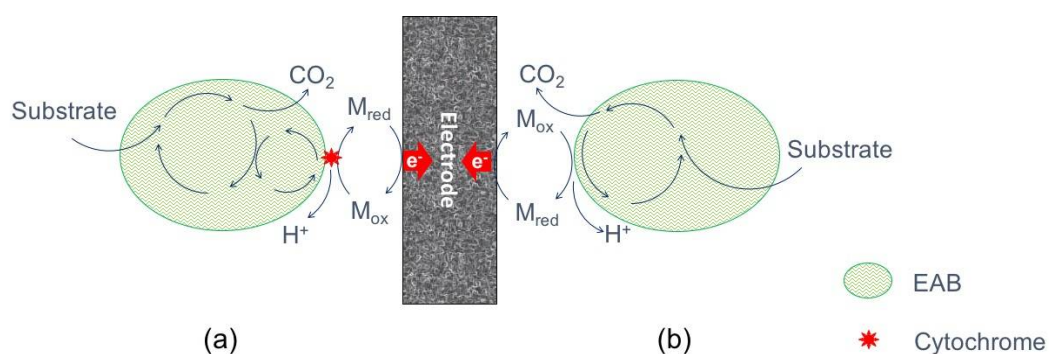


Figure 3. Simplified representation of mediated extracellular electron transfer (MEET) by secondary metabolites. (a) by outer cell-membrane cytochromes, and e⁻ shuttles. (b) by self-produced or external redox mediators.

The MEET process is also possible through the anodic reduction of primary metabolites derived of fermentation and anaerobic respiration processes (Figure 4). However, there is not so much evidence of exactly which kind of reduced products are involved, apart of the slow reaction rates with electrodes making it as an inefficient electron transfer mechanisms [26]. In anaerobic respiration, the sulphate reduction, leads to the formation of sulfide, which by interactions with

available electron acceptors (e.g. iron oxides or electrodes) becomes oxidized, allowing the electron transfer [45]. On the other hand, through fermentation processes, it is possible to obtain energy-rich reduced metabolites like hydrogen, ethanol, formate or ammonia, compounds that can be oxidized by microbial metabolism in the presence of electrocatalytic anodes, thus avoiding the oxidation of those metabolites by other biological processes [43].

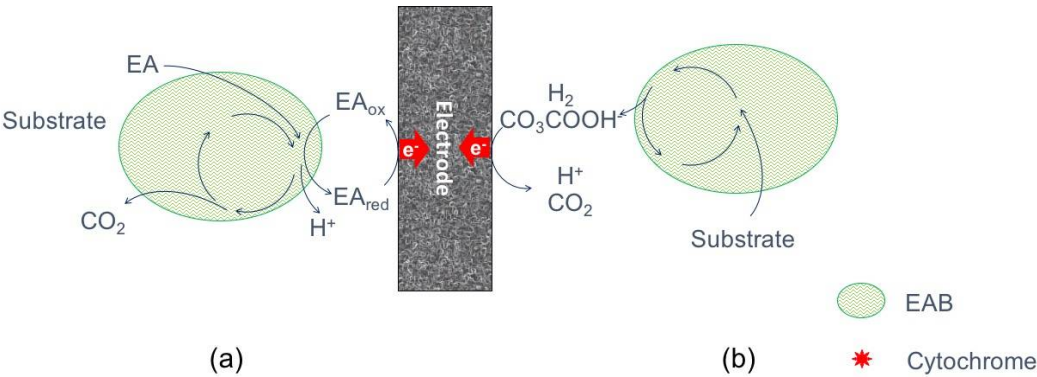


Figure 4. Simplified representation of mediated extracellular electron transfer (MEET) by primary metabolites. (a) by reduced terminal electron acceptors (EA), and (b) by oxidation of reduced fermentation products.

3. Electroactive bacteria and mixed biofilms formation

Electroactive bacteria (EAB), also known as exoelectrogens, electrogens, electricogens, exoelectrogenic or anode respiring bacteria, are microorganisms with the ability to conserve energy from electron transfer to an electron acceptor (e.g. a solid-state electrode), and play a key role in the current densities generation and energy efficiency performance of MET [1,46]. EAB have been found in marine/fresh water sediments [47–49], as well as in different substrates such as manure [50,51], aerobic/anaerobic wastewater treatment sludge [52–54], and wastewater of different origins [55,56].

The presence of EAB in different environments is associated to the inherent characteristics of the electroactive microorganism, whose majority are anaerobic or facultative anaerobic, with some species that can endure aerobic conditions [30]. The most extensive studied EAB are from *Geobacter* and *Shewanella* genus, due to their respiratory versatility for using different carbon sources and electron donors/acceptors [32,57–61]. In previous studies focused on MET performance, it has been identified the interaction of a wide variety of microbial communities, which through DEET processes leads to energy generation [4]. It has been established that as long as there are available substrates in a MET, methanogenic, fermentative and electroactive communities can interact and develop biomass in suspension, in the aqueous environment [62], as well as biofilms attached to an electrode [63].

The biofilm formation, relies on a self-produced hydrated extracellular polymeric substance, composed of polysaccharides, proteins, nucleic acids and lipids. This matrix provides stability, mediates the adhesion of biofilms to surfaces and allows the interconnection of biofilm cells [64]. Since some EAB are able to develop pili and nanowires [31,32], it is expected that those appendages lead to the establishment of nano-power grids, which enhance the biofilm stability, ease the electron transfer in long-range distances, contribute to the growth of biofilm thickness, thus increasing the current production [46]. Biofilms play an important role in electrochemical processes and energy production, since higher cell densities allow higher contact among cells, enhancing the electron transfer possibilities [65]. In biofilms composed by *Geobacter* species, it has been reported long-range electron conductions from cells, located at distances bigger than 50 μm respect to the electrode [66]. However, in biofilms surpassing a thickness threshold of 60 μm, there were no variations in current production, due to the minimal current contribution of the outer bacteria [58], derived of the inherent resistance of the biofilm matrix for electron transfer [46].

In MET applications for power harvesting, the mixture of EAB with redox mediator producers, fermentative and methanogenic populations, are considered undesirable. Those non-electroactive microorganisms could either release mediators that limits the current flow or could detour electrons through another pathways rather than allowing their use for current generation [67]. However, in environmental applications, pure microbial cultures are idyllic due to the complex nature of substances to treat [5]. Mixed populations are highly suitable when a MET will be fed with complex organic matrices like wastewater [8,68,69], since they are able to degrade complex compounds such as carbohydrates and proteins, into more simple and suitable structures like volatile fatty acids [30].

Compared with single-strain cultures, the use of mixed-culture bacteria in METs have some advantages like the no necessity of sterilization, their environmental adaptation, application in continuous processes, the higher robustness and electrical productivity [65,68]. In fact, there are reports of current densities (flow of electric charge per electrode surface area) of a MET inoculated with mixed consortia between 516 mA m⁻² (anaerobic digester sludge) and 1300 mA m⁻² (wastewater), which are higher in comparison with reports of single-strain inoculum with values between 44 mA m⁻² (*P. aeruginosa*) and 130 mA m⁻² (*S. oneidensis*) [69].

Good sources of mixed cultures are aerobic/anaerobic sludge, sediments, manure and domestic wastewater [30]. In a study using lab-scale MET treating real wastewater (COD = 12,020 +/- mg L⁻¹), have reached a maximal power density (rate of electric energy per electrode surface area) of 204 mW m⁻² - anode surface [56]. In addition, at the end of operation (day 278th), the microbial composition was characterized with PCR techniques and were found changes of the dominant species from 50% to 14% for *Actinobacteria*, from 17% to 57% for *Proteobacteria*, and from 33% to 29% for *Firmicutes*, being the *Proteobacteria* and *Firmicutes* genus recognized as EAB [56].

Other study with a lab-scale MET inoculated with activated sludge and fed with livestock wastewater (BOD₅ = 453 mg L⁻¹) [70], it was found a maximum power density of 197 mWm⁻² - cathode area. After 1 month of operation a microbial characterization of the system was done, finding that the electrodes were dominated by *Geobacter* species (72%), followed by other EAB belonging to *Desulfuromonas* (3,1%), *Hydrogenophaga* (3,1%), *Paludibacter* (3,1%) and *Proteiniphilum* (6,3%), all of them bacteria identified before in MFC [70].

4. MET for wastewater treatment

A large potential of energy is kept in wastewaters in the form of biodegradable organic matter. It is known that the energy contained in wastewaters cannot be correlated directly with COD values, but estimations point for 17.7 to 28.7 kJ g⁻¹ COD [71]. In the United States, wastewater treatment facilities consume around 15 GW of power (3% of nationwide power production), whereas 17 GW can be contained in wastewaters of different origins, amount that could supply the energy requirements for their treatment [27]. MET have shown a potential for wastewater treatment, since their COD removal rates can reach up to 90%, with coulombic efficiencies (fraction of electrons recovered as current versus the maximum possible recovery) higher than 80% [72]. It is estimated that in anaerobic digestion 1 kg COD can be converted to 4.16 kWh of power (1 kWh of usable energy in form of electricity), therefore if a MET is intended to compete as an alternative wastewater treatment technology with simultaneous energy generation, they must reach a similar substrate conversion rate [2].

Currently it is not possible for a MET to produce cost-effective energy [26], however, they constitute as an alternative technology to harvest energy directly from wastewater [73]. Some of the limitations for field application of METs include: installation costs, expensive electrode materials and low energy density generated [74]. From the wastewater perspective METs systems arises as an interesting alternative, since they are capable of oxidizing easily degradable organic matter [4]. MET are flexible platforms that by means of oxidation and reduction processes can treat wastewater at a rate of 7.1 kg COD m⁻³ (reactor vol. day⁻¹), and offer the advantage of saving costs like aeration and sludge disposal [75]. However it is also recommended that for high organic loaded/complex wastewater, MET should be complemented with other technologies to obtain fermented products that can be oxidized by electroactive bacteria [5,76].

4.1. Processes and innovative set-ups

The bioelectrochemical wastewater treatment can be accomplished by electrically coupling a microbial anode to a counter electrode (cathode) that performs a reduction reaction. As a result of this electrical connection between the anode and cathode it is promoted the flow of the electrons [77]. The implementation of METs for wastewater treatment has been based on the different reactions that can be catalyzed by EAB, therefore the MET operation can be classified as systems based on non-spontaneous reactions and spontaneous reactions.

3.1.1. Non-spontaneous reactions

Then non-spontaneous reactions occur when the Gibbs free energy of the reaction is positive and the theoretical cell voltage or electromotive force is negative, therefore power needs to be supply. Examples of METs that rely on non-spontaneous reactions are MEC and fluidized systems (Figure 5).

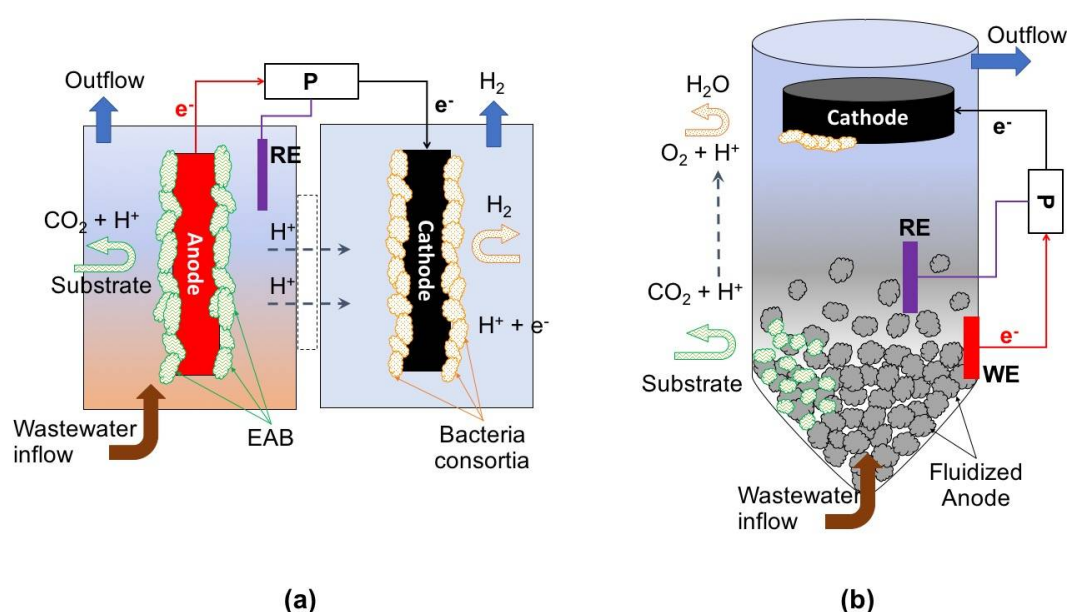


Figure 5. Examples of set-ups of MET for wastewater treatment. Non-spontaneous reactions: (a) MEC reactor, and (b) fluidized reactor. P = potentiostat; RE = reference electrode; WE = working electrode.

When electrical power is used to enhance the potential difference between the anode and the cathode, either to enable or increase the rate of the electrode reactions, the system is called microbial electrolysis cell (MEC). In a classical MEC, electroactive microorganisms use a solid-state anode as terminal electron acceptor for the oxidation of organic waste substrates to carbon dioxide, while simultaneously releasing protons to the solution. Electrons flow from the anode to the cathode through an external circuit while protons diffuse to the cathode through a proton-exchange membrane separating the two electrode compartments. At the cathode, in the presence of a suitable (bio)catalyst, the electrons combine with a soluble electron acceptor, generating a target product [45]. Likewise, electroactive bacteria can also use a cathode as the electron donor for reducing substrates presents in the wastewater like NO_2 , NO_3 , SO_4 , $\text{C}_2\text{H}_2\text{Cl}_4$, among others [78].

These reactions require the potential generated from substrate oxidation at the anode to be boosted with an external power supply to make the cathodic reaction thermodynamically feasible [45]. The energy can be supplied by a power source or a potentiostat depending on the selected mode of operation: galvanostatic (based on input current flow) or potentiostatic mode (based on a fixed potential between two electrodes). In galvanostatic mode only two electrodes are needed, but if

it is wanted to maintain the potential of one of the electrodes under a selected value (working electrode), it is necessary to work with a reference electrode in a 3-electrode configuration. The other electrode is called counter or auxiliary electrode and its potential is dependent upon the current flow circulating through the system. This configuration allows controlling the anodic or cathodic reactions, which is crucial for the study of the microbial-electrode interaction.

When the anode potential is controlled in a 3-electrode configuration, the organic matter oxidation is being performed at this working electrode. Some systems have been developed for this purpose, like classical MEC design, based on 2-chambered configuration, separated by an ion exchange membrane [11]. In other cases, the potential is fixed at the cathode, to solve the energy requirements and remove contaminants present in waters by reduction reactions, such as SO_4 from groundwater [79], and N from low COD effluents [80]. In addition, simpler systems have been developed to allow the scaling-up and implementation in wastewater treatment plants. An example of this is a bioelectrochemical denitrification MET system [81]. In these systems, the ion exchange membranes and the reference electrode have been removed, so that power is supply through a power source instead of a potentiostat. Bioelectrochemical denitrification presents several advantages compared to classical heterotrophic denitrification, for instance, the unlimited electron source of a cathode (supplied by an electric flux) while avoiding the need of adding external organic matter.

Other systems based on non-spontaneous reactions, rely on merging a classical fluidized reactor with a MET, like the so-called microbial electrochemical fluidized bed reactor (ME-FBR) [62]. The ME-FBR has been developed with the aim of maximizing the superficial area of the anode being available for electroactive microorganisms, and improving the kinetics of the catalysis by employing an environment with favorable mixing properties. These systems have been used to treat real wastewater and studies report that the ME-FBR was able to remove up to 95 % of the chemical oxygen demand (COD) in brewery effluent [82].

4.1.2. Spontaneous reactions

a). Power generation

Electric power generation using MFC is the most studied MET application. Organic compounds are oxidized by microorganisms using the anode as electron acceptor. The electrons flow through an external circuit to the cathode where oxygen is reduced [83]. Electrical energy can be recovered from the external circuit because the overall reaction, oxidation of organics and reduction of oxygen, is thermodynamically favorable [84].

Generally, MFC configurations are classified as either dual or single chambered designs (Figure 6). In a dual chamber configuration, both anode and cathode are immersed in a solution that ease either oxidative or reductive conditions, the protons flow through an ion exchange membrane and the electrons along an external circuit. In a single chamber, the anode and the cathode can be immersed on the same electrolyte or the cathode can be exposed to the atmosphere, in this configuration, the released protons migrate to the cathode through a polymer electrolyte membrane, and the electrons through an external circuit [30]. Some MFC innovations for wastewater treatment with simultaneous power generation are described below.

Based on the principles of generating conditions for dissolved oxygen gradient, by exposing the cathode to the atmosphere, a simple to install and operate floating all-in-one MFC system has been proposed [70]. In this system, a floater is fixed to a proton exchange membrane and to a carbon-cloth cathode with Pt catalyst; attached to the same floater, by means of a stainless-steel wire, are installed either carbon-cloth or carbon-brush anodes; the circuit is completed by an external stainless-steel wire and a resistor. Due to its characteristics, this type of system can be operated as plug-and-play device and installed directly in wastewater pre-treatment units (e.g. settling tanks). The system has the possibility of being scalable and customizable by enlarging the anodes, will allow the attachment of EAB, thus increasing the organic matter consumption. With this type of

configuration, it is possible to achieve a removal of BOD and COD up to 63% and 71% respectively [70].

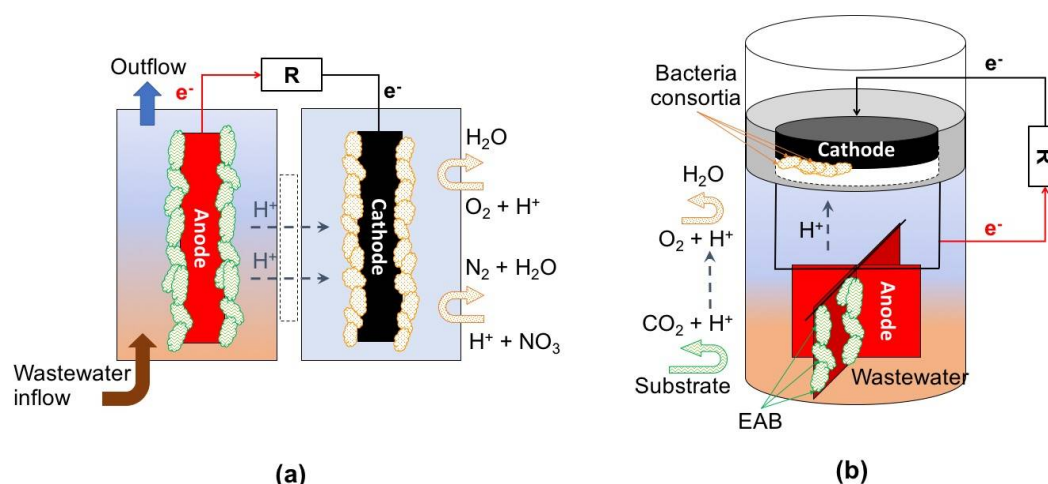


Figure 6. Examples of set-ups of MET for wastewater treatment. Spontaneous reactions: (a) typical dual-chamber MFC, and (b) single-chamber floating MFC reactor. R = resistor.

The efforts in developing innovative shapes and mixture of electrode material to enhance the performance of MFC, lead to the creation of annular single chamber MFC, or a spiral microbial electrochemical cell [85,86]. This type of MFC reactor is built with a Plexiglas cylindrical chamber, with a spiral anode electrode of graphite-varnished stainless-steel mesh, and a cylindrical concentric cathode composed either by a carbon cloth or stainless-steel mesh treated with Pt (0.5 mg cm^{-2}) to enhance electro-conductive properties of the material. This type of spiral configuration allows to increase the anodic surface area and at the same time reducing the space between electrodes, characteristics that led to obtain remarkable results in terms of wastewater treatment, reaching up to 91% of COD removal and a maximal power density of 20 W m^{-3} , and given the relative low cost of the implemented materials, it has a potential to be scaled-up.

Another innovative concept respect to traditional MFC configuration are the so-called tubular MFCs. A variety of this system is a vertical flow reactor composed of two perforated polypropylene tubes, acting as double shell, with an embedded layer of carbon cloth cathode exposed to air, a hydrogel layer acting as intermediate layer and an internal ion exchange membrane. At the interior of this reactor it is installed a concentric monolithic activated carbon anode, and the connection between anode and cathode is made by an external circuit with a resistor (1000Ω at starting-up; 150Ω at normal operative conditions) [87]. In experiments operating two tubular MFC units fed with synthetic wastewater and at different organic loading rates, it was possible to obtain COD removal rates between 51% and 82%, with a simultaneous energy production up to $1.75 \text{ Wh g}^{-1} \text{ COD}$. These results are promising for scaling-up this reactor in complementary modular systems for polishing effluents from treatment units, such as anaerobic digesters [88].

A similar setup called MFC stack, was developed using a PVC pipe as framework modular tubular air-cathode system, divided in different sections conforming independent anodic chambers [89]. Each anodic chamber has perforations on its body, it is wrapped on a layer of carbon fiber cloth containing MnO_2 , and packed with a cation exchange membrane. The air-cathode MFC sections are hydraulically connected by silicone gel tubes, and circuits are composed by titanium wires connecting anode and cathode with external resistor of 1000Ω ; this modular set-up has the advantage of being able to be operated either in series or parallel circuit mode. With this type of set-up has been reported a maximal removal rates of 84% for COD and 91% for NH_4 (at a loading rate of $1.2 \text{ Kg COD m}^{-3} \text{ d}^{-1}$; maximal power density = 176 W m^{-2} at 0.38 V and 43 mA), values that indicate its plausibility for treating wastewater with a simultaneous power production.

b). Short-circuit systems

Based on the principles of MET, but in contrast to the traditional MFC approach of simultaneous energy harvesting and pollutant depuration, microbial electrochemical snorkel (MES) concept was developed [90]. A MES system is a short-circuit-based set-up, i.e. are not intended to harvest electric current flows, therefore their configuration does not require complex electrochemical reactors with ionic exchange membranes or other separators (Figure 7). The design can be simplified to a single piece of conductive material or a full electroconductive granular material bed, that creates a direct electrochemical connection between anodic and cathodic zones [91]. A short-circuited system provides the highest currents, meaning that it ensures the highest rate for compounds oxidation. In the MES, one of the sides of an electrode plays the role of an anode and the other side the role of a cathode [90]. The anodic part should be exposed to anaerobic conditions and develop an electroactive biofilm over it, while the cathode is expose to the atmosphere, favoring the creation of redox potential gradient conditions.

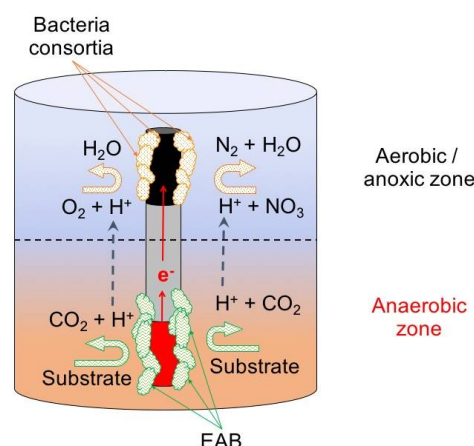


Figure 7. Example of MET configuration for wastewater treatment: microbial electrochemical snorkel (MES).

Due to the low electrical resistivity of the materials used as snorkels, the electrons are transported from the anodic to the cathodic zone that is characterized by having aerobic/oxic conditions; there electroactive and facultative bacteria use those electrons for the reduction of oxygen or other available electron acceptors like NO_2 , NO_3 , SO_4 or S_2O_3 . The concept has been tested in a laboratory scale set-up using glass bioreactors with 120 ml of wastewater (230 – 600 mg COD L^{-1}), and 20 cm titanium rod (20 cm length) covered with a colonized graphite felt anode in one extreme and a Pt cathode on the other. Operating under batch mode for 4 days, this set-up was able to reach a COD removal efficiency of 55 % (OLR discharge of 125 mg $\text{L}^{-1}\text{d}^{-1}$), being higher than a similar MFC used as control, showing its potential for wastewater treatment [90].

The use of conductive rods as subsurface snorkels has been investigated to accelerate the anaerobic oxidation of organic matter in marine and fresh water sediments. It has been demonstrated that their introduction in marine sediment promote a change in its redox condition, expressed by the oxidation of the sediments around the snorkels, and its implementation in fresh water has led to the inhibition of methane production after long trial periods [92]. The results suggest that subsurface snorkels could be a sustainable approach for redirecting the microbial respiration in subsurface environments, and profiles such systems as a potential alternative for degradation of organic contaminants and inhibition of methanogenesis in that type of environments [92].

The microbial electrochemical snorkels have also been tested for the removal of NO_3 from fresh waters in laboratory scale systems. The set-up consisted of a snorkel composed by zero-valent iron rode (25 cm length x 1 cm diameter), with an anodic carbon felt (0.3 cm thick x 7 cm diameter), inserted in a cylindrical reactor of 2 L, seeded with 600 mL of fresh water sediment, and fed with a

2.0 mg L⁻¹ NaNO₃ solution. In this set-up, the carbon felt ease the attachment of EAB that releases electrons from the consumption of substrates from the anaerobic/anoxic sediment. The electrons flow from the anode to the cathodic section of the rode located on its extreme, where denitrifying bacteria adsorbed in the rode, uses those electrons for the reduction of NO₃ of the overlaying solution. With this set-up, operating under batch conditions it was possible to remove up to 98% of NO₃ in 16 day [93]. This configuration has also shown its potential to maximize the removal of recalcitrant pollutants, like petroleum hydrocarbons [91].

4.2. Trends on MET for wastewater treatment

MET research has gained interest since the 1990s, not only for the possibility of power generation, catalysis of useful products, but also for its potential in wastewater treatment. Between year 1962 and 2017, were reported 7220 publications dealing with MET (Figure 8). From them, only 34 publications are reported before year 1997, but from that year on, the total number of publication increased notoriously to 1055 in 2017.

The numbers show a rising interest in exploring the potential of MET for power generation or catalysis of sub products of interest. In terms of wastewater treatment (MET+WWT), the first publication dates-back to year 2000, and up to year 2017, 1678 peer reviewed works have been published. Regarding the merging of MET and constructed wetlands technology (MET+CW), 73 references were identified, showing a growing pattern from 4 publications in year 2012 to 28 publications in year 2017. In comparison with the total amount of publications related with MET and MET+WWT, the MET+CW is still a new-born but promising research field as is denoted by the studies described below.

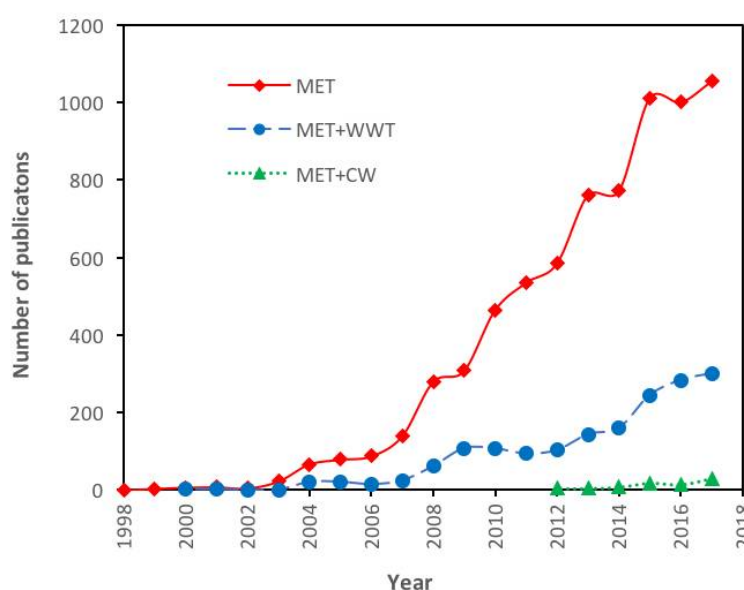


Figure 8. Trends in publications of METs, their implementation for wastewater treatment (MET+WWT) and their combination with constructed wetlands (MET+CW). Data obtained from Scopus® database. For MET publications, the search was limited to title, abstract and keyword (TITLE-ABS-KEY) using the terms “microbial fuel cell*”, “microbial electrochemical tech*”, “microbial electrochemical sys*”, “bioelectrochemical tech*” and “bioelectrochemical sys*”. For MET+WWT, the search was narrowed adding the terms “wastewater treat*” and “water treat*”. For MET+CW the search was narrowed again, adding the terms “constructed wetlands”, “treatment wetlands”, “engineered wetlands” and “artificial wetlands”. The search included publications in 14 different categories pre-set by the database: “article”, “conference paper”, “review”, “article in press”, “book chapter”, “note”, “conference review”, “editorial”, “short survey”, “erratum”, “book”, “letter”, “business article” and “undefined”.

5. CW-MFC coupling

The presence of redox gradients along a CW depth profile, with anaerobic zones at bottom and anoxic/aerobic zones at top, led to explore the possibility of combining them with MET. Similarly, to a conventional MFC, a CW-MFC include the installation of an anode located at the bottom in the anaerobic zone, and a cathode located at the top in anoxic/aerobic zone. On the anodic zone, the metabolic activity of electroactive bacteria, allow the consumption of organic compounds, releasing electrons that are transferred to the anode. From the anode, the electrons flow along an external circuit to the cathode, where they can be used in the reduction of O₂ or NO₃. To complete the charge balance, some configurations incorporate an ion separator, others simply allow them to flow in the bulk fluid. Likewise, in conventional MET for wastewater treatment, with a CW-MFC systems is indented to achieve a treatment performance at least as good as in a conventional CW, but with the added value of harvesting energy derived of the metabolic activity of EAB and their interaction with the electrodes installed in the set-up. A typical set-up of CW-MFC is presented in Figure 9.

The first experience reported with a CW-MFC was a laboratory scale reactor for the removal of methylene blue dye, COD and simultaneous power generation from synthetic wastewater (8000 mgL⁻¹) at different dye concentrations [23]. The reactor operated in batch mode along 96 h, achieved a maximal COD removal of 75% (at 1500 mg L⁻¹ of initial dye concentration), and maximal power density of 15.73 mW m⁻² and a maximal current density and 69.75 mA m⁻² (at 1000 mgL⁻¹ of initial dye concentration). Since that first study, the potential applications have been expanded from conventional pollutants like organic matter and nutrient, to more complicated compounds like pharmaceuticals. A selection of recent studies published about this merging technology is summarized in Table 1.

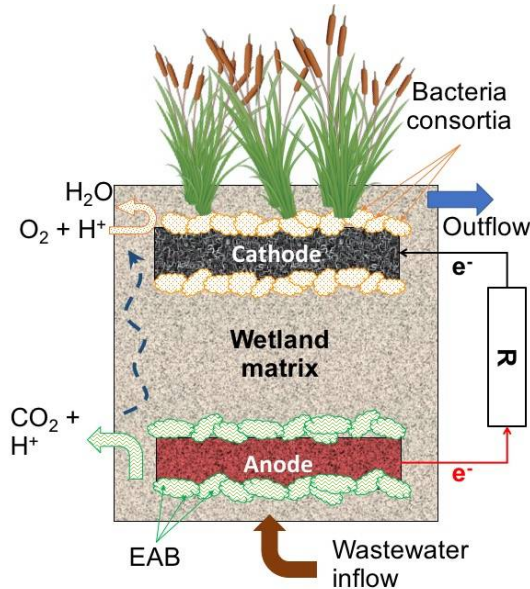


Figure 9. Typical CW-MFC configuration.

In the ambit of conventional pollutants, it was tested the removal of organic matter and nutrients along with electricity production from swine slurry with alum sludge-based constructed wetlands incorporating MFC [94]. The test included a laboratory scale set-up operating with simultaneous up-flow/down-flow feeding pattern, achieving removal efficiencies of 64% of COD, 75% of NH₄, 86% for TP, and reaching a maximal power density of 0.268 W m⁻³. With a CW-MFC up-flow set-up, it was tested the removal of COD, N, and energy generation from synthetic wastewater, experimenting different circuit connections, organic loads and electrode configurations [95]. After 1 day of hydraulic retention time (HRT) the average removal efficiencies were 99%, 46% and 96% for COD, NO₃ and NH₄ respectively, and a maximal power density of 93 mW m⁻³.

The performance of a laboratory scale down-flow CW-MFC reactor was tested for nitrate removal and bioenergy generation [96]. The reactor, operating in continuous mode with HRT

513 varying from 24 to 96h and with carbon fiber felt working as anode and cathode, reached removal
514 rates around 57% for COD and 80% for NO_3 , with a maximal power generation of 8.08 mW m^{-2} . One
515 issue that was highlighted in this work, was the neutral and slightly alkaline environment generated
516 in the reactor operating under closed circuit configuration, condition that promoted the richness of
517 the bacterial community, increasing the bioenergy generation in comparison with an open-circuit
518 operation with acidic conditions [96].

519 Regarding the treatment of more complex types of wastewater, it was tested the performance of
520 a CW-MFC to treat oil contaminated wastewater along with electricity generation, and compared
521 with single CW and MFC reactors [97]. Their design included a laboratory scale system operating in
522 an up-flow mode with a HRT of 3 day, with carbon brush as anode and copper plated carbon-felt as
523 cathode. This CW-MFC reached removal rates close to 95 % for oil, 73% for COD and 57% for TOC,
524 generating a power density of 102 mW m^{-2} . In this study, the plants seem to have an important role
525 in the performance of the cathode, which could be associated to the impact of the roots which
526 allowed some oxygen transfer to the cathode or the provision of suitable conditions for developing
527 of EAB communities.

528 The removal of antibiotics (tetracycline and sulfamethoxazole) and the simultaneous electricity
529 generation was tested in an upflow CW-MFC [98]. In their experiment, laboratory columns
530 operating with HRT of 2.5 days, were fed with synthetic wastewater spiked with different
531 concentrations of tetracycline and sulfamethoxazole (200 , 500 and $800 \mu\text{g L}^{-1}$), obtaining removal
532 rates above 99.5% for both antibiotics and a maximal power density of 57.8 mW m^{-2} , showing
533 potential as complementary technology for removal of pharmaceuticals.

534 The removal of B and the electricity generation was tested with a hybrid CW-MFC, composed
535 of one surface flow reactor and followed by a horizontal subsurface reactor [99]. The system was
536 operated in a continuous mode fed by 3 pulses per day with synthetic wastewater containing boron
537 in concentrations ranging from 2 to 32 mg L^{-1} . After a stable period of operation, this hybrid system
538 achieved removal rates of B around 63% and a power density generation of 78 mW m^{-3} (anode
539 working volume). Additionally, the removal of NO_2 and NO_3 was tested with removals of 19% and
540 47% respectively. This study also highlighted that the increase in B concentration have a negative
541 impact on the power and current densities generated by the system. Furthermore, enzymes like
542 dehydrogenase and urease were found closed to the rhizosphere, which seem to be strongly
543 correlated with the bioelectricity generation in the system, denoting the positive impact of the
544 presence of plants in this type of systems.

545 One of the most recent studies, tested the degradation of nitrobenzene from wastewater with a
546 single membrane-less CW-MFC, compared with the performance of a conventional MFC [100]. This
547 laboratory scale system was fed with synthetic wastewater containing nitrobenzene (concentrations
548 varying from 5 to 80 mg L^{-1}), with glucose as carbon source (concentration from 100 to 600 mg L^{-1}),
549 and operated under up-flow continuous mode with a HRT of 24h. The removal rates of nitrobenzene
550 and COD were around 92% and 78% respectively, promoting a maximal power density of 1.53 mW m^{-2} .
551 This study highlights that the high removal rates of nitrobenzene are the result of an appropriate
552 balance nitrobenzene:COD ratio and HRT ($1:16$ at $\text{HRT} = 24\text{h}$).

553 Leaving aside as objective the recovery energy and focusing only on the removal of pollutants,
554 an innovative development has been proposed by using electro-conductive biofilters (instead of
555 external circuits as previously tested CW-MFC), resulting in the development of the Microbial
556 Electrochemical-based Constructed Wetland (METland). In a METland system, the EAB are
557 stimulated to generate and transfer electrons to an electro-conductive material that act as an
558 unlimited acceptor (Figure 10) maximizing substrate consumption, instead of leaving free electrons
559 for methane generation and consequently to a decrease of microbial metabolism rates (as in
560 anaerobic system), due to the limited number of electron acceptors [101].

561

Table 1. Summary of selected studies of merging between constructed wetlands and microbial electrochemical technologies (2016 - 2018)

Target	Reactor characteristics	Matrix	Circuit ⁺	Max. Electrochemical performance ⁺⁺	Max. removal rates	Ref.
Wastewater treatment	<ul style="list-style-type: none">Type = METlandScale = laboratoryHRT = 0.5 to 4 dSubstrate = cokeFeed = horizontal flow-continuousPlants = N.A.	Pre-treated municipal wastewater: OLR variations from 2.0 to 12.7 g BOD ₅ m ⁻² d ⁻¹	Coke as single piece electrode. System operating as short-circuit filter (snorkel)	<ul style="list-style-type: none">CD = N.A.PD = N.A.CE = N.A.	<ul style="list-style-type: none">COD = 93%BOD = 99%NH₄ = 97%TN = 69%	[102]
Long term performance assessment of cathode position for CW-MFC	<ul style="list-style-type: none">Type = CW-MFCScale = pilotHRT = 2.6 dSubstrate = gravelFeed = horizontal flow-continuousPlants = <i>P. australis</i>	Urban wastewater: <ul style="list-style-type: none">COD = - 323 mg L⁻¹ (1st period) - 254.94 mg L⁻¹ (2nd period)NH₄-N = - 41.7 mg L⁻¹ (1st period) - 29.7 mg L⁻¹ (2nd period).	<ul style="list-style-type: none">Anode = GRCathode = GRExternal circuit = CuWResistor = 1000 Ω	<ul style="list-style-type: none">CD = - 1st period = 45.5 mA m⁻² - 2nd period = 138.8 mA m⁻²PD = - 1st period = 7.9 mW m⁻² - 2nd period = 14.5 mW m⁻²CE = N.A.	<ul style="list-style-type: none">COD = 61%NH₄ = 60%.	[103]
Azo dye degradation and electricity generation	<ul style="list-style-type: none">Type = CW-MFC (open and closed circuit)Scale = laboratoryHRT = 3 dSubstrate = gravelFeed = upflow - continuousPlants = N.A.	Synthetic wastewater: <ul style="list-style-type: none">Glucose = 50 mg L⁻¹Methyl Orange (MO) = 450 mg L⁻¹	<ul style="list-style-type: none">Anode = GAC + SSMAir cathode = GAC + SSM.External circuit = CuWResistor = 1000 Ω.	<ul style="list-style-type: none">CD = N.A.PD = 0.688 W m⁻³CE = 31.47%	<ul style="list-style-type: none">MO = - 75% (open circuit) - 87% (closed circuit)Dimethyl-p-phenylenediamine = - 84% (open circuit) - 96% (closed circuit)	[104]
Influence of substrate and electrode on performance of CW-MFC	<ul style="list-style-type: none">Type = single chamber CW-MFCScale = laboratoryHRT = 3 dSubstrate = gravelFeed = upflow - continuousPlants = N.A.	Synthetic wastewater: <ul style="list-style-type: none">COD = 200 mg L⁻¹X-3B = 166.7 mg L⁻¹Glucose = 140 mg L⁻¹	<ul style="list-style-type: none">Anode = AC + SSM.Cathode = AC + SSM.External circuit = TWResistor = 1000 Ω	<ul style="list-style-type: none">CD = N.A.PD = 0.2 W m⁻³CE = N.A.	<ul style="list-style-type: none">COD = 94% (glucose reactor)X-3B = 92% (glucose+X-3B reactor)	[105]
Wastewater treatment and electricity generation	<ul style="list-style-type: none">Type = CW-MFCScale = laboratoryHRT = 1 dSubstrate = gravelFeed = upflow - continuousPlants = <i>T. latifolia</i>	Synthetic wastewater: <ul style="list-style-type: none">COD = 624 mg L⁻¹NO₃ = 142 mg L⁻¹NH₄ = 40 mg L⁻¹	<ul style="list-style-type: none">Anode = AC + SSMCathode = AC + SSMExternal circuit = CuWResistor = 1000 Ω	<ul style="list-style-type: none">CD = N.A.PD = 93 mW m⁻³CE = 1.42%	<ul style="list-style-type: none">COD = 99%NO₃ = 46%NH₄ = 96%	[95]

Target	Reactor characteristics	Matrix	Circuit ⁺	Max. Electrochemical performance ⁺⁺	Max. removal rates	Ref.
Nitrate removal and bioenergy production	<ul style="list-style-type: none">Type = CW-MFCScale = laboratoryHRT = 1, 2 and 4 dSubstrate = quartz sandFeed = downflow - continousPlants = <i>Canna indica</i>	Synthetic wastewater: <ul style="list-style-type: none">COD = 215, 423 and 813 mg L⁻¹NO₃ = 20.3, 39.8 and 79.4 mg L⁻¹	<ul style="list-style-type: none">Anode = CFFCathode = CFFExternal circuit = CuWResistor = 1000 Ω	<ul style="list-style-type: none">CD = 53.74 mA m⁻²PD = 8.08 mW m⁻²CE = 0.01 - 0.11 %	<ul style="list-style-type: none">COD = 57%NO₃ = 80%	[96]
Wastewater treatment and electricity generation	<ul style="list-style-type: none">Type = MFC + DAS-CWScale = laboratoryHRT = 2.5 dSubstrate = DAS and PAC⁺Feed = upflow-continuousPlants = N.A.	Diluted swine wastewater: <ul style="list-style-type: none">COD = 500 mg L⁻¹TN= 40.5 mg L⁻¹NH₄ = 4.2 mg L⁻¹Reactive P. (RP) = 9.8 mg L⁻¹	<ul style="list-style-type: none">Anode = SSM buried in PAC/DASCathode = SSM + GGExternal circuit = TWResistor = 950 Ω	<ul style="list-style-type: none">CD= N.A.PD = 87.79 mW m⁻²CE = 1.2%	<ul style="list-style-type: none">COD = 81%TN = 45%NH₄ = 53%RP = 88%.	[106]
OLR and electrode locations impact over electrogenesis capacity	<ul style="list-style-type: none">Type = CW-MFCa. bottom anode-rhizosphere cathode (BA-RC)b. rhizosphere anode-air cathode (RA-AC)c. bottom anode-air cathode (BA-AC)Scale = laboratoryOLR = 9.2 to 92 g m⁻² d⁻¹Substrate = gravelFeed = upflow - continuousPlants = <i>Canna indica</i>	Synthetic wastewater: COD = 50 to 500 mg L ⁻¹	<ul style="list-style-type: none">Anode = GACCathode =<ul style="list-style-type: none">- GAC (a.)- graphite felt (b., c., and d.)External circuit = TWCurrent collector = SSMResistor = 500 Ω	<ul style="list-style-type: none">CD = N.A.PD = 10.77 mW m⁻² (a., at 27.6 g COD m⁻² d⁻¹)CE = 0.16–2.12%	N.A.	[107]
Treatment of oil contaminated wastewater and electricity generation	<ul style="list-style-type: none">Type = CW-MFCScale = laboratoryHRT = 3 dSubstrate = layers of AC, glass wool and gravelFeed = upflow - continuousPlants = N.A.	Oil contaminated wastewater: <ul style="list-style-type: none">COD = 520 mg L⁻¹BOD = 190 mg L⁻¹Oil = 235 mg L⁻¹TOC = 33 mg L⁻¹	<ul style="list-style-type: none">Anode: carbon brushCathode: Cu-plated CFFExternal circuit = CuWResistor = 1000 Ω	<ul style="list-style-type: none">CD = N.A.PD = 3868 mW m⁻³CE = N.A.	<ul style="list-style-type: none">COD = 73%TOC = 57%Oil = 95%	[97]

Target	Reactor characteristics	Matrix	Circuit ⁺	Max. Electrochemical performance ⁺⁺	Max. removal rates	Ref.
Removal of antibiotics (tetracycline - TC and sulfamethoxazole - SMX), development resistance genes and electricity generation	<ul style="list-style-type: none">• Type = CW-MFC• Scale = laboratory• HRT = 2.5 d• Substrate = sand and gravel• Feed = upflow - continuous• Plants = N.A.	Synthetic wastewater: <ul style="list-style-type: none">• TC = 200, 500 and 800 µg L⁻¹• SMX = 200, 500 and 800 µg L⁻¹	<ul style="list-style-type: none">• Anode = GAC• Cathode = GAC• External circuit = CuW• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = 30 mA m⁻²• PD = 0.0578 W m⁻²• CE = N.A.	<ul style="list-style-type: none">• TC effluent: 0.66, 1.1 and 1.65 µg L⁻¹• SMX effluent: 0.90, 1.70 and 2.40 µg L⁻¹	[98]
Effects of electrode gap on wastewater treatment and bioelectricity generation	<ul style="list-style-type: none">• Type = CW-MFC• Scale = laboratory• HRT = 3 d• Substrate = gravel• Feed = upflow - continuous• Plants = N.A.	Synthetic wastewater with: <ul style="list-style-type: none">• Glucose group = 300 mg L⁻¹ glucose• ABRX3 group = 300 mg L⁻¹ azo dye ABRX3 + 210 mg L⁻¹ glucose	<ul style="list-style-type: none">• Anode = GAC• Air cathode = GAC• External circuit = CuW• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = N.A.• PD = 0.38 W m⁻³• CE = N.A.	<ul style="list-style-type: none">• COD = 89% (glucose group)• Azo dye = 91% (ABRX3 group)	[108]
Electricity harvesting and methane mitigation	<ul style="list-style-type: none">• Type = CW- MFC (open and closed circuit operation)• Scale = laboratory• HRT = 1, 2, 3 and 4 d• Substrate = gravel• Feed = upflow - continuous• Plants = <i>S. alterniflora</i>	Synthetic wastewater: glucose = 0 to 2 mM	<ul style="list-style-type: none">• Anode = GAC• Cathode = GAC• Current collector: SSM• External circuit: TW• Resistor = 50 to 1000 Ω	<ul style="list-style-type: none">• CD = 187 mA m⁻²• (at HRT = 96h)• PD = 76.7 mW m⁻²• (at HRT = 72h)• CE = 14.9%	Methanogenesis suppression = 98% (at HRT = 96h)	[109]
Optimization of bioelectricity generation and wastewater treatment	<ul style="list-style-type: none">• Type = Vertical subsurface-flow CW-MFC (VSFCW-MFC)• Scale = laboratory• HRT = 2 d• Substrate = gravel• Feed = upflow - continuous• Plants = <i>P. australis</i>	Synthetic wastewater: COD = 200, 400 and 800 mg L ⁻¹	<ul style="list-style-type: none">• Anode = GAC• Cathode = GAC• Current collector = SSM• External circuit = TW.• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = 0.56 mA• PD = 0.15 W m⁻³• CE = 0.31 %	COD = 94%	[110]
Assessment of intermittent aeration (IA) and radial oxygen loss (ROL) for wastewater treatment and electricity generation	<ul style="list-style-type: none">• Type = CW-MFC• Scale = laboratory• HRT= 3 d• Substrate = gravel• Feed = batch• Plants = <i>Canna indica</i>	Synthetic wastewater: Glucose: 350 to 2000 mg L ⁻¹	<ul style="list-style-type: none">• Anode: graphite felt• Cathode: Pt coated carbon cloth• Separator: GW• External circuit: CuW• External resistor = N.A.	<ul style="list-style-type: none">• CD =- IA = 132.72 mA m⁻³- ROL = 181.36 mA m⁻³• PD =- IA = 31.04 mW m⁻³- ROL = 19.60 mW m⁻³• CE = N.A.	<ul style="list-style-type: none">• COD =- 78% (IA)- 72% (ROL)	[111]

Target	Reactor characteristics	Matrix	Circuit ⁺	Max. Electrochemical performance ⁺⁺	Max. removal rates	Ref.
Boron (B) removal and bioelectric production	<ul style="list-style-type: none">• Type = Hybrid CW-MFC• Scale = laboratory• HRT = 7d• Substrate = organic peat, sand and gravel.• Feed = horizontal - pulse• Plants = <i>T. latifolia</i>	Synthetic wastewater: Hoagland solution H ₃ BO ₃ (2 to 32 mg L ⁻¹)	<ul style="list-style-type: none">• Anode: GR (x2)• Cathode = Mg bar• Separator = glass wool• External circuit = CuW.• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = 105 mA m⁻²• PD = 78 mW m⁻³• CE = N.A.	<ul style="list-style-type: none">• B = 63%• NO₃ = 47%• NO₂ = 19%	[99]
Bioelectricity generation, contaminant removal and microbial community structure	<ul style="list-style-type: none">• Type = CW-MFC• Scale = laboratory• HRT = 24, 48 and 96 h• Substrate = quartz sand• Feed = downflow - continous• Plants = N.A.	Synthetic wastewater	<ul style="list-style-type: none">• Anode = CFF• Cathode = CFF• External circuit = CuW• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = 48.41 mA m⁻²• PD = 8.91 mW m⁻²• CE = 0.22 %	<ul style="list-style-type: none">• COD = 86%• NO₃-N = 87%	[112]
Effects of electrode material and substrate concentration on bioenergy output and wastewater treatment	<ul style="list-style-type: none">• Type = CW Air-cathode MFC (CW-ACMFC)• Scale = laboratory• HRT = 24, 48 and 96 h• Substrate = Quartz sand• Feed = downflow - continous• Plants = <i>Canna indica</i>	Synthetic wastewater: COD = 215, 423 and 813 mg L ⁻¹	Anode and air cathode pairs = a. CFF b. SSM c. GR d. FN <ul style="list-style-type: none">• External circuit = CuW• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = 17.3 mA m⁻² (a.)• PD = 1.78 mW m⁻² (d.)• CE = N.A.	<ul style="list-style-type: none">• COD = a. 49% b. 37% c. 52% d. 35%• NO₃ = a. 80% b. 69% c. 42% d. 84%	[113]
Energy capture and nutrients removal	<ul style="list-style-type: none">• Type = Tiered CW-MFC• Scale = laboratory• HRT = N.A.• Substrate = dewatered alum sludge• Feed = upflow (inner section); downflow (outer section)• Plants = N.A.	Synthetic wastewater: <ul style="list-style-type: none">• Glucose = 281 mg L⁻¹• NH₄CL = 57 mg L⁻¹• K₂HPO₄ = 18.23 mg L⁻¹• CaCl₂ = 11.5 mg L⁻¹• MgSO₄ = 12 mg L⁻¹	<ul style="list-style-type: none">• Anode: SSM packed in graphite gravel-size layer• Air-cathode: SSM in GAC layer• External circuit = CuW• Resistor = 500 Ω	<ul style="list-style-type: none">• CD = N.A.• PD = 2 W m⁻³• CE = 1.85%	<ul style="list-style-type: none">• COD = 88%• TN = 75%• NH₄ = 85%• PO₄ = 94%	[114]

Target	Reactor characteristics	Matrix	Circuit ⁺	Max. Electrochemical performance ⁺⁺	Max. removal rates	Ref.
Degradation of nitro-benzene form wastewater	<ul style="list-style-type: none">Type = CW-MFC (single membrane-less air cathode)Scale = laboratoryHRT = 24 hSubstrate = GravelFeed = upflow - continousPlants = Water hyacinth	Synthetic wastewater: <ul style="list-style-type: none">Nitrobenzene (NB) = 5 - 80 mg L⁻¹Glucose = 100 - 600 mg L⁻¹	<ul style="list-style-type: none">Anode = GBCathode = GB (placed at air-water interface)External circuit: CuWResistor = 1000 Ω	<ul style="list-style-type: none">CD = 8.52 mA m⁻²PD = 1.53 mW m⁻²CE = 16.4%	<ul style="list-style-type: none">COD = 78%NB = 92%	[100]
Influence of glass wool separator on CW-MFC bioelectricity generation performance	<ul style="list-style-type: none">Type = CW-MFCScale = laboratoryHRT = 1 dSubstrate = Dewatered alum sludgeFeed = upflow - continousPlants = N.A.	Synthetic wastewater: <ul style="list-style-type: none">CH₃COONa = 642 mg L⁻¹NH₄CL = 114 mg L⁻¹CaCl₂2H₂O = 16 mg L⁻¹MgSO₄ = 12 mg L⁻¹KH₂PO₄ = 14 mg L⁻¹Trace element sol. = 1 mL	<ul style="list-style-type: none">Anode = CFF (3 ≠ heights)Cathode = CFF (located at air-water interface)Separator = GW (only one column)External circuit = N.A.Resistor = 1000 Ω	<ul style="list-style-type: none">CD = N.A.PD = 66.22 mW m⁻²CE = N.A.	N.A.	[115]
Impact of substrate on wastewater treatment performance and bioelectricity generation of CW-MFC	<ul style="list-style-type: none">Type = CW-MFCScale = laboratory (3 different reactors: R1 - R3)HRT = 4 dSubstrate = sand (R1), zeolite (R2) and volcanic cinder (R3)Feed = upflow - continousPlants = <i>T. latifolia</i>	Synthetic wastewater: <ul style="list-style-type: none">Glucose = 200 mg L⁻¹NH₄CL = 150 mg L⁻¹KCl = 130 mg L⁻¹NaHCO₃ = 3,130 mg L⁻¹Micro-element sol. = 1mL	<ul style="list-style-type: none">Anode = GP (2 ≠ heights)Cathode = MgPSeparator = GWExternal circuit = CuWResistor = 1000 Ω	<ul style="list-style-type: none">CD = 16.1 mA m⁻² (for R2)PD = 26.12 mW m⁻² (for R2)CE = 1.64% (for R2)	<ul style="list-style-type: none">COD = 92%NH₄ = 93%NO₃ = 81%TP = 96%	[116]
Bioelectricity generation and microbial community development in a CW-MFC	<ul style="list-style-type: none">Type = CW-MFCScale = laboratoryHRT = 3 dSubstrate = sand and ceramsiteFeed = upflow - continousPlants = <i>P. australis</i>	Synthetic wastewater: <ul style="list-style-type: none">Sucrose = 53.48 mg L⁻¹(NH₄)₂SO₄ = 37.71 mg L⁻¹KNO₃ = 50.50 mg L⁻¹KH₂PO₄ = 6.58 mg L⁻¹Micro-element sol.	<ul style="list-style-type: none">Anode = TM + AC (cylinder)Cathode = TMSeparator = N.A.External circuit = CuWResistor = 1000 Ω	<ul style="list-style-type: none">CD = 16.63 mA m⁻²PD = 3,714 mW m⁻²CE = N.A.	<ul style="list-style-type: none">COD = 82%TN = 82%TP = 95%	[117]

Target	Reactor characteristics	Matrix	Circuit ⁺	Max. Electrochemical performance ⁺⁺	Max. removal rates	Ref.
Performance enhancement of CW-MFC with submerged plants	<ul style="list-style-type: none">• Type = CW-MFC• Scale = mesocosm (planted and non-planted)• HRT = 3 d• Substrate = sludge• Feed = surface flow - continous• Plants = <i>H. verticillata</i>	Synthetic wastewater: <ul style="list-style-type: none">• Glucose = 51.35 mg L⁻¹• (NH₄)₂SO₄ = 37.6 mg L⁻¹• KNO₃ = 40.57 mg L⁻¹• KH₂PO₄ = 7 mg L⁻¹• MgSO₄ = 10 mg L⁻¹• CaCl₂ = 10 mg L⁻¹• FeSO₄·7H₂O = 18.3 mg L⁻¹	<ul style="list-style-type: none">• Anode = CFB + TW• Cathode = CFB + TW (enclosed on polyethylene chamber)• Separator = N.A.• External circuit = TW• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = N.A.• PD = 558.50 mV (as power)• CE = N.A.	<ul style="list-style-type: none">• COD = 64%• NH₄ = 89%• NO₃ = 78%• TP = 94%	[118]
Effect of vegetation on treatment performance and bioelectricity generation in CW-MFC	<ul style="list-style-type: none">• Type = CW-MFC• Scale = microcosm (planted: M1 - M4; non-planted: M5)• HRT = 4 d• Substrate = coarse sand; gravel at inlet/outlet zones• Feed = upflow - continous• Plants = <i>T. latifolia</i> (M1), <i>T. angustifolia</i> (M2), <i>J. gerardii</i> (M3), <i>C. divisa</i> (M4)	Synthetic wastewater: <ul style="list-style-type: none">• Glucose = 200 mg L⁻¹• NH₄CL = 150 mg L⁻¹• KCl = 130 mg L⁻¹• NaHCO₃ = 3,130 mg L⁻¹• Micro-element sol. = 1mL	<ul style="list-style-type: none">• Anode = GP• Cathode = MgP• Separator = GW• External circuit = CuW• Resistor = 1000 Ω	<ul style="list-style-type: none">• CD = 33.8 mA m⁻² (for M2)• PD = 18.1 mW m⁻² (for M2)• CE = 8.28 ± 10.4% (for M2)	<ul style="list-style-type: none">• COD = 88% (M1)• NH₄ = 98% (M2)• NO₃ = 63% (M5)• TP = 97% (M2)	[119]

⁺ Activated carbon (AC), carbon fiber brush (CFB), carbon fiber felt (CFF), copper wire (CuW), dewatered alum sludge (DAS), foamed nickel (FN), glass wool (GW), granular activated carbon (GAC), granular graphite (GG), graphite plate (GP), graphite bar (GB), graphite rod (GR), magnesium plate (MgP), stainless steel mesh (SSM), titanium mesh (TM), titanium wire (TW) powder activated carbon (PAC). ⁺⁺ Coulombic efficiency (CE), current density (CD), power density (PD).

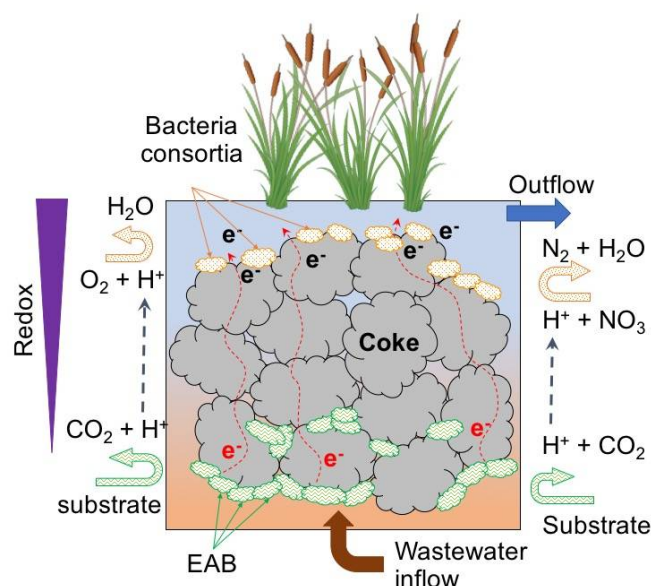


Figure 10. Conceptual illustration of METland set-up

METland system has been tested for the removal of organic matter and nitrogen with horizontal subsurface flow (HSSF) coke biofilters at laboratory scale at different hydraulic loading rates (HRT), ranging from 4 to 0.5 days [102]. The coke based biofilter showed removal rates of 91% for COD and for 96% for BOD₅ at the lowest HRT (0.5 d), and 97% for NH₄ and 69% for TN at 3.5 d HRT results that suggest that METland system can enhance biodegradation rates, thus allowing the reduction of the area requirements of classical CWs.

Conceptually this new system operates similarly to a microbial electrochemical snorkel – MES [90,91,120], which is composed of a single conductive material that allows the connection between anoxic zones (performing as anode) and oxic zones (performing as cathode). Like the CW-MFC technology, the METland technology is still in development, therefore several uncertainties exist regarding the dynamics involve in the removal of pollutants, and in its performance along time.

6. Challenges and future perspectives for CW-MFC systems

Based on the studies that merges CW and MFC, it is possible to identify that most of the reported systems have been designed and operated at laboratory scale, and their implementation as a suitable real scale system is still in development. The main challenges for scaling up the CW-MFC technology, are the same as those that face traditional MET for wastewater treatment. This constrains include the electrolyte resistance (ohmic), the charge-transfer resistance due to slow reaction rates on electrodes (kinetic), and the resistance caused by retarded diffusion (transport) [121]. This limitations result in low power densities and coulombic efficiencies that range between 9 to 72 mW m⁻² and 0.05–10.48% [109].

Additional limiting factors that must be considered include i) the internal resistance of a CW-MFC which increases linearly as the size and distance between electrodes increase [24]; ii) the over-potential during activation and the insufficient electrical contact between bacteria and anode [122]; iii) the competition between EAB and other microorganisms (e.g. methanogenic bacteria) for electrons or substrates leading to low coulombic efficiencies [109]; iv) the deterioration of cathode over time and the excessive growth of heterotrophic bacteria around it that plummet the concentrations of electron acceptors like O₂ [114]; as well as v) the high concentration of organic matter that could increase slightly the acidity inside the systems, limiting the growth of EAB and the diffusion of protons, therefore affecting the coulombic efficiency [113].

The constrains are also associated to the design of the circuits, and the inherent limitations of membranes and electrode materials to allow the flow of ions and electrons in the system [123]. Therefore efforts must be invested in the understanding of the processes involved in the release,

transfer and acceptance of electrons, and in minimizing the losses [124]. Additional efforts must be invested in determining optimal inoculums, substrate conditions, ionic strength of water, internal and external resistance of the systems, as well as innovative electrode spacing between them [122], all with the aim of designing and developing cost effective electrodes and circuits. Based on the reviewed publications of CW-MFC, it can be identified that the research priorities have a strong preference in terms of the enhancement of the power generation. However, up to now, the energy yields of the tested systems are not competitive in comparison with other alternatives such a biogas collection from conventional wastewater treatment facilities. That fact also constrains the ambition of scaling up the CW-MFC systems.

An approach that seems to be underestimated as research field, is the study and development of set-ups based on the merging of CW and MET that do not target the harvesting of energy, but rely on the explained bioelectrochemical principles to enhance the performance of CW. One option worth to be investigated, would be the merging of CW and MEC or MRC, that by setting potentials through an external power supply, makes possible to control the inner conditions of the system, allowing to overcome the lack of electron acceptors, therefore leading to the maximization of treatment efficiency. Other alternative approach would be the study of CW-MET set-ups that dispense of electrodes and external circuits, but uses electro-conductive materials and operating in short-circuit mode. Those proposed alternatives could lead to the enhancement removal processes inside CW, leading to the reduction of the required area to build a CW, therefore diminishing the footprint of the system.

7. Conclusions

Microbial electrochemical technologies, constitutes as a relative recent innovative approach for wastewater treatment, that a laboratory-scale level show remarkable results in terms in the removal of organic matter and other pollutants of interest, as well as for the recovery of potential energy store in chemical form in wastewaters.

Among the alternative MET configurations, CW-MFC constitutes as one of the most innovative set-ups, which merge the ground-breaking approach of MFC and the well know capabilities of constructed wetlands for wastewater treatment. This combination constitutes as an interesting and promising set-up among the different options constructed wetland technology, specially for the possibilities of using this set-up as a new option of intensified wetland system that could keep a high performance with a lower footprint.

However, there a still several challenges that must be overcome for scaling-up the technology, if is expected to develop a set-up able to compete with other wastewater treatment alternatives in terms of recovery energy, e.g. in form of biogas. Those efforts should be focused on the study and development of new materials, internal conditions of the systems, inoculums, configuration of systems, as well as to increase the understanding of the different processes involve in the release, transfer and use of electrons in these systems.

It would be interesting to focus major efforts in systems that take advantage of bioelectrochemical principles without the necessity of simultaneous power generation. An option for this would be to invest higher efforts on the research of CW operating with MEC or MERC set-ups, systems operating in short-circuit mode like snorkel-based METs, or like in innovative MET-CW setups such as METland systems.

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