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From microbial fuel cell (MFC) to microbial electrochemical snorkel (MES): maximizing chemical oxygen demand (COD) removal from wastewater

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The paper introduces the concept of the microbial electrochemical snorkel (MES), a simplified design of a “short-circuited” microbial fuel cell (MFC). The MES cannot provide current but it is optimized for wastewater treatment. An electrochemically active biofilm (EAB) was grown on graphite felt under constant polarization in an urban wastewater. Controlling the electrode potential and inoculating the bioreactor with a suspension of an established EAB improved the performance and the reproducibility of the anodes. Anodes, colonized by an EAB were tested for the chemical oxygen demand (COD) removal from urban wastewater using a variety of bio-electrochemical processes (microbial electrolysis, MFC, MES). The MES technology, as well as a short-circuited MFC, led to a COD removal 57% higher than a 1000 Ω -connected MFC, confirming the potential for wastewater treatment.

Keywords: urban wastewater treatment; electrochemically active biofilms; electrochemical snorkel; microbial fuel cell

Introduction

Aerobic bacteria require oxygen-containing environments for growth. In wastewater treatment units, the aerobic tanks must consequently be thoroughly aerated, using compressors that are responsible for the main part of energy costs. Important efforts are being undertaken to design systems that can reduce or eliminate the need for aeration. Indeed, MFCs involving EABs are regarded as a promising technology to clean organic matter from wastewater providing electrical current and simultaneously decreasing the need for tank aeration. An MFC is basically made up of two chambers, anode and cathode, separated by a proton/cation exchange membrane (Erable et al. 2010). EABs growing on anodes, oxidize the organic matter and produce electrons and protons in the anode chamber. Electrons collected on the anode are transported to the cathode by an external circuit, and protons are transferred through the membrane internally. Thus, a potential difference is produced between the anode and cathode chamber due to dissimilar liquid solutions. Electrons and protons are consumed in the cathode compartment by reduction of oxygen.

The performance of MFCs is characterized by power/current curves which represent the power (P) provided as a function of the current (I). These curves are obtained by varying the electrical resistance of the external circuit (Figure 1). For very high resistance, or even open circuit, the voltage (V) is maximal, but the

current and consequently the power are nil ($P = U.I$). Decreasing the resistance leads to a voltage decrease and then a current increase resulting in a bell-shaped curve. When the resistance is very low, or when the cell is connected in short circuit, an MFC provides maximum current but power is reduced to zero because there is no voltage. For energy generation, the optimal functioning point for an MFC is the point of maximal power (point a in Figure 1). In contrast, it has been rarely reported that, with the objective of optimizing wastewater treatment, the optimal functioning point is at short circuit (point b in Figure 1). A short-circuited MFC provides the highest currents, meaning that it ensures the highest rate for organic matter oxidation. If the bell-shaped power/current curve were perfectly symmetrical, shifting from the maximum-power point to the maximum-current point should double the rate of wastewater treatment (100% increase). As shown in Figure 1 relating to previous work with an air-cathode MFC inoculated with a marine biofilm (Erable and Bergel 2009), a 60% increase in current was observed when shifting from point (a) to point (b). A few attempts have been reported with such a strategy (Kim et al. 2008; Srikanth et al. 2010). Higher substrate removal efficiency has been observed with a short-circuited MFC compared to a resistance-connected MFC but gains remained quite low, probably due to kinetic limitations for organic matter breakdown, and/or because a large part of chemical oxygen demand (COD) removal was due to non-electrochemical

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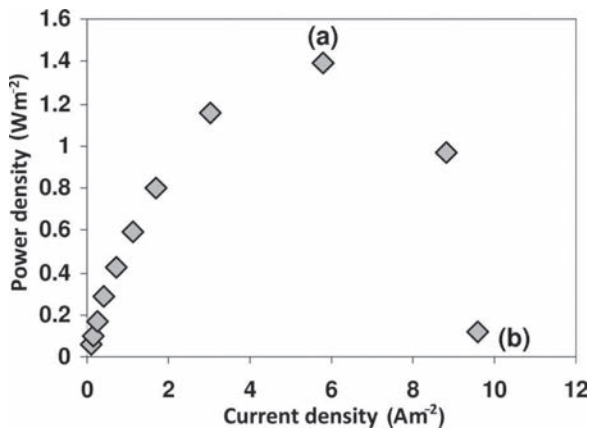


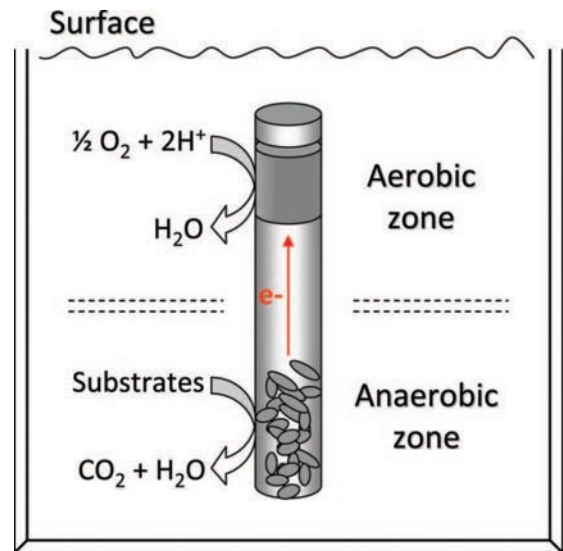
Figure 1. Polarization curve of a microbial fuel cell (adapted from Erable et al. 2009a).

pathways that used soluble electron acceptors (ie low columbic efficiency) and/or to the rate of removal being limited by kinetics.

If the ultimate goal is no longer to produce current but only to maximize the oxidation of organic matter, the system does not require complex electrochemical reactors with membranes or any other type of separators. The system can be simplified to two connected electrodes, or even a single piece of conductive material, as illustrated in Figure 2. The result is a basic device that the authors propose calling a microbial electrochemical snorkel (MES) because it works exactly like a snorkel that allows the anode respiring bacteria to transfer to oxygen the electrons produced by their metabolism. Unlike MFCs, an MES does not divert energy to produce electricity but it ensures maximum efficiency for the oxidation of organic matter.

Technically, the MES concept can simply consist of the following (Figure 2): (i) a conductive material acting as anode, which must be colonized by an EAB, (ii) a conductive material acting as cathode, that ensures electron removal to the final electron acceptor and allows bacteria “snorkelling” to oxygen for example. The same scheme can be applied to final electron acceptors, other than oxygen, eg nitrates, nitrites, sulfates, and thiosulfates. The cathodic reaction can be abiotic, or also benefit from the development of an EAB (biocathode).

Any combination of conductive materials can be used to construct an MES. The anode and cathode materials can be different or not, for instance graphite for the anode and stainless steel for the cathode. Stainless steel has sometimes been claimed to be more effective than basic carbon materials for development of an oxygen-reducing EAB (Dumas et al. 2008a; Jadhav and Ghangrekar 2008). The same material can



Electrochemical snorkel

Figure 2. Microbial electrochemical snorkel (MES) concept. In this example, the MES consists of a graphite rod in which the down part plays the role of an anodic site and the top part the role of a cathodic site. An electroactive biofilm should form on the anodic part exposed to anaerobic conditions, while a catalyst should be deposited and/or an EA biofilm should form on the cathodic part exposed to the aerobic zone. The system should be immersed vertically in the treatment tank.

be used for both anode and cathode. A specific catalyst can also be deposited on the cathode. Most of the results obtained so far from the field of MFC research can be exploited to design MESs, as for example, the use of a floating air-cathode (Song et al. 2010). In turn, the MES concept affords new easy-to-handle devices that can be very helpful in improving research on MFCs.

The purpose of this work was to assess the feasibility and quantify the efficiency of the MES concept for the treatment of urban wastewaters. In the initial steps of MFC research, simple substrates like acetate and glucose were commonly used, but substrates have recently grown in complexity with the aim of treating a wide range of real wastewaters or utilizing different sources of biomass (Rozendal et al. 2008; Cercado-Quezada et al. 2010; Pant et al. 2010). These authors explained that studies should now focus on real wastewater for (i) improving the degradation of complex materials and (ii) controlling the microbial reactions occurring in the microbial electrochemical systems. In this framework, real wastewater collected from an urban wastewater treatment plant was used. Wastewaters were used both as inoculum to form the EAB on graphite-based anodes and also as the medium

to be treated. The procedure for forming microbial anodes was optimized and then the colonized anodes were used for wastewater treatment. The efficiency of COD removal was compared with (i) an electrolysis process performed at -0.1 V vs SCE, (ii) an air-cathode MFC that classically operated at maximal power ($R = 1000 \Omega$), a short-circuited air-cathode MFC ($R = 0 \Omega$), and (iv) the MES concept consisting of a 20 cm titanium rod with EAB colonized graphite at its lower end and platinum mineral catalyst at its top end.

Material and methods

Wastewater and COD measurement

Wastewater was collected after mesh filtration at the inlet of an urban wastewater treatment plant (Brax, near Toulouse, France). The initial organic load of wastewater varied from 230 to 600 mg COD l^{-1} depending on the day and the season of collection. The wastewater was deoxygenated with oxygen free N_2/CO_2 (80/20%) gas for 10 min to create an anaerobic environment prior to use.

The COD was measured in duplicate using standard methods (APHA and WPCF 1998). Coulombic efficiencies were calculated as reported by Logan et al. (2006).

EAB optimization

Graphite felt anodes (RVG, Carbone Loraine) of 40 cm^2 estimated surface area were colonized under electrode polarization at -100 mV vs SCE in glass bio-electrochemical reactors fed with 500 ml wastewater (Erable et al. 2010). Polarization of anodes was achieved with a multipotentiostat (VMP2 Bio-Logic SA, software EC-Lab v.8.3) using a 10 cm^2 platinum mesh as auxiliary electrode and a saturated calomel electrode (SCE, Radiometer Analytical, TR100) as reference. When indicated, wastewater was supplemented with 1 g l^{-1} acetate or with a microbial suspension of electrochemically active (EA) microorganisms. These EA microorganisms were collected from a wild EAB established on a graphite electrode. The EAB was obtained after 3 weeks from a wastewater/activated sludge suspension (ratio 2:1) on a graphite electrode polarized at -100 mV vs SCE. Cyclic voltammetry was performed *in situ* on the microbial anodes after suspending polarization. The potential was scanned 3 times between the open circuit potential (OCP) and $+300$ mV vs SCE at a scan rate of 1 mV s^{-1} .

When several microbial anodes were formed in parallel in the same reactor (section 3.4) a N-stat system (Biologic SA) was coupled to the multipotentiostat to polarize each working electrode and to measure independently the current generated on

each surface, as described elsewhere (Dumas et al. 2008b).

Electrolysis cell, MFC and MES

Microbial electrolysis in dual-chamber MFCs

A dual-chamber MFC consisted of 2 compartments of 0.5 l each separated by a proton exchange membrane (Nafion[®]). The anodic chamber was hermetically sealed, with no gas flow. The previously described microbial anode (working electrode) was placed vertically at the bottom of the anodic chamber and electrical contact was made with a titanium wire. The cathode (auxiliary electrode) was a 20 cm^2 estimated surface area platinum (Pt) mesh positioned in the cathodic compartment, which was filled with 50 mM phosphate buffer pH 8.0 and constantly fed with air by an aquarium-type pump.

Experiments were performed at constant potential (chronoamperometry) using a multi-potentiostat (VMP2 Bio-Logic SA, software EC-Lab v.8.3). All potentials were reported against a saturated calomel standard reference electrode (SCE) placed in the anodic compartment. The potential of the microbial anode was fixed at -100 mV vs SCE during chronoamperometry and current was followed over time.

Air-cathode MFC

Two types of air-cathode MFCs were used. In both cases, the cathode was a 0.5 mg Pt cm^{-2} carbon-felt air-breathing cathode (Paxitech, France) with an estimated surface area of 12.5 cm^2 . The volume of the anodic chamber was either 500 ml or 120 ml, in order to compare different electrode surface/reactor volume ratios (ie 8 $m^2 m^{-3}$ and 33 $m^2 m^{-3}$). For each architecture, efforts were made to set the microbial anode parallel to the cathode separated by a distance of 2 cm.

Electrochemical polarizations were performed by varying progressively after 15 min stabilization the external resistance over a range from 1 to $10^6 \Omega$. Preliminary evaluation of air-cathode MFC performance showed that the maximum power density delivered by the air-cathode MFC was achieved with a resistance of 1000 Ω (polarization curve not shown). Wastewater treatment was evaluated in a MFC connected with a 1000 Ω resistance (P_{max}), connected directly in short-circuit (I_{max}).

Microbial electrochemical snorkel

A basic MES device was designed consisting of a 20 cm titanium rod with a colonized graphite felt anode at its lower end and platinum at the top.

Platinum was chosen to maximize the electrochemical reduction rate of dissolved oxygen. Wastewater treatment tests were carried out in glass bioreactors 40 mm in diameter containing 120 ml wastewater. The MES system was placed vertically in the bioreactor with the platinum side exposed to the surface. Experiments were carried out in fed batch mode at room temperature ($20 \pm 2^\circ\text{C}$) with urban wastewater. Three consecutive batches were studied.

Results and discussion

Microbial anode optimization

Three bio-electrochemical reactors, each containing a 40 cm^2 graphite felt electrode, were filled with different sources of mixed microbial populations, viz. raw wastewater, wastewater supplemented with 10 mM acetate, and wastewater supplemented with a suspension of EAB and 10 mM acetate. The anode potential was maintained at -100 mV vs SCE for 8 days to promote settlement of EA species on the electrode surface (Erable et al. 2009a, 2009b).

When the reactor was filled with raw urban wastewater, it took approximately 3 days for the current to increase to a maximum of 0.7 A m^{-2} (Figure 3a). Addition of acetate (Figure 3b) did not affect the initial increase rate of current, which corresponded to the phase of electroactive biofilm (EAB) growth, but the high load of easily-available organic matter (ie acetate) maintained the growth phase for an extra day. A higher maximum current of 1.25 A m^{-2} was reached. The addition of a microbial suspension collected from a previous EAB into the wastewater led to a rapid (<1 day) and intense (up to 1.75 A m^{-2}) current increase (Figure 3c).

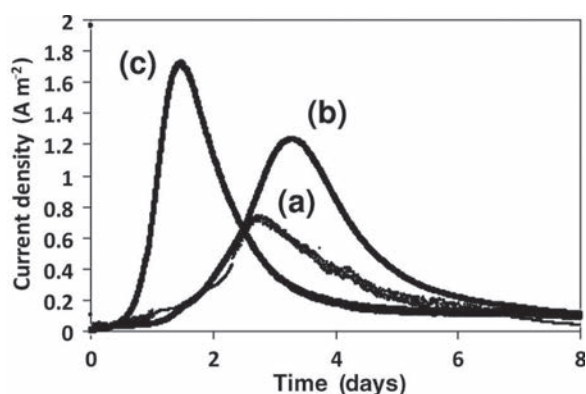


Figure 3. Current generated by a graphite felt anode polarized at -100 mV vs SCE in urban wastewater (a), in urban wastewater supplemented with acetate (b), in urban wastewater supplemented with a suspension of electrochemically active bacteria (c).

Various adaptive evolution strategies have been reported to optimize the design of microbial anodes. It has been shown that following enrichment of a mixed culture on a graphite anode surface electricity generation can be optimized by imposing a constant electrode potential (Cho and Ellington 2007; Aelterman et al. 2008; Parot et al. 2008). A previous study that dealt with microbial anodes formed from marine sediment has confirmed that forming microbial anodes under polarization reduces the start-up time and allows the steady-state performance to be reached rapidly (Erable et al. 2009b) as observed here. Using as inoculum an EAB collected from an established bioanode has also been demonstrated to accelerate electrode colonization by EA microorganisms (Rabaey et al. 2004; Liu et al. 2008; Erable and Bergel 2009). Here, coupling polarization and inoculation with a previously formed EAB had a substantial effect, both reducing the start-up phase and increasing the maximum current.

Microbial anodes used further in this study were consequently prepared from urban wastewater supplemented with an EAB suspension (Figure 3c). Five microbial anodes were formed following this procedure. Basically, after polarization for 1 week, the anodes showed an open circuit potential ranging from -560 to -510 mV vs SCE and sustained an average current density of $1.8\text{ A m}^{-2} \pm 0.2\text{ A m}^{-2}$ at -100 mV vs SCE (data not shown). Cyclic voltammeteries, which were performed *in situ* after suspending polarization, confirmed the reproducibility of the performance of the different anodes.

COD removal by microbial electrolysis at -0.1 V vs SCE

A microbial anode was inserted in the anodic chamber of a dual-chamber MFC and its performance, in terms of electricity production and COD reduction, was evaluated by microbial electrolysis at -100 mV vs SCE (chronoamperometry). The choice of evaluating the performance at -100 mV vs SCE was based on several publications reporting anode potentials close to this value when a MFC works in short-circuit (under zero voltage) (Scott et al. 2007; Min and Angelidaki 2008). The anodic chamber was fuelled with real urban wastewater and COD removal was measured periodically. Each time the electrical current fell to zero, the urban wastewater was renewed in the anodic compartment.

The results remained quite similar from one batch to the other both in terms of generated current and COD removal (Figure 4). The current increase was slightly steeper during successive batches. This phenomenon has often been noted in the literature and has been explained by a bacterial enrichment in EA species

inside the anodic biofilm (Liu et al. 2008; Erable et al. 2009b). Nevertheless only weak effects on the performance of the maximum current densities were observed for the successive batches. Between 0.49 and 0.74 A m⁻² were observed depending on the batch experiments. The corresponding COD removal rates were reproducible and the legal limit for water discharge (125 mg CODL⁻¹) was attained in less than 5 days for each batch. However, no precise relationship between the initial COD load of the effluent and the maximum current density could be established.

The reproducibility of the results obtained here strengthens the interest in working with a microbial anode colonized under a fixed electrode potential coupled to inoculating with an EAB suspension. Indeed, such high reproducibility is not generally obtained when working with non-polarized MFCs. For instance, it has been reported that improving the reproducibility of successive batches performed with MFC requires running experiments in cycles to limit

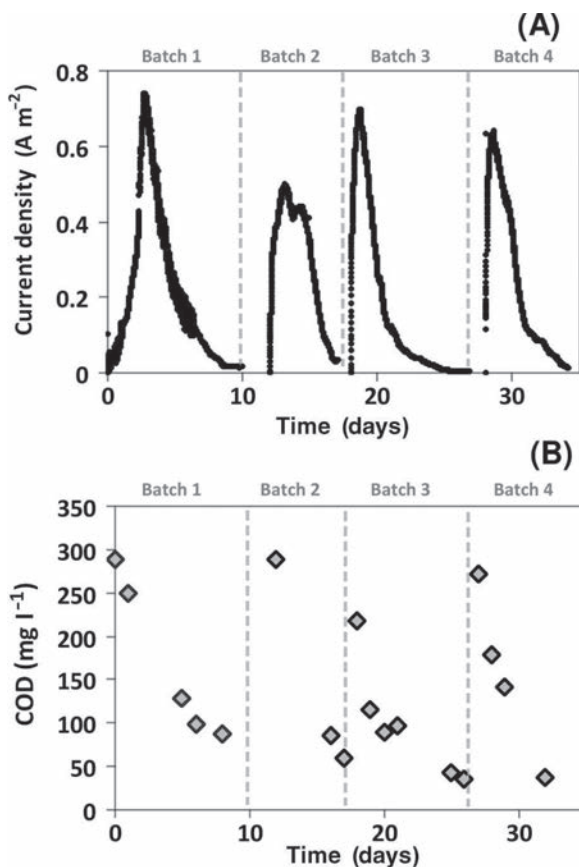


Figure 4. Current density (A) and COD removal (B) with a graphite felt microbial anode polarized at -100 mV vs SCE (microbial electrolysis) during successive raw wastewater batches.

the variability in the microbial communities (Rabaey et al. 2005). A recent study (Larrosa et al. 2009) has demonstrated that a minimum of four replicates was required to detect reproducible COD removal with MFCs.

Coulombic efficiencies between 61 and 74% were calculated for the different batch experiments. That means that between 26 and 39% of the initial COD load was not oxidized through electron transfer to the graphite anode. The COD not involved in current generation may be: (i) consumed by competitive degradation pathways that use other soluble electron acceptors (eg oxygen, nitrates, sulfates, organic molecules) or planktonic cells or cells in a biofilm, (ii) and/or used for growth and reproduction. Heterotrophic bacteria typically require 40–60% in aerobic and 15–20% in anaerobic conditions of the removed COD to produce new cells (Rodrigo et al. 2010).

The overall COD ([COD]_{total}) removal is thus the sum of:

$$[\text{COD}]_{\text{total}} = [\text{COD}]_{\text{electricity}} + [\text{COD}]_{\text{competitive metabolisms}} + [\text{COD}]_{\text{bacterial growth}} \quad (1)$$

To estimate the amount of COD oxidized by planktonic bacteria, a control experiment without microbial electrode was conducted in parallel with a fifth batch performed identically to the four previous batches (Figure 5). The rate of COD degradation in the control experiment was low, about 16 mg COD l⁻¹ day⁻¹, while it reached 80 mg COD l⁻¹ day⁻¹ with the electrode. This experience confirmed the essential

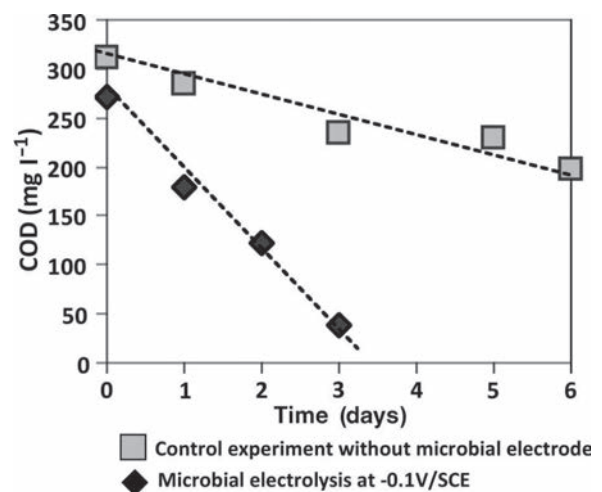


Figure 5. COD removal from wastewater with a graphite felt microbial anode polarized at -100 mV vs SCE (microbial electrolysis). The control experiment was a sealed anodic compartment without an electrode.

contribution, in terms of degradation, of the microbial electrode. The proportion of COD removed by the non-EA microorganisms is estimated at 20% of the initial COD load. In this case, the part used for the growth of bacterial cells and the maintenance of the biofilm EA was only 6 to 19% of the initial COD load (Equation (1)). The smallness of this quantity is logical considering that the EAB was already running for 40 days (5th batch) and was in stationary growth phase. The COD degradation observed in the control experiment certainly corresponded to a predominantly aerobic oxidation rather than anaerobic digestion because the control reactor as well as the experimental reactors were not completely sealed and slow oxygen transfer was possible. Similar control experiments were carried out in strictly sealed reactors deoxygenated by N_2/CO_2 (80/20%) in parallel to the other experiments. In these cases the COD decreased significantly more slowly.

Optimizing the MES concept with a short-circuited MFC

A freshly prepared microbial anode (40 cm^2) was transferred into the 500 ml anodic compartment of an air-cathode MFC. The microbial anode and the abiotic air-cathode were connected in short-circuit ($R = 0\ \Omega$). The MFC was fed in batch mode with urban wastewater (initial COD = 600 mg l^{-1}) or urban wastewater supplemented with acetate (initial COD = 1500 mg l^{-1}). The COD load of wastewater from the treatment unit was two times higher than previously measured (600 mg l^{-1} instead of 300 mg l^{-1}). A control experiment under strict anaerobic conditions and without microbial electrode was performed in parallel with the same wastewater.

The short-circuited MFC reached the acceptable limit for discharge (125 mg COD l^{-1}) in 4 days, ie it ensured an average oxidation rate of $110\text{ mg COD l}^{-1}\text{ day}^{-1}$, while the anaerobic control still contained about 95% of its initial organic load after 4 days (Figure 6A). The COD oxidation rate observed in the short-circuited MFC was thus higher than that achieved with the microbial anode polarized at -100 mV vs SCE ($90\text{ mg COD l}^{-1}\text{ day}^{-1}$) (Figure 4). The mixed potential of the two electrodes connected in short-circuit was -20 mV vs SCE (day 4). Indeed, the higher potential of the microbial anode in the MFC device promoted the oxidation reactions. Putting the anode and cathode in short circuit forces the anode to the highest possible value of potential, which promotes the oxidation reactions. Indeed, a way to optimize an MES device consists of increasing the kinetic of the cathode and/or its surface area. Both actions displace the operating potential of the device towards higher

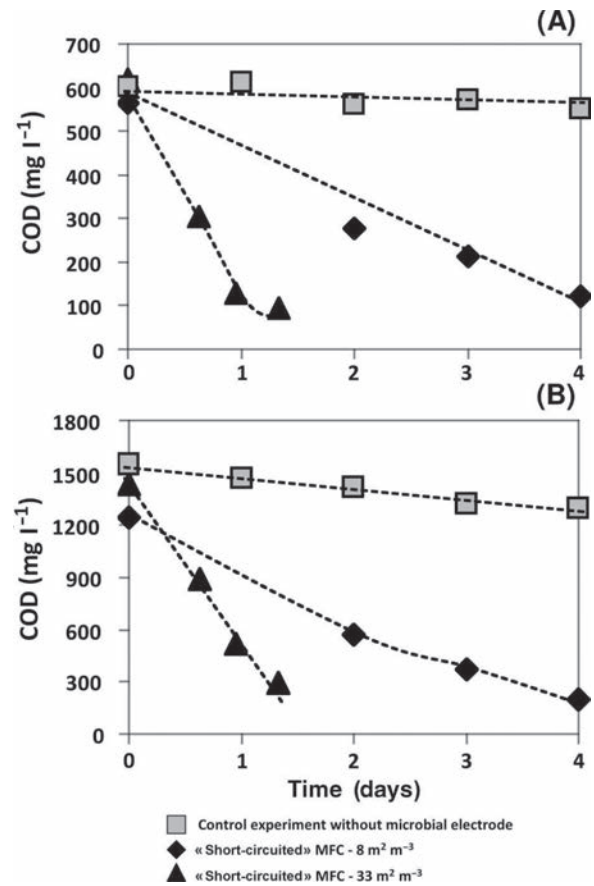


Figure 6. COD removal from wastewater (A) and acetate enriched wastewater (B) by “short-circuited” MFCs. Two ratios of anode surface/reactor volume ($\text{m}^2\text{ m}^{-3}$) were tested.

values and consequently accelerate the oxidation reactions on the anode side.

Acetate is a simple substrate and is often used as carbon source supplement to enrich and maintain EA bacteria (Cho and Ellington 2007). In parallel, substrate mixtures contained in real wastewater lead to the development of a more diverse microbial population. These mixed populations help convert complex substrates into simpler molecules that could be more easily oxidized by anode respiring bacteria (Pant et al. 2010). Regarding the acetate enriched wastewater (Figure 6B), after 4 days the total COD load dropped from 1500 to 240 mg COD l^{-1} (84%), ie an average oxidation rate higher than $300\text{ mg COD l}^{-1}\text{ day}^{-1}$. In the same time, the organic load in the control experiment without microbial anode only decreased from 1500 to $1320\text{ mg COD l}^{-1}$ (12%).

In general cases, when the reactor architecture ensures a homogeneous distribution of the electrical field, the production rate of an electrochemical process is directly controlled by the [electrode surface area/reactor volume] ratio. Increasing this ratio directly

increases the rate of product generation or substrate consumption. In this framework, a new experiment was performed by reducing the volume of the anodic compartment from 500 to 120 ml, meaning that the ratio [electrode surface area/reactor volume] was increased by a factor of 4 (from 33 to 8 m² m⁻³). The size of the electrodes and the inter-electrode distance were not changed. The degradation rate of the COD load in wastewater was 4 times faster with the ratio 33 m² m⁻³ and the legal target of 125 mg COD l⁻¹ was then reached within 1 day (oxidation rate > 500 mg COD l⁻¹ day⁻¹) with urban wastewater (Figure 6A). This system could also be used as a basis to design a continuous process that could achieve the legal COD removal target with a residence time of < 1 day. The residence time is a key factor in the design of wastewater treatment units. Using the process described in this paper that does not require any aeration, the legal discharge values for COD were attainable within 3 to 7 h.

Basic microbial electrochemical snorkel (MES)

The validity of the MES concept for urban wastewater treatment was finally checked by running four experiments in parallel, viz. (1) an anaerobic control with a sealed reactor; (2) an air-cathode MFC with a 120 ml anode compartment classically connected with a 1000 Ω resistance (operation at maximal power); (3) an air-cathode MFC with a 120 ml anode compartment that was short-circuited according to the MES concept; (4) a compact MES device composed of a 40 cm² graphite felt anode connected to a 10 cm² platinum mesh cathode

The four microbial anodes used in each device were prepared as described in Section 3.1 by polarizing them in the same reactor to ensure that each electrode was exposed strictly to the same medium. All the microbial anodes reached a maximal current density about 1.6 A m⁻² after polarization for 5 days and showed similar current-potential curves with a relative difference of < 5% (data not shown). The four experiments were run in parallel in order to achieve strictly identical experimental conditions (temperature, wastewater).

The wastewaters used in this part of the study had COD loads between 290 and 330 mg l⁻¹ depending on the batch series (1–3). The goal was to reach the legal limit for water discharge of 125 mg COD l⁻¹ corresponding in this case to ~35% of the initial COD load. COD removal was measured in each reactor after 24 h.

As reported in Figure 7, the results were reproducible for the three successive batches. The low degradation (15%) observed in the anaerobic control was due to the slow metabolism of the anaerobic

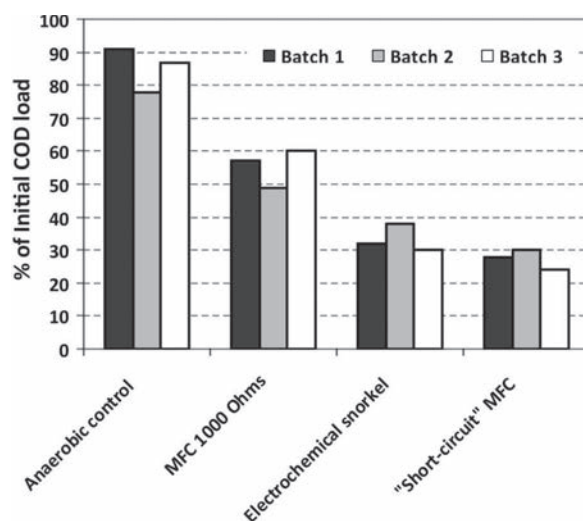


Figure 7. Comparison of different anaerobic processes for wastewater treatment after 24 h, with three successive batches.

bacteria. The MFC connected with a 1000 Ω resistance ensured 50% removal of the initial COD load after 24 h. The short-circuited MFC and the compact MES ensured around 72% and 69% COD removal and in both cases the legal limit for water discharge was reached in < 24 h. Considering that an average 15% of COD removal is due to planktonic degradation (control batches) the 1000 Ω-connected MFC ensured 35% of COD removal and the MES systems around 55%. The MES system represents a gain of around 57% with respect to the 1000 Ω-connected MFC. This value corresponds to the prediction in Figure 1 on the basis of current characteristics of MFC. It confirms the high interest in the concept for COD.

Conclusion

The combination of polarizing the electrode and enriching the wastewater with EA species had a very positive effect on improving the performance and the reproducibility of microbial anodes. The comparative study of different methods for the treatment of urban wastewater (microbial electrolysis, MFC, MES) validated the MES concept and showed its potential. This technology, which aims at maximizing the reaction rates by elimination of current generation, provided a degradation performance far higher than the MFCs that operated at optimum power. This demonstration opens a new avenue for the design of extremely simple electro-microbial devices for wastewater treatment.

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