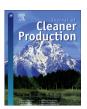
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Resources recovery in the dairy industry: bioelectricity production using a continuous microbial fuel cell



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ABSTRACT

The increasing food demand and the exhaustion of non-renewable fuels provide new market opportunities in the agro-farming sector. Biological systems designed to add value to useless organic subproducts and to generate off-grid electricity may be one of the most interesting outcomes. Therefore, the capacity of some microorganisms to transfer electrons generated during organic carbon oxidation directly to an anode in a so-called microbial fuel cell (MFC) might be an asset in a sustainable management context. In this regard, the main goal of the present work was to evaluate the performance of a continuous MFC applied in a dairy industry. A maximum voltage of 576 mV was produced during continuous operation, corresponding to a power density of 92.2 mW m $^{-2}$ or 1.9 W m $^{-3}$. MFC was able to remove 1298 \pm 617 mg L $^{-1}$ of chemical oxygen demand (COD) at a hydraulic retention time of 8.4 h, and the maximum COD removal (63 \pm 5%) was achieved after 20 days of continuous operation. In addition, the coulombic efficiency average was around 10.5 \pm 10% with a maximum of 24.2 \pm 1.5%. In average, the MFC was able to extract a specific energy of 8.95 \times 10 $^{-2}$ kW h kg $^{-1}$ COD with a maximum output of 20.53 \times 10 $^{-2}$ kW h kg $^{-1}$ COD. In conclusion, the MFC technology is a valuable option for simultaneous wastewater treatment and energy recovery and deserves to be tested and scaled-up in the dairy industry.

1. Introduction

The access to food in quantity and quality is one of the key issues affecting human development and is a main driver for the circular economy growth and human equity. The increasing shortage of resources, namely water and energy, is highly challenging for science and technology. Nowadays, research on innovative, cost-effective and competitive industrial processes are necessary to boost food production and the quest for sustainable technologies to process agro-industrial wastewaters is a step forward (Aydiner et al., 2016; Almuktar et al., 2015).

In response to this ambition, several efforts are being pursued trying to explore decentralized and clean energy sources. One of those possibilities is the use of microbial fuel cells (MFCs) (Logan et al., 2006; Martins et al., 2010). The MFC technology is based on the ability of some carbon-oxidizing microorganisms to transfer

electrons directly to an anode in anaerobic conditions (Peixoto et al., 2013; Logan et al., 2006). The standard design consists of an electrochemical cell with anodic and cathodic compartments separated by a proton exchange membrane. In the anode compartment, the conversion of different carbon sources is carried out through catalytic reactions, which involve anaerobic electroactive microorganisms (Franks and Nevin, 2010). Several environmental factors influence MFC performance, namely, inoculum type, carbon source, concentration, feed rate, pH, temperature and reactor configuration (Ren et al., 2014a; Cheng et al., 2006). Therefore, for full scale applications in wastewater treatment. continuous flow MFC could be more suitable than batch or fedbatch for a higher COD removal and power generation (Sevda et al., 2015; Zhuang et al., 2012; Rahimnejad et al., 2011). In continuous MFC operation, the maximum chemical oxygen demand (COD) removal is in the range of 70% and 99% (Pasupuleti et al., 2015; Rahimnejad et al., 2011). However, the hydraulic retention time (HRT) was higher (up to 48 h) than those adopted in conventional wastewater treatment systems (4–12 h) (Sevda et al., 2015; Ahn and Logan, 2013; Aelterman et al., 2006; Min et al.,

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2005). In conclusion, there have been few studies on wastewater treatment using MFC in a HRT range of ~10 h or less (Kim et al., 2015).

A cleaner and more competitive agro-industrial management is essential in order to face energy and water scarcity (Almuktar et al., 2015). In particular, dairy industry is one of the most significant examples of biodegradable organic based wastewaters that endanger surface waters quality. Therefore, a technology that achieves wastewater treatment with simultaneous bioenergy recovery will represent an asset towards an eco-efficient industry (Aydiner et al., 2016; Pant et al., 2010; Oh et al., 2010). Dairy wastewater is a complex organic mixture rich in carbohydrates, proteins and fats, presenting a significant biological oxygen demand (BOD) and COD (Karadag et al., 2015; Elakkiya and Matheswaran, 2013; Demirel et al., 2005). Biological processes are the standard in dairy wastewater treatment plant designs, often complemented by physico-chemical processes for fat and grease abatement or nutrient removal (Karadag et al., 2015; Martín-Rilo et al., 2015; Demirel et al., 2005). Lagoon systems are the preferred low-cost approach, but the performance is rather low and odour problems may occur (Bhatia and Goyal, 2014). On the contrary, activated sludge processes present a good performance but the energy consumption and sludge production are significant drawbacks (Demirel et al., 2005), Anaerobic filters (Lim and Fox, 2011) and upflow anaerobic sludge blanket reactors or related concepts (Ramasamy et al., 2004; Demirel et al., 2005) are used also. These reactors may have some performance limitations and being driven by biogas production requires further conversion processes to produce electricity (Passeggi et al., 2012). Therefore, there is a substantial gap that requires innovative designs towards a circular economy and clean production in the dairy industry (Kubota and da Rosa, 2013).

In electricity driven world, MFCs are probably the comprehensive answer for such a demand in the agro-food industry, being a more efficient answer than biogas production, especially when we compare the net electrical efficiency of anaerobic digestion (10%—15%) with the coulombic efficiency of MFCs (20%—80%) (Mardanpour et al., 2012; Pant et al., 2010; Oh et al., 2010). In addition, MFCs are not affected by high concentrations of volatile fatty acids that are known to inhibit conventional anaerobic digestion processes (Oh and Martin, 2009; Hawkes et al., 2007; Logan and Regan, 2006). However, to date only a few attempts tried to apply MFC technology in the framework of industrial dairy wastewaters, especially in a continuous flow mode. Therefore, the goal of the present work is to assess the performance of a continuous MFC for dairy wastewater treatment and energy valorisation.

2. Material and methods

2.1. MFC setup and operation

A dual chamber MFC was assembled by connecting two compartments of transparent poly methylmethacrylate, with equal dimensions (12 cm \times 8 cm \times 5 cm). The two compartments were physically separated by a proton exchange membrane (Nafion Membrane 117, DuPont Co., USA), sealed with a silicone rubbers, and to keep it tight, rubber gaskets and stainless steel screws were used. Total volume of each chamber was 480 mL with approximately 350 mL of liquid volume. The electrodes, both carbon Toray TP-090 (QUINTECH, USA) sheet (6 cm \times 6 cm = 72 cm²), were connected to an external resistance using copper coated wires (2 mm diameter). The circuit was closed with a fixed resistance of 500 Ω , according to the results obtained by Elakkiya and Matheswaran (2013). In Fig. 1 it is depicted a schematic diagram of continuous MFC setup.

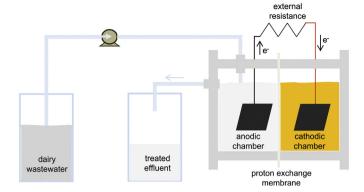


Fig. 1. Schematic diagram of continuous MFC setup.

In order to promote biofilm formation on anode surface and to produce power density, 350 mL of pre-screened municipal wastewater (with an average chemical oxygen demand (COD) concentration of $500\pm100~\text{mgL}^{-1}$, conductivity of $790\pm20~\mu\text{S cm}^{-1}$ and pH 7 ± 0.2) was used as a batch stirred anolyte at room temperature ($\approx22~^\circ\text{C}$). After sampling, the wastewater was deoxygenated with nitrogen gas and kept at $4~^\circ\text{C}$ until use (Peixoto et al., 2013). When necessary, wastewater was replaced to avoid organic carbon limitations on bioelectricity production. In the cathode compartment and to avoid oxygen limitation, 50 mM of hexacyanoferrate in phosphate buffer (50 mM) at pH 7, was used as a catholyte solution.

After the steady state was reached (i.e: when similar values of power density were obtained along three consecutively cycles), the flow regime was changed from fed-batch to continuous and a dairy wastewater was used to feed the MFC at a flow rate of 1 L d⁻¹. A synthetic feed was prepared in order to simulate the average composition of a dairy wastewater (1500–5000 mgCOD L⁻¹; Elakkiya and Matheswaran, 2013; Mardanpour et al., 2012), and no precise mode was in place regarding synthetic effluent preparation because wastewater COD values in the dairy industry present significant variations due to process operations and due to discontinuity in the production cycles of different products (Farizoglu and Uzuner, 2011). Therefore, the synthetic effluent was prepared by adding ~100 mL of low fat pasteurized milk to ~5 L of tap water, without macro or micronutrient supplementation.

2.2. Electrochemical and chemical analysis

Bioelectricity production was measured by recording every 30 min the voltage between anode and cathode. Data were collected automatically and stored in a computer by a USB-9215A BNC connector data logger (National Instruments) and a data acquisition software (Labview 6.0) (Martins et al., 2014). Electrochemical analysis was performed according to Martins et al. (2010). Briefly, the current intensity (I) was calculated according to the Ohm's law (Equation (1)), where V is the voltage and R the resistance. The current density (j) was calculated as depicted in Equation (2), where A is the projected surface area of the anode electrode. The power density (P) is calculated as the product of current intensity and voltage divided by the projected surface area of the anode (Equation (3)). The polarization curve, describing the voltage and the power density as a function of the current density (Peixoto et al., 2013) was recorded using a series of resistances in the range of 71.1 k Ω to 50 Ω during biofilm formation and the stable phase of bioelectricity production along the continuous operation (Peixoto et al., 2013). The internal resistance of the MFC (Rint) was calculated from the slope of the polarization curve in the region

dominated by Ohmic losses (Peixoto et al., 2013). The open circuit voltage (OCV) was measured at infinite resistance.

$$I = \frac{V}{R} \tag{1}$$

$$j = \frac{I}{A} \tag{2}$$

$$P = \frac{IV}{A} \tag{3}$$

Treatment performance was assessed by COD removal, using chromate as the oxidant agent, as described in Standard Methods (A.P.H.A et al., 1998). Coulombic efficiency (CE) was calculated according to Equation (4), where Ct was the total coulombs calculated by integrating the current over time (Equation (5)), and Cth was the theoretical amount of coulombs available based on the COD removed in the MFC over the same amount of time, calculated by Equation (6).

$$CE = \frac{Ct}{Cth} \times 100\% \tag{4}$$

$$Ct = \sum I \, \Delta t \tag{5}$$

$$Cth = \frac{Fb(COD_{in} - COD_{out})Q\Delta t}{M}$$
 (6)

I is the current intensity, Δt is the time interval, F is Faraday's constant, b=4 is the number of electrons exchanged per mole of oxygen, CODin and CODout are the influent and effluent COD, Q is the flow rate, and M=32 is the molecular weight of oxygen (Ren et al., 2014a; Logan et al., 2006).

3. Results and discussion

3.1. MFC operation

Voltage variations between anode and cathode were monitored during biofilm formation. Voltage started to increase after 14 days of incubation with municipal wastewater and a stable value (431 \pm 22 mV) was obtained after 6 feeding cycles. In terms of power density, the maximum value obtained during biofilm formation was around 52 \pm 5 mW m⁻². After the 26th day of operation, the MFC reactor flow regime was switched to continuous and the synthetic dairy effluent was added. The voltage variation and power density along time are depicted in Fig. 2.

During continuous operation, voltage remained between 400 mV and 500 mV for approximately four days, which corresponded to 45 mW m⁻² and 69 mWm⁻², respectively. The voltage dropped due to fouling of the feeding tubes and cleaning was done by hydraulic washing increasing the flow rate to the maximum value (~10 mL min⁻¹) and by switching the flow direction. Thereafter, the feed was renewed and COD was determined. The voltage started to increase and reached a new maximum, 576 mV (92 W m⁻²). During this period, a new polarization and power curve assessment were carried out (Figs. 3 and 4). Based on those results, it was decided to change the external resistor from 500 Ω to 80 Ω . This change had the purpose of the optimize power production once that the optimum external resistance is the resistance for which the maximum power density was obtained (Martins et al., 2014).

However, after a short period where power density increased from 92 mW m^{-2} to 246 mW m^{-2} , it started to decrease, probably

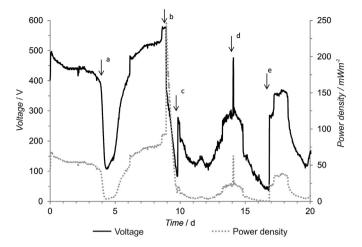


Fig. 2. Voltage (V) and power density (P) variation along MFC continuous operation; a) addition of new feed solution; b) addition of new feed solution and changing the external resistor from 500 Ω to 80 Ω ; c) changing the external resistor from 80 Ω to 500 Ω ; d) addition of new feed solution; and e) addition of new cathodic solution.

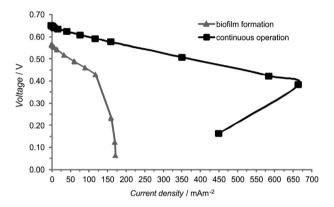


Fig. 3. Polarization curves obtained in the stable phase of biofilm formation and in continuous MFC operation.

due to a high electron discharge promoted by the lower resistor (Mardanpour et al., 2012; Venkata Mohan et al., 2010). At lower external resistances, the electrons flow more rapidly through the circuit compared to higher external resistances (Mardanpour et al., 2012). A higher wastewater oxidation rate by microorganisms is expected with a large amount of oxidized electron carriers at low external resistances (Venkata Mohan et al., 2010).

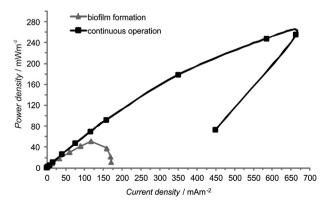


Fig. 4. Power curves obtained in the stable phase of biofilm formation and in continuous MFC operation.

To reverse the voltage decrease, the external resistor was switched to 500 Ω . However, power density maintained the descending trend for two days and only partially recovered afterwards. At day 40 (13 days after continuous operation), a new feed solution was added and voltage increased up to 474 mV (62 mW m $^{-2}$) decreasing afterwards, probably due to cathodic solution limitations. A new cathodic solution was prepared and voltage increased again until 369 mV (37 mW m $^{-2}$), decreasing afterwards. The fair performance in power production could be due to the membrane fouling on the anode driven by the organic concentration (Elakkiya and Matheswaran, 2013). Moreover, recent studies have shown that both high power densities and low effluent COD concentrations is difficult to achieve in MFC under continuous flow conditions (Kim et al., 2015; Elakkiya and Matheswaran, 2013; Akman et al., 2013).

3.2. Polarization and power curves

The open circuit voltage (OCV) corresponds to the maximum voltage that is possible to obtain from the MFC under a set of experimental conditions. The measured OCV was 562 mV during biofilm formation and 649 mV during continuous operation. These values are in the range of those reported in literature for similar setups (500–800 mV) (Peixoto et al., 2013; Logan, 2010). The polarization curves depicted in Fig. 3 presents the voltage as a function of current density obtained during biofilm formation and reactor operation with resistances varying from 71.1 k Ω to 50 Ω . The results reveal a current density increase from 174 mA m⁻² during biofilm formation up to 665 mA m⁻² when in continuous operation.

The shape of the curves confirmed the prevalence of ohmic losses generated by one or several of the following factors: membrane resistance, wastewater electrolyte resistance and bacterial metabolism. In the present work, the initial slope of polarization curves, usually observed due to activation losses, were not observed. This revealed a mature biofilm in both cases, where direct electron transfer may predominate as well as interspecies electron transfer instead of mediated processes. The sharp final voltage drop, due to mass transfer limitations in the electrode surface, was evident in the polarization curve obtained during biofilm formation. During continuous operation, internal mass transfer limitations were not significant because the MFC was continuously fed with new substrate (Aelterman et al., 2006). However, at higher current densities, power disruption was noticed in the polarization curve that corresponds to overshoot of the system (Fig. 3). This phenomenon can provide some additional insights about biofilm electrical stability that discharges the electrons faster in the presence of lower external resistances (Mardanpour et al., 2012; Venkata Mohan et al., 2010). Nevertheless, this process is not well understood. Probably, biofilm stability might be compromised in continuous operation, being acclimation and biofilm development considered the probable causes (Logan, 2012). This limitation can be overcome through biofilm stabilization using a controlled electric potential during MFC biofilm growth phase and operation (Zhu et al., 2013). In addition, this fact might explain the efficiency loss in terms of voltage and the observed power oscillations (Fig. 2). Furthermore, the variation of output power with the external resistance can be attributed to polarization losses (i.e., activation, ohmic and concentration) across MFC (Logan et al., 2006). Thus, based on the slope of voltage vs. current density curve in the ohmic losses zone, it was also possible to verify that the internal resistance of the MFC, either in batch or in continuous flow, was very low, 15 Ω and 5 Ω , respectively. These values were lower than expected when compared with published results (55 Ω) (Mardanpour et al., 2012; Venkata Mohan et al., 2010), However, this reveals a good biofilm adhesion to the electrode surface and the predominance of direct electron transfer to the electrode. A further explanation for these low values of internal resistance might be the cathodic solution that was used in the present work. In MFCs using pH buffers, it was verified a proton transfer increase and an internal resistance reduction (Elakkiya and Matheswaran, 2013: Fan et al., 2007).

The maximum power density obtained with respect to the electrode area was 255 mW m $^{-2}$, corresponding to a current density of 665 mA m $^{-2}$ (external resistor = 80 Ω), and this was observed during continuous operation (Fig. 4). The maximum power density increased 5 times by changing the flow regime of the reactor, compared with the 51 mWm $^{-2}$ obtained in the power curve during the biofilm formation in batch mode. This result might be due to the high COD concentration of the influent and to the decrease of reaction by-products in anodic chamber that could inhibit bacterial activity. Indeed, the main disadvantages associated with batch MFCs are the substrate depletion and by-product toxicity (Ren et al., 2014b; Rahimnejad et al., 2011).

When comparing the maximum power densities during biofilm formation and continuous operation with the power densities obtained experimentally, it was possible to observe a significant correlation. When the MFC was operated in the batch mode during biofilm adhesion and formation, the maximum power density was 57 mW m⁻². This value was very similar to the one obtained in the power curve, 51 mW m⁻². During continuous operation with the dairy effluent and in the period immediately before the determination of the polarization and power curve, the observed power density was around 90 mW m⁻², but in the polarization curve, a maximum of 255 mW m⁻² was obtained. This result motivated the external resistance switch and contributed to a power density increase up to 228 mW m⁻². However, as described previously (section 3.1.), this value was not retained and started to decrease afterwards.

3.3. Organic matter processing

The MFC performance regarding the organic matter degradation expressed by COD removal is presented in Table 1.

In average, the MFC was able to reduce 1298 \pm 617 mg L^{-1} of COD at an hydraulic retention time of 8.4 h. COD removal rates were lower than other ones presented in MFC literature (Elakkiya and Matheswaran, 2013; Mardanpour et al., 2012; Velasquez-Orta et al., 2011; Venkata Mohan et al., 2010), probably due to shorter hydraulic retention time when compared to long fed-batch cycle times (12-72 h) (Ren et al., 2014a; Mardanpour et al., 2012). To date, COD removal rates in MFCs operated with dairy wastewater varied from 55 \pm 11% (average of the present study) up to 95% (Elakkiya and Matheswaran, 2013). In addition, during continuous operation, the coulombic efficiency average was around $10.5 \pm 10\%$ with the maximum of 24.2 \pm 1.5%. The high standard error of coulombic efficiency might be the result of substrate concentration oscillations, as well as the presence of different electron acceptors and reactor chamber designs (Hu, 2008). A higher organic load during initial phases of operation might also result in lower coulombic efficiency, mainly due to the organic matter utilization by other microbial processes towards growth and physiological balances generating other by-products (Butti et al., 2016; Venkata Mohan et al., 2010; Pant et al., 2010). The average energy production, expressed as kWh \mbox{kg}^{-1} COD removed, was 8.95×10^{-2} and the maximum value was obtained between the day 32 and 36, $20.53 \times 10^{-2} \text{ kWh kg}^{-1}$ COD. A comparison of type, inoculum and MFC performance fed with dairy wastewaters is presented in Table 2.

As can be seen in Table 2, up to now, the best results obtained with a MFC operated with dairy wastewater were achieved in a

 Table 1

 COD and coulombic efficiency values in feed and output.

Time elapsed after the start of operation in continuous/day	Feed COD/mg ${\rm L}^{-1}$	Output flow COD/mg ${\rm L}^{-1}$	COD removal/%	CE/%
5	2417 ± 148	1026 ± 60	58 ± 2	4.1 ± 0.1
8	1513 ± 148	922 ± 120	39 ± 8	11.3 ± 0.9
13	1920 ± 247	786 ± 123	59 ± 6	24.2 ± 1.5
20	3299 ± 25	1222 ± 175	63 ± 5	2.2 ± 0.1

(mean value \pm standard deviation).

Table 2Comparison of MFC performance using dairy wastewater as substrate.

MFC type	Inoculum	Operation mode	Maximum power density/W m ⁻³	CE/%	Reference
Dual chambered	Activated sludge; Municipal wastewater treatment plant	Continuous	5.1	24	This study
Dual chambered	Activated sludge; Dairy wastewater treatment plant	Fed-batch	2.6	17	Elakkiya and Matheswaran 2013
Single chambered	Anaerobic sludge; Industrial wastewater treatment plant	Fed-batch	1.1	14	Venkata Mohan et al., 2010
Single chambered	Activated sludge; Dairy wastewater treatment plant	Fed-batch	20.2	27	Mardanpour et al., 2012
Single chambered	Anaerobic sludge; Municipal wastewater treatment plant	Batch	0.4	3	Velasquez-Orta et al., 2011

single chambered MFC inoculated with aerobic activated sludge from a dairy wastewater treatment plant (Mardanpour et al., 2012). revealing the importance of inoculum selection and acclimation. The maximum power density and CE were both higher in Mardanpour et al. (2012), but those values were achieved under a fed-batch cycle of 72 h, contrasting to the lower HRT (8.4 h) obtained in the present study with a conventional MFC configuration. Regarding the coulombic efficiency, the values achieved in the reported fed-batch work were similar to the ones obtained in the present study. Nevertheless, the present work innovates because it was carried out in a continuous flow mode displaying higher energy conversion efficiency than the fed-batch and batch mode operation (Pasupuleti et al., 2015). Comparing the power densities, the present values are in the same range of previous works. Therefore, MFC could provide the appropriate level of treatment for effluent reuse in crop irrigation, namely in vulnerable water scarcity zones, as is the case in the Mediterranean area. In addition, MFC might also provide enough power for low-energy applications, namely real-time environmental monitoring (Peixoto et al., 2011).

4. Conclusions

Linear models of production and consumption are increasingly challenged. In this regard, the present study disclosed the MFC as a resource recovery technology in the dairy industry, combining wastewater treatment and off-grid electricity production. The following conclusions can be drawn from this research:

- 1) the maximum voltage obtained by the two chamber MFC during continuous operation was around 576 mV, corresponding to a power density of 1.9 W ${\rm m}^{-3}$;
- 2) the maximum power density was 246 mW m $^{-2}$ with an external resistance of 80 Ω ;
- 3) COD removal rates were 55 \pm 11%, corresponding to a removal of 1298 \pm 617 mg L⁻¹ COD, at a hydraulic retention time of 8.4 h;
- 4) the maximum columbic efficiency obtained by the present study was of 24.2 \pm 1.5%, corresponding to an energy production around 20.5 \times 10⁻² kW h kg⁻¹ COD removed.

MFC applications in the dairy industry require further optimization in order to increase power voltage and to reduce retention times. In spite of this, the MFC experimental results encourage a more ambitious agenda when is to be designed for by-products

reuse in the agro-food industry. Effective and low-cost construction materials and straightforward reactor designs, as well as power recovery by the development of more efficient voltage boosting circuits are among the main technological challenges that are to be addressed further for an eco-efficient wastewater treatment with decentralized energy production.

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