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Scratching and transplanting of electro-active biofilm in fruit peeling leachate by ultrasound: re-inoculation in new microbial fuel cell for enhancement of bio-energy production and organic matter detection

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Abstract

Objective An electro-active biofilm of Fruit Peeling (FP) leachate was formed onto the Carbon Felt (CF) bio-anode in a Microbial Fuel Cell (MFC), after functioning for a long time. The electro active-biofilm thus formed was then scratched by ultrasound and re-inoculated in a new leachate to be transplanted onto the bio-anode. This procedure allowed the microbial electron charge transfer and therefore the enhancement of the bio-energy production of the fuel cell.

Results By using the repetitive mechanical biofilm removal, re-suspension and electrochemically facilitated biofilm formation, the voltage was substantially increased. In effect, the voltage of the 1st G of biofilm, rose gradually and reached its maximum value of

65 mV after 10 days. Whilst the 2nd generation allowed to obtain the maximum voltage 276 mV and without any lag time. The DCO abatement using the 1st G biofilm was 68% greater than the 3rd G 26%. Besides, the electrochemical impedance spectroscopy characterization and cyclic voltammetry of bio-anode with 2nd G biofilm confirmed the ability of electro-active biofilm formation on a new support. The biofilm transplanted showed thus greater kinetic performance, with reduced lag time demonstrating the interest of the selection that took place during the formation of successive biofilms.

Conclusions Despite the transplantation of the electro-active biofilm onto the bio-anode, the MFC still

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produced relatively lower power output. Nevertheless, it has been tested successfully for monitoring and detecting the oxidation of sodium acetate substrate in the very wide concentration range 0.0025–35 g/l.

Keywords Carbon felt · Microbial fuel cell · Electroactive bio-film · Scratching-transplantation · Acetate detection

Abbreviations

CF	Carbon felt
CG	Carbon graphite
GS	Garden soil
GS-MFC	Garden soil microbial fuel cell
FP	Fruit peeling
FP-MFC	Fruit peeling microbial fuel cell
1st	First generation microbial fuel cell
G-MFC	
1st G	First generation biofilm
2nd G	Second generation biofilm (re-inoculated biofilm)
3rd G	Third generation biofilm (re-inoculated biofilm)
MFC	Microbial fuel cell
SS	Stainless steel
Gs	Generations

Introduction

Owing to preservation of environment, industrial wastewaters that contain a great deal of organic matter, require stronger treatment (Mathuriya and Sharma 2009). In general, managers of industrial units choose the promising low cost technology of Microbial Fuel Cells (MFCs), for the treatment of their effluents (Huang and Logan 2008). These innovative electrochemical devices, convert organic wastes into electricity by oxidation (Tingry et al. 2013).

A MFC consists of two compartments (anodic and cathodic) separated by a cation exchange membrane. In the anodic compartment, the micro-organisms form the biofilm that oxidises the organic matter, known as biocatalyst (Santoro et al. 2017). Whilst, the cathodic compartment uses metal or nano-particles catalysts such as platinum, nickel, etc..., to reduce oxygen. Moreover, these devices need rich environment of

great microbial diversity that can convert different organic compounds into sustainable and renewable energy.

Performance optimisation of MFC for wastes treatment and sustainable clean energy generation was investigated to make easier the commercialisation of this new technology (Sedighi et al. 2018). Amongst the innovative applications utilizing MFCs, we can cite the treatment of domestic wastewaters (Puig et al. 2011), the reduction of sludge produced by biological treatment (Kim et al. 2007), treatment of dairy industrial effluent (Manohar et al. 2008), and more recently biotreatment of vinasse (Ottoni et al. 2019), removal of salts and elimination of heavy metals traces from wastewater (Champavert et al. 2017; Yahiaoui et al. 2020).

Upon the oxidation of organic matter by biofilm (bio-catalyst), three mechanisms between the bacterial population and electrodes, can occur: Direct transfer using cytochromes (Holmes et al. 2004; Cheng et al. 2006; Fapetu et al. 2016); indirect transfer using mediators either exogen or endogen, such as dyes (Nam and Park 1999) and phenazines (Rabaey et al. 2004) and finally electron transfer by means of nanowires (Gorby et al. 2006).

The biofilm formation mechanisms in electro active microorganisms leading to improved electrocatalytic rates for applications in bioelectrochemical systems, were addressed (Angelaalincy et al. 2018). The screening of sediment and wastewater samples to be used as anolytes in a MFC for microbial electron transfer activity, was recently investigated (Aiyer et al. 2019). The inoculation of a reactor with a scraped-off biofilm collected from a running MFC, improved the electrochemical performance of the new biofilm with respect to that was used as inoculum (Cheng et al. 2011). The successive scratching and re-inoculation steps using lower polarisation potentials, showed an increase in current density using paper waste MFC (Ketep et al. 2013). The consecutive selection and acclimatization of wastewater inoculum based, mixed culture microbial biofilms demonstrated an alternative biofilm removal, re-suspension and electrochemically facilitated biofilm formation.

It was shown that the bio-electrocatalytic current density of the secondary biofilm formation was much greater than a primary biofilm in a MFC (Liu et al. 2008). Furthermore, it has been demonstrated that once a biofilm was formed on a given anode (1st G),

then was transferred to a new sterile leachate and made in contact with a new electrode support, it continued to grow allowing the formation of the 2nd G (Rivalland et al. 2015). Moreover, the biofilm transfer can be achieved by suspension of the support-electrode in buffered solution, handshaking and scraping of any remaining biofilm (Kim et al. 2014).

In view of this bibliography, we followed the scratching/transplanting method utilized by Ketep et al. (2013) using two different backgrounds: garden soil and natural fruit peeling leachates, for comparing and testing the advantage of biofilm re-inoculation. In effect, we tested the benefits of re-inoculation of bacterial biofilm formed on electrode surfaces using fuel cell device without prior polarization of bio-anode. The purpose of the present paper was to perform the scratching/re-inoculation of bacterial biofilm, for increasing bio-energy of MFC and oxidizing organic matter contained in wastewater. We characterized the three successive biofilm generations by Scanning Electronic Microscopy (SEM), Cyclic Voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS). Besides, despite its lower power output, we utilized this MFC for monitoring and detecting the oxidation of sodium acetate in a very wide concentration range. Finally, we assessed the current response of the MFC-based detector to various concentrations of substrate in the effluent.

Experimental

Inoculum and microbial fuel cell

Effluent samples were prepared from 500 g of Fruit Peelings (FP) mixed with potassium chloride solution 60 mM (conductivity = 8.85 mS cm⁻¹ and pH 4.8). Another leachate was prepared similarly using Garden Soil (GS) (conductivity = 11.72 mS cm⁻¹ and pH 5.2). They were used without further filtration as inoculum source for microbial reactor.

As illustrated by Fig. 1a, the MFC consisted of two glass spherical half-cells 75 mL, connected to each other by cationic exchange perfluorinated membrane (Nafion-117; Dupont, Ward Hill, MA, USA), 2 cm in diameter. The Carbone Felt (CF) and Stainless Steel (SS) sheet were used as bio-anode and cathode respectively. They were thus, placed inside the anolyte and catholyte of the MFC. The anolyte contained the

leachate fed continuously with sodium acetate solution (20 mM), whereas the catholyte contained potassium ferricyanide K₃[Fe(CN)₆] 20 mM. The top end of the anolyte was tightly sealed with paraffin film to reduce oxygen penetration. Owing to polarization and in order to render the biofilm electroactive, an external resistor (1000 Ω) to complete a closed external circuit loop and Copper wires were used to conduct electrons between electrodes. The electroactive biofilm was therefore formed on the surface of the electrode within 10 days and the evolution of the cell voltage with time, was then measured with the digital voltmeter (APRI-LIA M890C+) placed in parallel.

Scratching/transplanting biofilm

The aim of the present investigation was to select the electro-active bio-film and to eliminate the non-electroactive bacterial species that consumed the fuel uselessly without producing any valuable energy.

In our experiments, the successive biofilm were formed in the FP effluent which was sterilized to remove planktonic microorganisms. As a matter of fact, the primary biofilm was formed from FP leachate in running MFC. It was then scratched-off from the bioanode by ultrasound and used as inoculum in another MFC to form the secondary biofilm. Besides, in order to optimize the operating conditions for electroactive biofilm development, and stabilize the substrate, sterilized sodium acetate solution was added daily. Under this condition, the pH of the bulk was controlled by the evolution of the effluent itself. The experimental protocol was compared to others, for allowing the formation of the primary biofilm.

In reference to literature, we review below the previous methods so far utilized for scratching and transplanting of electro-active bio-film onto carbon carbon-based materials, which are as follows:

a-As suggested by Rivalland et al. (2015), the biofilm formed on an anode (in 1st G), it was then transferred to a new sterile leachate and made in contact with a new electrode support. It resulted in the growth of the 2nd G biofilm. The same procedure was followed to form the 3rd G biofilm generation.

b-As recommended by Kim et al. (2014), and Doyle et al. (2017), the biofilm transfer can be achieved by suspension of the support-electrode in buffered solution, handshaking and scraping of any remaining biofilm.

Fig. 1 **a** Scheme of MFC, **b** sonication method steps

c-As advised by Ketep et al. (2013) and Yates et al. (2017), the primary biofilm was scrapped-off from bio-anode using sonication in Ringer solution, then transferred into sterilized leachate to be re-inoculated onto a new electrode.

Accordingly, we adopted in the present work the method followed by Ketep et al. (2013). In effect, after forming the electro-active biofilm in the MFC, the electrode bearing this biofilm was subject to sonication suspended in Ringer's solution (30 ml) for 15 min in three replicates, 5 min each in order to preserve the bacterial cells already scratched. This makes it possible to recover more cells without exposing the unhooked cells to a long duration of sonication (Fig. 1b). The primary biofilm was formed onto CF bio-anode in MFC during more than ten days without supplying any electrical polarisation. The biofilms formed from the successive re-inoculations were then performed in identical experimental conditions.

The primary electroactive biofilm formed in 1st MFC, was detached from the initial inoculated electrode by ultrasound in 30 ml physiological sterilised Ringer solution. This volume was added to a new sterilized leachate to be inoculated and used as the anodic chamber for a new electrochemical cell called 2nd MFC. The primary biofilm were removed from the anode surface and used to inoculate a new electrochemical reactor filled with sterilized FP leachate. The new biofilm (i.e. 2nd G) was formed on the new sterilised carbon felt electrode: the same steps were followed to form 3rd G.

The scanning electron microscope JSM-6610LA was used to examine the anodic electrode surface

before the MFC experiment and after the biofilm re-formation.

Electrochemical characterisation

The cyclic voltammetry curves were recorded at scan rate 10 mV/s and electrochemical impedance spectroscopy diagrams were recorded in range (100 kHz to 100 mHz) using the potentiostat–galvanostat (PGZ-301). The temperature was maintained at 25 °C. The electrochemical glass cells of 80 mL were filled with FP leachate containing primary biofilm (1st G), secondary (2nd G) or tertiary biofilm (3rd G). The lid and the reactor-body were sealed with a clamping ring. The working electrodes were flat 1.27 cm in diameter and 5 cm in length CF (99%, Alfa Aesar) screwed onto 5 mm diameter, 1 cm long graphite rods that ensured the electrical connection. The new CF electrodes were pre-treated by soaking in hydrochloric acid 1.0 M overnight to eliminate possible metal ion contamination, 20 min rinsing with distilled water (10 times) then immersed in sterilized water 48 h and allowed to dry in an oven (50 °C) at least one hour. Auxiliary electrodes were stainless steel flat cleaned by sterilization in Pasteur oven (Heraeus INSTRUMENTS Vacutherm). Potentials were controlled and expressed versus a saturated silver chloride electrode. The working electrode (i.e. bio-anode) was kept as close as possible to the reference electrode in front of the platinum auxiliary electrode.

Results

Voltage evolution of MFCs

In previous investigations, the first 1st G of biofilm was obtained naturally by connecting directly the bio-anode to cathode in closed circuit configuration using an electric resistance (Huang et al. 2014; Cabezas et al. 2015). Accordingly, the same procedure was, therefore followed by us to obtain the 1st G of biofilm, where the voltage increased gradually and reached its maximum value of 65 mV after 10 days. As for the 2nd G, the voltage started with 150 mV without lag time and attained its highest value of 276 mV. However, the voltage of the 3rd G was lower than that of the 2nd G but was still higher than the 1st G.

By comparing the temporal progression (Fig. 2) of MFCs using the electrodes of the 1st and 2nd Gs, the following comments may be made:

- During the first period (0–100 h), the MFC of 2nd G allowed a starting voltage greater than 10 mV, right up the 1st hour, which explained the rapid formation of electroactive biofilm in the presence of the 1st G biofilm scratched (tested in sterile environment and electrode). Between 100 and 200 h, the improvement of the voltage of the 1st G MFC was noticed, due to the formation of the electroactive biofilm. After 200 h, the variations of the voltage of the two MFCs were identical which resulted in the stable formation of the electroactive biofilm on both electrodes.
- Throughout 10 days, the temporal variations of the three generations show that the FP-MFC of the 2nd

Fig. 2 Evolution voltage curves of 1st G MFC using: GS and FP leachates

G recorded an initial electric voltage higher than 5 mV and a rapid progression from the second operating day (Table 1). There was an increase of the cell voltage, upon the addition of the fuel. Really, the voltage was much higher, when the medium had been changed and the fuel had been added simultaneously.

Moreover, the two MFCs of 2nd G (GS and PF) have been compared between each other. For both MFCs, an initial voltage greater than 5 mV was observed with a rapid progression from the second operating day. In addition, during the voltage evolution, two antagonist behaviours were observed. The GS-MFC (1st G) started right up from the beginning, whilst the FP-MFC did so after 5 days. For the 2nd G (i.e. after biofilm transplantation), the opposite occurred, showing a remarkable difference in lag time of 2 days for GS-MFC (Fig. 3).

Primary, secondary and tertiary biofilms formed in MFC

Akoğlu (2020) have recently carried out an investigation on the biofilm formation obtained from lactic acid bacteria. The biofilm was utilized advantageously and beneficially as the starter culture, for the formation of the new biofilm. On this basis, our primary biofilm collected from a MFC prototype, was removed from the anode surface by ultrasound and was then used to inoculate a new electrochemical reactor filled of sterilized FP leachate. As shown in Fig. 4, the new biofilm (2nd G) was therefore formed on the new sterilized CF electrode. The initial lag period was in contrast reduced with respect to that obviously observed with the primary biofilm; the bioenergy performances were thus amply better. Consequently, the secondary biofilm was used to inoculate the third electrochemical reactor. The 3rd G showed a

Table 1 Values of voltage (mV) of FP-MFC

Duration (h)	Sterilized leachate	1st G	2nd G	3rd G
33	< 1	5	150	52.2
66	< 1	65	201.3	82
266	< 1	20	276	220

Fig. 3 Comparison between 2nd electricity generation GS and FP MFCs; **a** evolution curves and **b** bar-chart values

remarkable performance as well; it provided maximum power density about 21.77 mW m^{-2} .

In summary, forming the primary biofilm as inoculum led to the secondary biofilm that provided the significant power density which was four times higher than that given with the primary biofilm (Fig. 5). It was besides possible to form the tertiary biofilm from the secondary biofilm as inoculum. The power density yielded with the tertiary biofilm was slightly lower than that observed with the secondary one. These results are in quite good agreement with previous investigations in the improvement of bio-electro-catalytic performance of mixed culture biofilms by consecutive electrochemical selection. A comparison of primary and secondary biofilms

Fig. 4 Evolution voltage curves of FP-MFCs: 1st G; 2nd G and 3rd G

Fig. 5 Comparison between FP-MFCs of 1st and 2nd Gs; **a** Evolution curves and **b** Bar-chart values

revealed that the secondary biofilm usually exceeds the primary one (Liu et al. 2008).

Furthermore, as shown in Fig. 6, SEM images (magnification 50 μ m) confirm reformation of biofilms resulting from sonication of successive FP generations: a) pristine CF fibres, b) biofilm of 1st

G, c) biofilm of 2nd G, d) biofilm of 3rd G. Qualitatively, the scratching/transplanting of our biofilm by ultrasound seems to be in agreement with the experience described by Zhao et al. (2015), where it was shown that delaying polarization of carbon cloth bioanode, brought about biofilm architecture that was

Fig. 6 SEM images (magnification 50 μm) confirm reformation of biofilms resulting from sonication of successive generations: **a** pristine CF; **b** biofilm of 1st G; **c** biofilm of 2nd G; **d** biofilm of 3rd G

more heterogeneous and thinner as compared to homogenous and thick biofilm resulting in full polarization.

Electrochemical characterization

Impedance spectroscopy

The impedance spectra of the different MFCs are represented according to the Nyquist diagram. They are represented in Fig. 7 given below, for the different electrodes (pristine, 1st G, 2nd G and 3rd G). In general, their shapes are semi-circles which intersect the axis of the real at the two points R_e and R_{tc} corresponding to the electrolyte and transfer resistances respectively. Obviously, the variations of the curves are different, reflecting the duration of operation of the MFC, in the presence of the bacterial biofilm. However, the MFC without biofilm has the highest resistance, the pristine work electrode gives a relatively higher resistance, and finally that of the 3rd G has the lowest resistance due to the electro-activity of the bacterial biofilm where the electronic conduction is better. This theory is verified in the case of electrodes immersed in the juice solution of autumn peelings fruits. For GS leachate, the electrode carrying the biofilm of the 1st G has a higher resistance than the electrode of the 2nd G: it is assumed in this case that the electroactive biofilm has not taken enough time to rebuild itself for train the 2nd G. In order to make it clear, a zooming has been provided inside the

figure where the curves overlapped. The values of real impedance of the three generations and the pristine electrode are therefore in the following order:

$$Re(Z)_{3rd\ G} < Re(Z)_{2nd\ G} < Re(Z)_{1st\ G} < Re(Z)_{pristine}$$

As it is shown by the above inequalities, the thicker and fully developed the electroactive biofilm, the lower the charge transfer resistance was. The biofilm-free MFC yields the highest resistance, the sterile gives a relatively lower resistance, and finally the third generation leads to the lowest resistance due to the electro-activity of the bacterial biofilm where electronic conduction becomes much pronounced. Qualitatively, our results are comparable to those previously reported by Doyle et al. (2017), though they used polarization for the biofilm growth.

Cyclic voltammetry (CV)

The quality of the tertiary bio-anode can be assessed from CV curves recorded at day 10, when the current was maximal. The scan rate of 10 mV/s was consequently low enough to represent the stationary characteristics of the electrode. The voltammograms recorded in the absence of substrate confirmed that the current was due to acetate oxidation. The voltammograms (Fig. 8) of the sterile electrodes show a hysteresis of the electrodes before use, due to generation of capacitive currents. As a consequence of the increase of the interface capacitance with time, the

Fig. 7 **a** Impedance Nyquist diagram of different stages of bioanodes: pristine, 1st G and 2nd G of biofilm: GS. **b** Nyquist diagrams of bio-anodes inoculated with fruit peeling biofilms of:

Pristine/sterile leachate (black circle); 1st G (red circle); 2nd G and (blue inverted triangle); 3rd G (asterisk)

electron transfer also increases, as indicated by the voltammograms of the electrodes of 1st and 2nd Gs. These observations are in accordance with the voltammograms which give clearly the redox compounds

contained in the biofilms that can be addressed by the electrode (Fricke et al. 2008; Harnisch and Freguia 2012).

Fig. 8 Voltamograms of bio-anodes inoculated with FP biofilms of: Pristine/sterile leachate (black line); 1st G (red line); 2nd G (blue line); 3rd G (pink line)

Chemical oxygen demand (COD)

COD was determined for assessing the degradation of organic matter by MFC. In effect, its value for FP leachate before the treatment was about 466 mg L⁻¹. After 10 days, it decreased down to 147, 280 and 345 mg L⁻¹ for 1st, 2nd and 3rd G FP, respectively. The corresponding COD abatements were, therefore tabulated by using (Eq. 1). The values are summarized in Table 2. As it is illustrated, the COD abatement was thus in favour to 1st FP-MFC, while the energy production was better with 2nd and 3rd Gs (i.e. after transplanting). The organic matter degradation was higher with scratched biofilm from the 1st G because the initial leachate contained not only the direct contact electrode biofilm but also planktonic microorganisms that helped in the COD abatement. In agreement with the results obtained recently using the MFC for the treatment of vinasse where the inoculum concentration promoted a different variation in relation to the measures of COD and power density (Ottoni et al. 2019).

$$\text{COD abatement (\%)} = 100 \times (C_i - C_f^G) / C_i \quad (1)$$

where C_i and C_f^G are the initial and final COD respectively.

Table 2 Values of COD abatement of FP-MFC for the three generations

Generation	1st G	2nd G	3rd G
COD abatement (%)	68.45	39.91	25.96

Polarization and power characteristics of MFC

The characteristic curves of polarization and power of the MFC were used to evaluate the electrical performance of the cell under load. The polarization curve was obtained using different values of the resistance ranging between 10 Ω and 10 M Ω . As shown in Fig. 9, the polarization curve i.e. voltage versus current density presents three distinct regions. The first region represents the activation over voltage that results from the energy loss during the initiation of the Ox/Red reactions and electron transfer between the bacterial cell and the anodic surface. In the beginning, the MFC creates a short-circuit current density at the highest voltage. The second region shows an ohmic linear drop caused by the electrolyte. The third region displays a second over voltage that yields the maximum open-circuit current density due to the loss of concentration occurring during the diffusion phenomenon. On the other hand, the power curve i.e. power density versus current density provides the maximum energy that can be delivered by the MFC. The power density is obtained as the product of output voltage and current density. The power density versus current density provides a curve having a first ohmic linear part, which increases by reaching the maximum power point. It then falls as the current density increases. As investigated in previous work, the power density of another MFC increased in the same manner with increasing values of current density, and reached optimal values upon the addition of substrate (Zerrouki et al. 2018).

Fig. 9 Power density curves of MFCs using different concentrations of substrate (sodium acetate)

Discussions

Comparison between three successive bio-film generations

By using the scratched biofilm, it was possible to reform an electro-active biofilm on a new electrode in a sterilized medium. The comparison of the impedance spectra and cyclic voltamograms of the sterile electrode and the 2nd G biofilm electrode confirmed the ability of the electroactive biofilm reformation on a new support. Rivalland et al. (2015) reported that the electrochemical monitoring of biofilm generations collected from French Guiana mangrove sediments, revealed the bacterial selection occurring at the anode for three distinct generations. Indeed, the first biofilm generation produced a stable current density reaching about 18 A/m² while 2nd and 3rd Gs yielded current densities of about 10 A/m², making in evidence that the 1stG biofilm is more electroactive than the 2nd G biofilm.

Besides, the importance of refreshing the medium along with the addition of the fuel, was confirmed by recorded higher voltage peaks. Ketep et al. (2014) demonstrated that the bio-anodes formed upon the addition of acetate, led to the highest current densities (6 A/m²) but were then unable to oxidize the raw effluent efficiently (0.5 A/m²). In contrast, the bio-anodes formed without acetate addition, were fully able to oxidize the organic matter contained in the effluent, giving up to 4.5 A/m² in continuous mode. Bacterial communities showed less bacterial diversity for the acetate-fed bio-anodes compared to those formed in raw effluents. By using the biofilm off-hook, it was possible to reform an electro-active biofilm on a new sterilized electrode in a sterilized medium. Sedighi et al. (2018) investigated the relation between the power generation of MFC and the COD removal in optimised conditions. They concluded that optimising both parameters simultaneously, made them compromising and therefore reducing the MFC efficiency.

MFC-based bio-detector

MFC-based biosensors are considered to be the next generation bio-sensing technology for environmental monitoring (Sun et al. 2015), in particular the inclusion of microbial activity, test of biochemical demand and detection of toxicants (Yang et al. 2015).

Although small power output of MFC constraints its application for directly operating electrical devices, great progress has been achieved in its utilization as a biosensor for monitoring of water quality and detecting of air quality (Cui et al. 2019) as well as determination of biodegradable organics (Lorant et al. 2019). So, beyond the small energy production harvested, our MFC was used also to develop a bio-detector using a two-compartment cell. In the anode compartment, the biocatalyst was renewed for each sample analysis by substituting the old microbial consortium with an equal amount of fresh one. The sodium acetate substrate was utilized as a target for detection. The biofilm was involved to degrade the substrate by producing electrons to be used as source of electric energy.

During the first week, the biofilm was cultured and formed in the MFC inoculated with the initial concentration of substrate 35 g/l. At the end of the first experiment, the newly formed biofilm was well recovered as needed on the same electrode. It was then used to inoculate the new MFCs using disparate values of substrate concentrations i.e. 0.0025, 0.025 and 0.25 g/l spanning more than fifteen thousands of order, to ensure the low and high detection limits. These limits were investigated by using values of maxima of power density curves that highlight the performances of the MFC.

As it is observed in Fig. 9, the peak of the maximum power seems to be proportional to the concentration of substrate consumed by the bacterial consortium. Thus the MFC can play the role of biosensor used as an organic matter detector. Besides, in order to prove this detection, the variation of the current density delivered by the MFC versus the fuel concentration (sodium acetate) is plotted. Unfortunately, it is shown to be not quite linear, because the concentration range is too wide; values are of completely different orders of magnitude (35 g/l is 14 000 times higher than 0.0025 g/l). Hence, a logarithmic scale plot yields a smooth straight which passes approximately through all the data points. As shown in Fig. 10, the straight line highlights the linearity of the curve in logarithmic scale.

Furthermore, the MFC with replaceable consortium could be used as a biosensor for on-line motoring of organic matter as it has been previously highlighted by Cui et al. (2019) and Sumaraj and Ghangrekar (2014). As pointed out by Sun et al. (2019), more efforts are

Fig. 10 Current density versus logarithm of concentration of substrate: a typical characteristic of biosensor

needed in the future to accelerate the response of the biosensor. Following this recommendation, our MFC-based detector may be accordingly successful for the fulfilment of this requirement. This interesting biological electric device has been tested using the micro-organism from waste media, and used for detecting organic matter detection, spanning the concentration range 0.0025–35 g/l. It has, therefore, detected the lowest concentration of substrate (i.e. 0.0025 g/l) achieving the lowest current density 0.0011 mA/cm². The higher the concentration, the more the output current that is directly proportional to the logarithm of the value of the input concentration (i.e. 0.11 mA/cm² at 35 g/l), hundred times greater. Moreover, according to Sun et al. (2019), the MFC-based biosensor for selective monitoring of acetate during anaerobic digestion, operated in a closed loop where the increase in acetate concentration at the anode was a function of the electric field generated by the MFC. The resulted electric current obviously increased linearly in the restricted concentration range of sodium acetate, less than 20 mM (i.e. ≤ 1.65 g/l). However, in our case, the current density was produced just by injecting the substrate into the anode compartment. It was really proportional to the logarithm of sodium acetate concentration. As a consequence, the MFC was able to detect wider concentration range of sodium acetate (i.e. 0.0025–35 g/l). Our MFC could therefore be used analogously in very wide concentration range for detecting of organic matter, as well as a radio frequency logarithmic detector power meter.

Conclusions

Taking our results into account, we can conclude that the MFC technology is highly promising for the

treatment of wastewater and the production of low bioenergy using two distinct inocula: Garden Soil and Fruit Peeling. Furthermore, in the present investigation, we have demonstrated the possibility to improve the energy production of a MFC by using different biofilm generations. The scratching of the biofilm from the electrode was achieved by ultrasound. Its re-inoculation into a new leachate enhanced the energy performance of the MFC using the 2ndG biofilm. These results were confirmed by the impedance spectra and the voltammograms. In addition, the COD abatement was thus in favour to 1stG-MFC, however, the energy production was better with 2nd & 3rd Gs, after biofilm transplantation. Besides, the linearity of maximum current density versus concentration of substrate on logarithmic scale makes in evidence that the MFC could be used as a biosensor for the detection of the waste organic matter. It can be widely used in very wider concentration range detection for monitoring oxidation of matter, as it is the case of logarithmic detector power meter for detecting radio frequency signals.

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