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MICROBIAL FUEL CELLS AS AN ALTERNATIVE FOR THE TREATMENT OF WASTEWATER IN CONNECTION WITH ENERGY GENERATION

CÉLULAS COMBUSTÍVEIS MICROBIANAS COMO ALTERNATIVA PARA O TRATAMENTO DE ÁGUAS RESIDUAIS RELACIONADAS À GERAÇÃO DE ENERGIA

PILAS DE COMBUSTIBLE MICROBIANAS COMO ALTERNATIVA PARA EL TRATAMIENTO DE AGUAS RESIDUALES RELACIONADAS CON LA GENERACIÓN DE ENERGÍA

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RESUMO

As preocupações com a proteção ambiental aumentaram nos últimos anos, levando à procura por novas fontes de energia renováveis para minimizar os danos antropológicos à atmosfera e aos corpos hídricos. As Células Combustíveis Microbianas estão inseridas nesse contexto, pois podem reduzir a carga orgânica de um efluente concomitantemente à produção de bioeletricidade. Este estudo investigou três diferentes fontes de microrganismos, avaliando parâmetros como concentração da fonte de carbono e temperatura na eficiência energética. A corrente elétrica gerada pela atividade microbiana na oxidação da matéria orgânica foi monitorada adicionalmente com a condutividade iônica e o pH do meio. A Demanda Química de Oxigênio também foi determinada para avaliação da remoção de matéria orgânica. A Célula Combustível Microbiana inoculada com Lodo Ativado apresentou maior corrente elétrica em comparação a outros estudos da literatura e foi observada alta influência da condutividade na eficiência do sistema. Também foi avaliada a atuação da temperatura na eficiência energética, sendo observados melhores resultados nas condições de 36 °C. Como conclusão, as células a combustível microbianas podem operar tanto em 4,0 gL^{-1} (DQO mgO_2 3886,728) quanto em 3,0 gL^{-1} (DQO mgO_2 3124,573).

Palavras-chave: Células Combustível Microbianas (CCM). Bioeletroquímica. Eficiência energética. Demanda Química de Oxigênio (DQO).

ABSTRACT

Concerns over environmental protection have increased in recent years, leading to a search for new renewable energy sources for minimizing anthropological damage to both atmosphere and water bodies. Microbial Fuel Cells are inserted in such a context, since they can reduce the organic load of an effluent concomitantly with the production of bioelectricity. This study investigated three different sources of microorganisms, evaluating parameters such as carbon source concentration and temperature in energy efficiency. The electrical current generated by microbial activity in the oxidation of organic matter was monitored additionally with ionic conductivity and pH of the medium. Chemical Oxygen Demand was also determined towards an evaluation of the removal of organic matter. The Microbial Fuel Cell inoculated with Activated Sludge showed higher electrical current in comparison to other studies from the literature and high conductivity was observed to have a significant influence on system efficiency. The effect of temperature on energy efficiency was also evaluated, with better results observed at 36°C. As a conclusion, Microbial Fuel Cells can operate at both 4.0 gL^{-1} (COD mgO_2 3886.728) and 3.0 gL^{-1} (COD mgO_2 3124.573).

Keywords*:* Microbial Fuel Cells (MFC). Bioelectrochemistry. Energy Efficiency. Chemical Oxygen Demand (COD).

RESUMEN

La preocupación por la protección del medio ambiente ha aumentado en los últimos años, lo que ha llevado a la búsqueda de nuevas fuentes de energía renovables para minimizar el daño antropológico tanto a la atmósfera como a los cuerpos de agua. Las pilas de combustible microbianas se insertan en tal contexto, ya que pueden reducir la carga orgánica de un efluente concomitantemente con la producción de bioelectricidad. Este estudio investigó tres fuentes diferentes de microorganismos, evaluando parámetros como la concentración de la fuente de carbono y la temperatura en la eficiencia energética. Además, se monitoreó la corriente eléctrica generada por la actividad microbiana en la oxidación de la materia orgánica con la conductividad iónica y el pH del medio. También se determinó la Demanda Química de Oxígeno para una evaluación de la remoción de materia orgánica. La Celda de Combustible Microbiana inoculada con Lodos Activados mostró mayor corriente eléctrica en comparación con otros estudios de la literatura y se observó una alta influencia de la conductividad en la eficiencia del sistema. También se evaluó el efecto de la temperatura en la eficiencia energética, con mejores resultados observados a 36°C. Como conclusión, las pilas de combustible microbianas pueden funcionar tanto a 4,0 gL^{-1} (DQO mgO_2 3886,728) como a 3,0 gL^{-1} (DQO mgO_2 3124,573).

Descriptores*:* Pilas de Combustible Microbianas (CCM). Bioelectroquímica. Eficiencia energética. Demanda Química de Oxígeno (DQO).

INTRODUCTION

Most energy sources currently used derive from fossil fuels, which are responsible mainly for the production of greenhouse gases (Singh et al., 2019). Therefore, concerns over the finding of new renewable sources of energy have substantially increased. Another obstacle faced in the 21st century is water scarcity, aggravated by population growth and industrialization, since human activities produce effluents that, when drained into water bodies without adequate treatments, can contaminate them and even cause eutrophication (Zhou et al., 2020; Priya et al., 2021). The conventional wastewater treatment consumes large amounts of energy, thus emitting high quantities of greenhouse gases (Singh et al., 2019). The process also requires the removal of nutrients, such as phosphorus (P) and nitrogen (N),

which demands biological treatments. Similar problems can be overcome by the use of Microbial Fuel Cells (MFC), since they generate bioelectricity while effluent is being treated. An MFC can be defined as a bioelectrochemical system that uses microorganisms to catalyze the oxidation of organic matter, thus converting chemical energy into electrical one. The process is an alternative to eliminating part of the effluents and wastes released to the environment.

Regarding energy production, MFC operates as a system with an anode and a cathode compartment separated by a polymeric membrane permeable only to protons (H+). Therefore, anaerobic microorganisms oxidize organic matter at the anode, whereas the cathode works aerobically, requiring the presence of O_2 or another electron acceptor. On the other hand, the polymeric membrane prevents the transfer of those electron acceptors (e^-A) to the other compartment (Rachinski et al., 2010; Logan et al., 2008).

MFC functions basically through the oxidation of the organic substrate at the anode, generating CO_2 and intermediate compounds, which will depend on the substrate used and the microbial community. The process results in the production of electrons (e^-) and protons (H^+) (reaction 1). Electrical energy is generated from the passage of those electrons through an external circuit, before they reach their e^-A. Protons migrate to the cathode through the membrane (Nafion® membrane is the one generally used) and, together with the electrons that travel through the external circuit, reduce the oxygen to water on the electrode surface (reaction 2). At the end of the oxidation-reduction reaction, CO_2 is released at the anode and H_2O is released at the cathode, according to reaction 3, and energy is formed (Rachinski et al., 2010; Logan et al., 2008). An example of such a reaction is the oxidation of a carbohydrate (e.g., glucose, which generates electrons and protons due to the oxidative process, according to the reactions:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24e^- + 24H^+ \quad (1)$$

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \quad (2)$$

$$C_6H_{12}O_6 + O_2 \rightarrow 6CO_2 + 2H_2O \quad (3)$$

MFC efficiency is still considerably low; therefore, it must be improved towards a larger energy production. Temperature is a fundamental parameter, since the bacterial kinetics and reactions necessary for the system to function depend on it (Hori, 2015). Therefore, the optimal temperature for a higher efficiency will depend on the species of microorganism used – in general, it ranges between 25 and 40 ^oC, since most species are mesophiles. pH is also crucial for the growth of microorganisms; it is associated with the functioning of enzymes, affecting metabolism and microbial growth, as well as the transfer of protons (Hori, 2015). Its value must be close to 7.0, obtained with the use of buffers (e.g., phosphate buffer). Passos et al. (2014) reported microorganisms produced several organic acids, decreasing the pH of the reactor and hindering cell growth in certain glucose concentrations.

Many factors, including types of microorganisms and substrates, electrode materials, pH, temperature, Hydraulic Retention Time (HRT), MFC geometry, and use of catalysts on the cathode can interfere with the functioning of an MFC (Slate, 2019). Penteado et al. (2016) demonstrated the influence of the relationship between nutrients and Chemical Oxygen Demand (COD) on the treatment of wastewater from the wine industry. They observed P and N supplementation in that effluent was not efficient for removing COD, probably due to the presence of recalcitrant materials, whose biodegradation is difficult (Penteado et al, 2016). The principle of the method for determining COD consists of the chemical oxidation of organic matter, obtained by a strong oxidant - potassium dichromate ($K_2Cr_2O_7$), in this case - in an acidic medium at high temperatures, where the organic matter is converted into CO_2 and H_2O during the COD process.

MATERIALS AND METHODS

The experimental procedure, divided into two steps, is described in what follows.

1. SCC - Single Chamber Cell

The experiments began with the development of a simpler system for facilitating the understanding of previously studied data. The first MFC system adopted was a glass container with both anode and cathode in the same compartment (SCC) and connected by a 100 Ω external resistance. Stainless steel wire was used as a cathode electrode in the form of a spiral, whereas the anode was formed by seven fractions of carbon fabric of close to 1.5 x 1.5 cm dimensions for increasing the area where microorganisms are located and enabling a greater biofilm formation. The carbon fabric was subjected to a pre-treatment, described in the cathode electrode section below.

After the assembly of the SCC, Dacar sludge (from the treatment of wastewater from a poultry slaughterhouse, Tietê - SP) previously macerated and a 1.0 g L^{-1} sucrose solution in a 1:9 ratio (sludge/food) were added for a 0.2 L total volume. N_2 gas was then bubbled for 15 minutes in the cell for the removal of O_2 so that the chamber would become anaerobic. The circuit was closed by a resistance and the system was placed in a bath for control of temperature at 34ºC. The process was conducted in a single batch and the electrical current was checked daily for a week.

2. DCC - Dual Chamber Cell

The main part of this work consisted in assembling three H-shaped Double Chamber Cells. Two were kindly donated by prof. Dr. José J. Linares from the Chemistry Institute at UNB – one was made of glass and the other was made of Polyvinyl Chloride, PVC, which was replicated in the

Electrochemistry and Chemistry Materials Laboratories in UFT - Gurupi. The Microbial Fuel Cell used for the experiments three sludge types (Activated Sludge and Anaerobic Bacterium, both from Pará state; and Municipal Slaughterhouse from Gurupi – Tocantins state). These MFC systems were in H-type, being that these two-chamber, anode and cathode, separated by proton exchange Nafion® membrane 117. The membrane pre-treatment and the development of the catalytic layer applied to the cathode were also conducted with the help of UnB and according to the methodology described in what follows.

Nafion® ***Membrane treatment:*** The Nafion® membrane was submerged in a 3.0% v/v hydrogen peroxide (H_2O_2) solution for one hour after boiling had begun and subsequently washed in deionized water at 95 °C for 1h. It was then submersed in a 0.5 M sulfuric acid (H_2SO_4) solution for one hour after it had started to boil and washed as in the previous step.

Cathode Electrode: Catalytic layers containing a 0.5 $mgPt.cm^{-2}$ charge were prepared and applied to the carbon fabric (area = 9.0 cm^2). One was obtained with Pt/C 20% and the other was provided with Pt/C 30%; both were anchored to a Pt plate electrode of each MFC. The third layer employed a Pt plate electrode without carbon fabric and catalytic layer. Its use required a pre-treatment, which consisted of its immersion in phosphate buffer (pH 7) at 65ºC for 45 minutes. The buffer was prepared with 0.5 M phosphoric acid (H_3PO_4) and addition of monobasic sodium phosphate (NaH_2PO_4) until the desired pH, monitored by a PH-5000 pH meter. The fabric was washed again with isopropyl alcohol. The Pt/C catalyst layer was deposited on a carbon fabric surface through the addition of 5.0% Nafion® and isopropyl alcohol (quantities provided in Table 1). The solution was homogenized in an ultrasound bath for 30 minutes and then dried in an oven at 90ºC for 3 h.

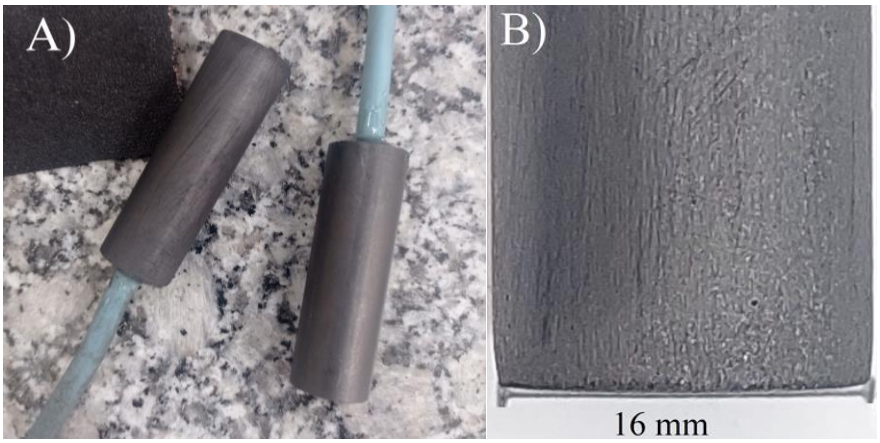
Table 1 - Reagents used for the production of the catalytic layer.

	20%Pt/C	**30%Pt/C**
Catalyst (mg)	22.5	15.0
Nafion® 5% (µL)	57.6	38.4
Isopropyl alcohol (mL)	1.0	1.0

Source: Authors (2023).

Anodic Electrode: The anodic material was graphite with cylindrical configuration (geometric area of 25 cm^2), where it was sanded with sandpaper for increasing the surface roughness and favoring anchorage, thus facilitating the adhesion of microorganisms present in the sludge (see Fig. 1).

Figure 1 - (A) Left: sanded graphite electrode; right: non sanded graphite electrode (B). Magnification: 10 X.



Source: Authors (2023).

Electrolyte Solutions used in MFC in two compartments: both composition and concentration of the synthetic wastewater in the buffered medium and phosphate buffer solutions used in the cathode and anode compartments are listed in Table 2.

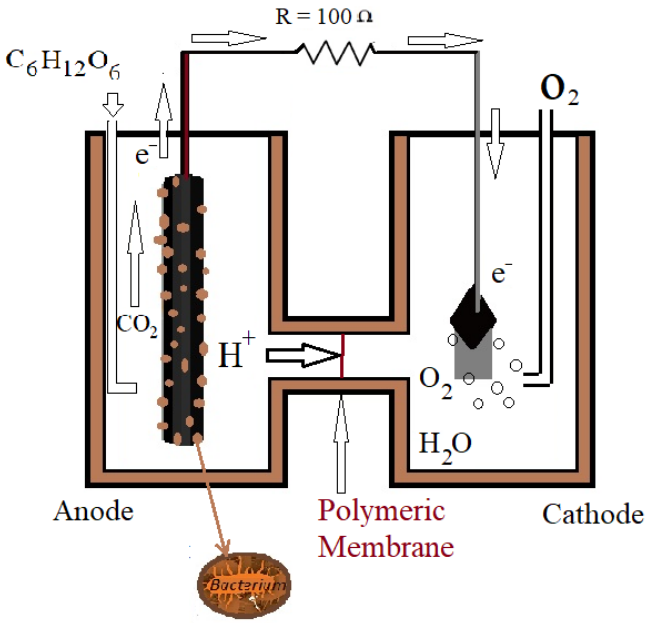
Table 2 - Composition and concentration [C] of the synthetic wastewater in a buffered medium and phosphate buffer solutions used in the cathode compartment.

Synthetic Food								
Component	Sucrose	$(NH_4)_2SO_4$	$NaHCO_3$	$MgCl_2$	$CaCl_2$	$ZnSO_4$	$(NH_4)_2Fe(SO_4)_2$	KH_2PO_4
[C] (g L^{-1})	4.0-3.0	0.761	1.061	0.358	0.321	0.049	0.112	0.441
Phosphate Buffer								
Component	NaH_2PO_4				Na_2HPO_4			
[C] (g L^{-1})	3.67				2.75			

Source: Authors (2023).

Assembly of MFC: the anode compartment of the two-chamber MFCs was separated from the cathode one by a previously treated Nafion® polymer membrane permeable only to protons, preventing the passage of other compounds between the parts. An external connection was then made with stainless steel wires for the passage of electrons between the electrodes and a 100Ω resistance enabled the calculation of the voltage produced by the system, from the current measured, by Ohm's law (Logan, 2008). After the assembly of one the MFCs, the electrodes were in contact with those solutions in an open circuit for 24 hours, starting the MFC operation. Fig. 2 illustrates the H-type MFC system.

Figure 2 - Schematic representation of the H-type MFC system.



Source: Authors (2023).

One day after the assembly was the period necessary for the microorganisms to acclimatize and the external circuit was then closed. In the first attempt, a problem occurred in the preparation of the phosphate buffer from H_3PO_4 and with the addition of NaH_2PO_4, leading to an error in the pH value (close to 2), noticed only on the 2nd day. After three days, all MFC had been assembled; however, monobasic sodium phosphate and dibasic sodium phosphate salts were used at the concentrations shown in Table 3, which also provides the specifications of the synthetic food used as food. The buffer at pH 6.7 was used too to control the pH in the food, together with sodium bicarbonate ($NaHCO_3$), and allow good movement of protons.

The system was operated in a semi-continuous process and 15 mL of the total anode volume were removed and fed with the same volume of synthetic food. Before and after feeding, the pH was checked and, when necessary, adjusted to 6.8 (± 0.3) and oxygen was bubbled into the cathode compartment for ensuring aerobic conditions for the oxygen reduction reaction. The temperature of the MFCs was kept constant at 27 °C. During the study, the concentration of the carbon source in the food was varied, as shown in Table 3 (the sucrose concentrations used were 4.0 and 3.0 g L^{-1}); therefore, the hydraulic retention time was 13.3 days throughout the experiment.

Table 3 - Type of microorganism sources used Microbial Fuel Cell.

		Anode		Cathode	
MFC	**Sludge type**	**Sludge (mL)**	**Food (mL)**	**Phosphate Buffer (mL)**	**Electrode**
1 – AS	Activated Sludge - PA	40	160	200	Pt/C 20%
2 – MS	Municipal Slaughterhouse - TO	40	160	200	without Pt/C
3 – AB	Anaerobic Bacterium - PA	40	160	200	Pt/C 30%

Source: Authors (2023).

Measurements of Parameters

i) Electric current

The electrical current (*i*) of the MFC experiment was monitored daily for 45 days by an ET-1002 Minipa digital multimeter. However, only MFC - AS was maintained until the end of the experiment - MFC - MS and MFC - AB were dismantled on the 34th and 18th days, respectively.

ii) pH

Both pH and current were monitored daily during the 45 days of the experiment. Part of the 15 mL of the sample taken before feeding was used and measured by a pH-5000 pH meter.

iii) Electrical Conductivity

Conductivity was monitored daily by an MCA 150 TECNOPON conductivity meter similarly to the measurement of pH.

iv) Chemical Oxygen Demand - COD

Addition of solutions and sample to COD: a 2.5 mL aliquot of the sample was placed into a COD tube by a pipette and 3.5 mL of a sulfuric acid + silver sulfate solution and 1.5 mL of a digestion solution (potassium dichromate + mercuric sulfate) were then added.

During the preparation of the blank, 2.5 mL of distilled water shook it well for complete homogenization. Dilutions were necessary for food and samples sucrose concentrations of 3.0 or 4.0 $g.L^{-1}$ so that the value did not exceed 1.0 $g.L^{-1}$. After measurement in a spectrophotometer, the absorbance value was multiplied by the dilution factor.

v) Sample digestion

The digester equipment was turned on until reaching the desired temperature (150 °C). The COD tubes were then introduced and the operating time of the biodigester was set to two hours. After room temperature had been reached, the tubes were positioned in a place protected from light for the reading of the spectrophotometer.

vi) Spectrophotometer

The COD calibration curve was built on the spectrophotometer and the reading was conducted in absorbance at 610 nm wavelength for all tubes, always zeroing the equipment with the digested distilled water sample (blank). The calibration curve for potassium biphthalate (at 100, 200, 300, 500 and 1000 mg L^{-1} concentrations) was generated, obtaining the following equation:

$$[\text{COD mgO}_2] = \frac{\text{ABS} + 3{,}78 \times 10^{-3}}{7{,}61 \times 10^{-2}}$$

where

[COD mgO_2] – sample COD value;

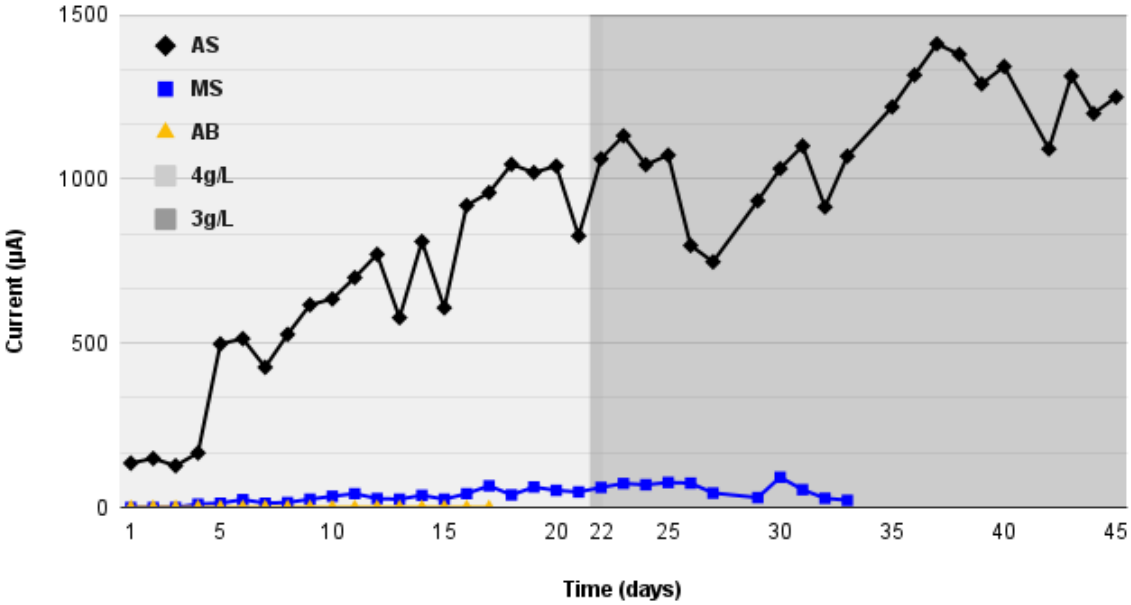
ABS – Absorbance read on the spectrophotometer at 610 nm wavelength.

RESULTS AND DISCUSSION

The electrical current (*i*) for SCC was monitored every day for approximately a week, obtaining an i = 2.1 μA low average, as expected, since both electrodes were in the same compartment, causing the medium's own e^-A to compete with the anode, thus losing part of the electron flow. No adequate microbial growth was observed because the food met only the need of Carbon (C) requirement, while Nitrogen (N) and Phosphorus (P) are strictly necessary for cell growth. The lack of important metals for various cellular functions also hampered the functioning and growth of the microorganisms present. Such problems were resolved in the feed for the other MFCs H-type. The current (*i*) in the MFC experiments was monitored daily for 45 days, leading to the results shown in Fig. 3, which also describes the variation in food concentration during the study.

Synthetic food is a source of nutrients produced in the laboratory for studies of the effects of macro and micronutrients on the development of the system. Despite being interesting for bench studies, synthetic food does not match the real effluent, since it has complex components – recalcitrant ones, in many cases - thus hampering the degradation of microorganisms (Khandaker et al., 2021). Differently from compounds on a laboratory scale, the concentrations of the aforementioned ones are not standardized, causing a variation in energy efficiency and removal of real organic matter. This fact could be observed in the experiment between the 26^{th} and 28^{th} days, i.e., the energy production dropped due to contamination in the food, which may have changed its properties. A possible presence of other microorganisms may have contributed to the reduction because of competition for nutrients from the environment with electrogenic beings.

Figure 3 - Electric current generation as a function of number of days at different food concentrations.



Source: Authors (2023).

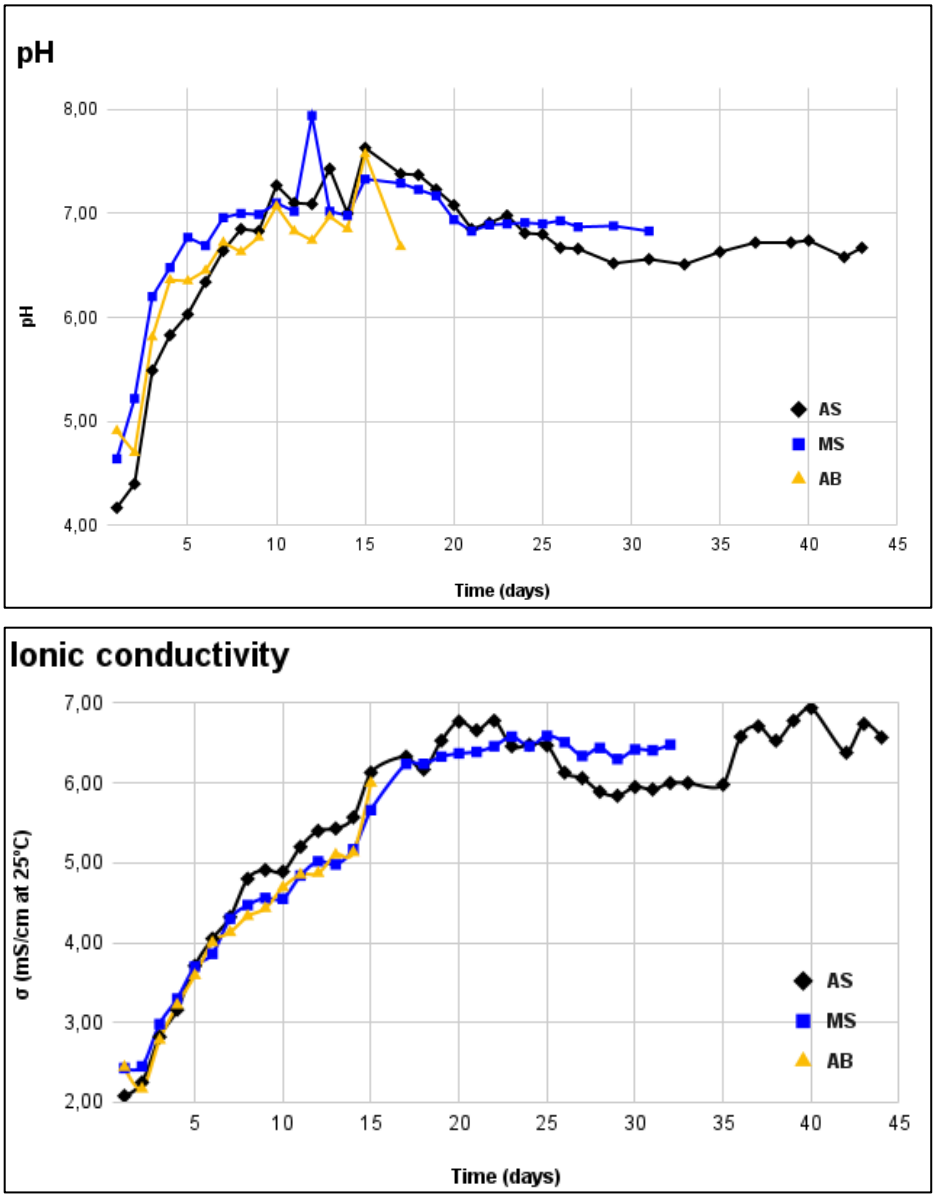
According to Fig. 3, there was a desirable energy production at both food concentrations, with peaks of 1,045 µA and 1,132 µA at concentrations 4.0 g L^{-1} (COD mgO_2 3886.728) and 3.0 g L^{-1} (COD mgO_2 3124.573), respectively. The COD present in the aliquots removed could not be measured, since the analysis method adopted was adjusted for high concentrations. However, the literature reports estimates of 92.0% to 94.4% removal of COD at room temperature (Penteado , 2016; Ye et al., 2020) using HRT of one day or less. The present work evaluated a HRT of 13.3 days, which may imply an increase in COD removal efficiency, given that, in this way, there is a load rate minor organic (Santos et al., 2016).

This study also evaluated the effect of temperature variation on the energy efficiency of MFC. Room temperature was maintained (28±1 °C) during most of the process; however, it was increased to 36±1 °C on the 34th day. Consequently, with a higher temperature, the Activated Sludge MFC obtained a greater current production, reaching a peak of 1,412 µA, which was the highest throughout the study (Fig. 3).

The use of different sludge enabled verifying the efficiency of energy production is completely dependent on the source of microorganism, since each MFC showed a substantially different current production under the same conditions. As displayed in Figure 2, the MFC inoculated with Activated Sludge had the highest energy generation, highlighting a greater community of electrogenic microorganisms. Moreover, a rich microbial community metabolizes different types of compounds more effectively, since it has more metabolic pathways capable of degrading complex substances. On the other hand, the MFC of sludge from the municipal slaughterhouse showed lower current numbers, for such a material tends to be treated with microbial control mechanisms, thus resulting in a less rich

microbiological population, but still with electrogenic species. Finally, the system containing the Anaerobic Bacteria source did not obtain an electric current, indicating those microorganisms cannot transfer electrons and are probably fermentative.

Figure 4 - Conductivity and pH as a function of time in days results for the three MFCs.

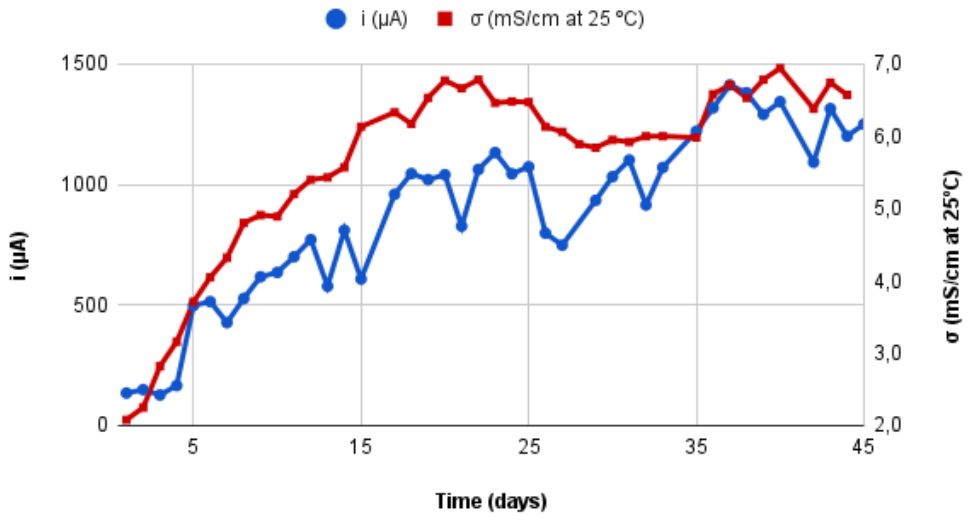


Source: Authors (2023).

Conductivity and pH were also measured during the experiment (see Fig. 4 for the results). Both variables exhibited a similar behavior, with an increase in the first days and a more stable behavior from the middle of the day experiment. Such a similarity is due to the use of phosphate buffer, which can maintain the pH and obtain good conductivity. This can be seen mainly during the first 10 days, since, at this point, the buffer concentration was adjusted to the value displayed in Table 2. Furthermore, the pH showed a 6.64 average value in MFC-AS and 6.68 and 6.46 in MFC-SM and AB, respectively. Those values are within the range in which most living beings live and perform their metabolic functions at their maximum, since pH is important for the functioning of enzymes and proteins. However, during the first assembly, pH dropped drastically to 2 and MFC-AS managed to obtain a small amount of current, highlighting the presence of acidophilic organisms. In contrast, the other microbial cells obtained no current under this condition.

Another factor to be reported is the effect of the electrolytic medium conductivity on the energy efficiency, more easily observed at MFC-AS (Fig. 5). Therefore, when the synthetic food correction was conducted for the phosphate buffer in the first 10 days, conductivity, hence, production of electrical current considerably increased. Furthermore, the increase in current accompanied the increase in conductivity throughout the experiment, since an increase in ionic conductivity expresses an increase in the concentration of ions, such as H^+, reducing internal resistance and ohmic losses due to greater ease of charge transfer (Penteado, 2016).

Figure 5 - Current and conductivity behavior for MFC-AS.



Source: Authors (2023).

CONCLUSIONS

This study showed MFCs are an alternative for energy production concomitantly with effluent treatment, functioning well at both 4.0 g L^{-1} (COD mgO_2 3886.728) and 3.0 g L^{-1} (COD mgO_2 3124.573). Although the level of organic matter removal could not be measured, studies have confirmed its good efficiency. The sources of microorganisms fully influence the level of energy production, since a rich community of electrogenic microorganisms is required. Activated Sludge used as a microorganisms source in MFC promoted a greater current production at both concentrations of synthetic food and temperatures investigated. The highest current peak occurred in the cell with 36 ºC±1 temperature and food at 3.0 g L^{-1}, reaching 1,412 µA (~1.5 mA). Furthermore, ionic conductivity showed an important factor for energy efficiency, facilitating charge transfer.

Finally, MFCs are a good system for reducing energy expenditure in effluent treatment and can serve as a pre-treatment of conventional treatment systems, or even treat effluents from remote locations not reached by the conventional treatment.

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