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Scientific Paper

Dual-chamber microbial fuel cell with denitrifying biocathode for the treatment of cassava processing wastewater

Célula a combustível microbiana de dupla câmara com biocátodo desnitrificante para o tratamento de águas residuárias do processamento da mandioca

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ABSTRACT

The aim of this study was to assess the feasibility of using cassava wastewater as a substrate for dual-chamber microbial fuel cells (MFCs) operating with denitrifying biocathodes. Two configurations related to the ion exchange membrane used were evaluated: one with an anion exchange membrane (MFC-A) and the other with a cation exchange membrane (MFC-C). Both bioreactors were operated in sequential batch mode. Furthermore, a low-cost platform based on Arduino technology was also proposed to enable continuous measurement and recording of voltage data from the MFCs. The highest voltage values were observed in the first days of MFC operation, with readings reaching approximately 350 mV (0.41 W·m ^{3}) and gradually decreasing after 100 days of operation to 243 mV (0.20 W·m ^{3}) and 125 mV (0.05 W·m ^{3}) for the MFC-A and MFC-C, respectively (mean values for the last 20 days of operation). In both MFCs, the chemical oxygen demand reduction and nitrogen removal were over 98% after reactor stabilization, with no noticeable nitrite accumulation. The experimental results indicated superior performance when MFC was equipped with an anion exchange membrane. The results presented here demonstrate the feasibility of using cassava wastewater as a viable substrate for MFCs equipped with a denitrifying biocathode, allowing for efficient wastewater treatment and simultaneous electricity generation.

Keywords: microbial fuel cell; autotrophic denitrifying biocathode; cassava processing wastewater; arduino differential voltmeter; ion exchange membrane.

RESUMO

O objetivo deste estudo foi avaliar a viabilidade de utilizar água residuária do processamento da mandioca (manipueira) como substrato para operação de células a combustível microbianas de câmara dupla (MFCs) equipadas com biocátodos desnitrificantes. Duas configurações relacionadas à membrana de troca iônica utilizada foram avaliadas: uma com uma membrana de troca aniônica (MFC-A) e outra com uma membrana de troca de catiônica (MFC-C). Ambos os biorreatores foram operados em modo de batelada sequencial. Além disso, uma plataforma de baixo custo baseada na tecnologia Arduino foi proposta para permitir a medição e o registro contínuo de dados de potencial elétrico das MFCs. Os valores de potencial elétrico mais altos foram observados nos primeiros dias de operação das MFCs, com leituras atingindo aproximadamente 350 mV (0,41 W·m^{-3}) e diminuindo gradualmente após 100 dias de operação para 243 mV (0,20 W·m^{-3}) e 125 mV (0,05 W·m^{-3}) para a MFC-A e a MFC-C, respectivamente (valores médios para os últimos 20 dias de operação). Em ambas as MFCs, a redução da demanda química de oxigênio (DQO) e a remoção de nitrogênio foram superiores a 98% após a estabilização do reator, sem acúmulo perceptível de nitrito. Os resultados experimentais indicaram um desempenho superior quando a MFC estava equipada com uma membrana de troca aniônica. Os resultados apresentados aqui demonstram a viabilidade de utilizar água residuária do processamento de mandioca como um substrato para MFCs equipadas com um biocátodo desnitrificante, permitindo um tratamento eficiente de águas residuárias e geração simultânea de eletricidade.

Palavras-chave: célula a combustível microbiana; biocátodo desnitrificante autotrófico; manipueira; voltímetro diferencial em arduino; membrana de troca iônica.

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INTRODUCTION

The processes involved in the production of cassava (*Manihot esculenta* Crantz) flour and starch often generate substantial quantities of liquid waste, commonly known as cassava processing wastewater (CPWW). These effluents are typically characterized by a high potential for pollution due to their high organic and nutrient content, as well as high toxicity resulting from the presence of linamarin, a cyanogenic glycoside that can be enzymatically converted to cyanide (Cruz *et al.*, 2021). The chemical oxygen demand (COD) of CPWW is known to exhibit significant variability. Costa *et al.* (2022) reported an average CPWW COD concentration of 56.7 (± 35.5) g COD·L^{-1} in their analysis of effluents from flour industries across various Brazilian states. The average COD concentration for CPWW derived from the starch industry was found to be 11.6 (± 9.5) g COD·L^{-1}, which is generally lower than that of the flour industry effluent. This is likely attributed to the higher degree of dilution with process water in the starch production process.

Despite the potential for numerous applications that could render its use feasible, CPWW is often directly discarded into the environment without undergoing any treatment (Cruz *et al.*, 2021; Costa *et al.*, 2022). Due to its high content of organic matter and nutrients, CPWW presents great potential as a substrate for obtaining value-added bioproducts and bioenergy, including but not limited to biofuels, biosurfactants, organic acids, polysaccharides, and aromatic compounds. Zhang *et al.* (2016) have extensively discussed the potential applications of CPWW in these areas.

Methanogenic anaerobic digestion (AD) is currently one of the most widely employed technologies for treating CPWW. However, some CPWW characteristics can pose challenges to the process, thereby limiting its efficiency. The high carbon-to-nitrogen ratio (C/N), high acidity, and elevated easily biodegradable carbohydrate content of CPWW are known to contribute to the instability of the AD process. These factors often require the addition of an alkalinity source to maintain optimal process conditions and ensure the efficient conversion of organic matter to biogas. Furthermore, the presence of cyanide in CPWW can significantly increase the inhibition of methanogens, further complicating the establishment and stabilization of AD processes, as reported by Cruz *et al.* (2021) and Costa *et al.* (2022). Despite the potential benefits of AD for treating CPWW, the aforementioned challenges often limit its effectiveness, resulting in unsatisfactory COD removal. These limitations have been highlighted in a recent review by Cremonez *et al.* (2021). Therefore, it is imperative to explore and develop more robust and effective strategies for the treatment of this residue.

An alternative strategy to address the challenge of CPWW treatment and energy generation is the use of this waste as a substrate for power generation in microbial fuel cells (MFCs) (Kaewkannetra; Chiwes; Chiu, 2011; Naseer *et al.*, 2021). MFCs are bio-electrochemical devices that utilize the biological oxidation of organic matter or other biodegradable compounds to generate electricity. The most common type of MFCs are dual-chamber systems, which consist of an anode and a cathode chamber, similar to conventional electrochemical cells. In the anode chamber of MFCs, microorganisms carry out the oxidation of organic matter in the absence of oxygen, leading to the production of CO_2, protons (H^+), and electrons. The electrons are transferred directly or indirectly to the anode and move toward the cathode through the electrical circuit, while the protons diffuse toward the cathode through an exchange membrane that separates the anode and cathode chambers. In the cathode chamber, oxygen (if aerobic cathode MFCs are used) reacts with the H^+ and electrons on the cathode surface, resulting in the formation of water (Logan *et al.*, 2006; Logan; Rabaey, 2012).

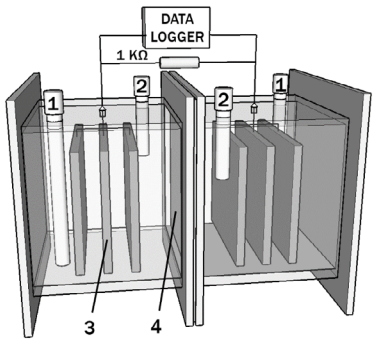
However, the use of abiotic aerobic cathodes in MFCs often requires the use of a metallic catalyst, such as platinum, which can significantly increase the cost of the reactor and limit its economic feasibility. This limitation can be minimized by using microorganisms as catalysts in a configuration known as biocathode, which eliminates the need for external metallic catalysts (Lefebvre; Al-Mamun; Ng, 2008; Li *et al.*, 2014). The use of biocathodes allows for the integration of both cathodic and anodic processes for wastewater treatment. Under anaerobic conditions in the cathode chamber, compounds such as nitrate, sulfate, and fumarate act as electron acceptors for the electrons generated in the anode chamber (Lefebvre; Al-Mamun; Ng, 2008; Rahimnejad *et al.*, 2015). The study conducted by Clauwaert *et al.* (2007) is considered to be one of the earliest investigations into the simultaneous removal of carbon and nitrogen using MFCs with cathodic denitrification. Since then, numerous researchers have explored the potential of this MFC configuration, including Lefebvre, Al-Mamun, and Ng (2008), Virdis *et al.* (2009), Puig *et al.* (2012), and Zhao *et al.* (2017).

Given the aforementioned considerations, the objective of this study was to assess the feasibility of using dual-chamber MFCs with denitrifying biocathodes for the treatment of CPWW, providing simultaneous removal of organic matter and nitrogen. Two MFC configurations were evaluated based on the type of ion exchange membrane used, one with a cation exchange membrane (CEM) and the other with an anion exchange membrane (AEM).

METHOD

MFC design and operation

Two dual-chamber MFCs with 0.15 L working volume in each chamber were used (Figure 1). Both MFCs were constructed using poly(methyl methacrylate) and differ from each other in terms of the ion exchange membrane used. MFC-A was equipped with an AEM (AMI-7001—Membrane International Inc.), and MFC-C was equipped with a CEM (CMI-7000—Membrane International Inc.). The effective working area of the membranes at the interface between chambers was 30.25 cm^2. Each individual chamber was fitted with a set of three interconnected stainless-steel mesh electrodes, fashioned in a rectangular-shaped envelope measuring 4.5 × 5 cm, and filled with 3 g of activated carbon. During the



Source: author (2024).

Figure 1 – Graphical representation of MFCs used. Highlighted discharge ports (1), feed ports (2), electrodes (3), and the ion-exchange membrane (4).

operational period of the MFCs, a constant external resistance of 1,000 ohms was maintained (Figure 1).

To inoculate the anode chambers, a mixture of 50 mL of anaerobic sludge obtained from a sewage treatment plant at a local shopping mall and 50 mL of liquor from an anodic chamber of a MFC fed with synthetic culture medium was added (kindly provided by the Environment Biotechnology Laboratory from the Federal University of Santa Catarina). The final volume was adjusted to 150 mL by adding 50 mL of CPWW diluted to 10 g COD·L^{-1}, which aimed to simulate effluents from starch industries. The anodic chambers were then continuously fed with the diluted CPWW throughout the experiment. The pH of the diluted CPWW was adjusted to 7.0 using sodium bicarbonate immediately prior to feeding.

For the cathode chamber inoculation, a mixture of 50 mL of sludge obtained from a bench-scale heterotrophic denitrifying reactor and 50 mL of the same liquor used to inoculate anodic chambers was used. The final volume was adjusted to 150 mL by adding 50 mL of effluent obtained from a bench-scale nitrifying reactor that was fed with the same CPWW used in the anodic chamber. This nitrified CPWW used had an average nitrate concentration of 973 mg NO_3^--N·L^{-1} and negligible levels of nitrite and COD. This nitrified CPWW was used to feed the cathode chambers throughout the study, resulting in a COD:N ratio of approximately 10:1 between the anode and cathode chambers. The pH value was adjusted to 7.0 prior to feeding.

A sequencing batch strategy was employed to feed the reactors, wherein a fixed volume of medium, referred to as the exchange volume (EV), was exchanged on a daily basis. Table 1 presents the operational conditions for both the anode and cathode chambers.

Electrochemical characterization

The output voltage of the MFCs was measured and recorded using a differential voltmeter. The voltmeter was developed with an Arduino microcontroller (Arduino Uno R3) that was equipped with a data logger module (Adafruit Data Logger Shield) and an analog-to-digital converter module (Adafruit ADS1115), as depicted in Figure 2. The ADS1115 module was capable of providing differential voltage measurements between its input pins, which allowed for the monitoring of two MFCs per module via the I^2C bus. Since the module has four input pins, up to six MFCs could be monitored simultaneously using three ADS1115 modules. Data acquisition was conducted at 5-min intervals, and a daily average was obtained from these measurements.

To calculate the electrical current, Equation 1 was used, and the power generated by the MFCs was then calculated using Equation 2. To obtain the volumetric power density, the power calculated was normalized by dividing the power value by the total volume of the MFC, which took into account both the anode and cathode chambers:

$$I = V/R \quad (1)$$

where:

I—Current (A);

V—Measured tension measured at the MFC (V);

R—External resistance (ohm).

$$P = I \times R \quad (2)$$

where:

P—Power (W);

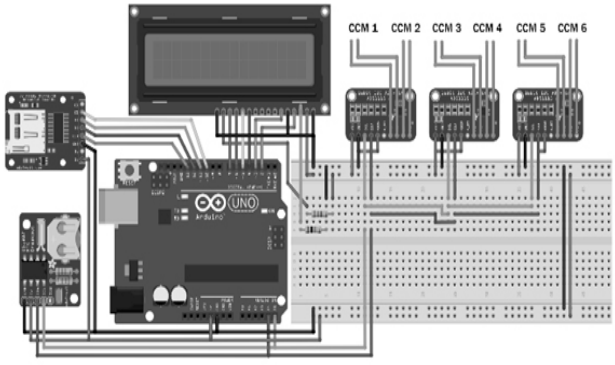
I—MFC current (A);

R—External resistance (ohm).

To determine the internal resistance values, polarization and power curves were generated at the end of the operating period (Fan; Li, 2016). To generate these curves, 10 different external resistances were used (52, 56, 61, 70, 89, 122, 165, 254, 405, and 1,000 ohms), with 1-h intervals between each condition. The coulomb efficiency was calculated using the method described by Logan *et al.* (2006).

Analytical determinations

The pH of the discharged effluent was measured daily using a portable pH meter (Akso, model AK88). To monitor the reduction in COD in the MFCs, the colorimetric method 5220-D from Standard Methods (APHA; AWWA; WEF, 2017) was used. The concentration of nitrate and nitrite was determined using the salicylic acid method (Cataldo *et al.*, 1975) and the Nitriver 2 analytical kit (HACH), respectively.



Source: author (2024).

Figure 2 – Schematic representation of the data acquisition system (differential voltmeter) developed using the Arduino platform.

Table 1 – Operational conditions applied to the MFCs.

Phase	EV (mL·d^{-1})	HRT (d)	OLR(an) (g COD·L^{-1}·d^{-1})	NLR(ct) (g NO_3^--N·L^{-1}·d^{-1})
I	20	7.50	1.33	0.13
II	27	5.56	1.80	0.18
III[a]	40	3.75	2.67	0.26

[a]Condition applied only to the MFC-A; EV: exchange volume; HRT: hydraulic retention time; OLR(an): organic loading rate applied to the anode chambers; NLR(ct): nitrate loading rate applied to the cathode chambers.

RESULTS AND DISCUSSION

Table 2 presents a synthesis of the results found regarding energy generation and the reduction of COD and nitrogen content. More detailed information about the results can be found in the subsequent sections.

The concentrations of ammonia and nitrite were below the limit of quantification throughout the entire trial, both for the influent and effluent of the cathodic chambers. In the samples derived from the anodic chambers, nitrogen species were not monitored.

Power generation

The initial stage of the operation period, spanning from 5 to 10 days, exhibited the highest voltage values, with a peak value of approximately 350 mV (Figure 3), resulting in an electrical current of 0.35 mA and a volumetric power density of 0.41 $W \cdot m^{-3}$.

Subsequent to this initial period of operation, the MFC-C demonstrated a gradual decline in power generation, achieving stabilization at 125 mV (±7 mV) on the 50th day of the experiment, resulting in a current of 0.125 mA and a volumetric power density of 0.05 $W \cdot m^{-3}$. In contrast, the MFC-A demonstrated a slower decline in voltage, reaching a mean value of 243 mV (±29 mV) during the final 20 days of operation, corresponding to a current of 0.24 mA and volumetric power density of 0.20 $W \cdot m^{-3}$. Nonetheless, the MFC-A exhibited a slow and constant decline in power generation until the conclusion of the experiment.

It is noteworthy that the increments in feed loading rate, resulting in increased substrate surplus and cyanide input into MFCs, did not affect the current generation. These findings are consistent with earlier research that has shown the ability of MFCs to withstand relatively elevated levels of cyanide (Chang *et al.*, 2005; Kaewkannetra; Chiwes; Chiu, 2011). Kim *et al.* (2004) reported that concentrations of up to 1.5 mM of cyanide can even enhance current generation by blocking the terminal oxidase at the respiratory chain, reducing inhibitory effects caused by the presence of electron acceptors with higher redox potential, such as oxygen and nitrate.

These results bear some resemblance to those reported by Kaewkannetra, Chiwes, and Chiu (2011), who investigated the utilization of CPWW as a substrate in an MFC with upward flow and an aerobic cathode, using glass wool to segregate the anodic from the cathodic zone within the reactor. In their work, the highest voltage values obtained were between 120 and 180 mV, with an external resistance of 100 ohms. Conversely, our results demonstrated slightly higher voltage values, particularly in MFC-A, with an external resistance of 1,000 ohms. However, when considering the voltage values obtained from the polarization curve with an external resistance of 122 ohms, we observed similar values to those reported in the previous study, approximately 180 and 120 mV for MFC-A and MFC-C, respectively.

Regarding the polarization and power curves obtained from MFCs (Figure 4), both devices showed the highest volumetric power density values under the lowest resistance tested, so it was not possible to determine the maximum volumetric power densities achievable by the MFCs. The highest volumetric power density observed was 1.51 $W \cdot m^{-3}$ (153 mV/9.86 $A \cdot m^{-3}$) and 0.42 $W \cdot m^{-3}$ (81 mV/5.22 $A \cdot m^{-3}$) for the MFC-A and MFC-C, respectively. The internal resistances of the MFCs were determined from the slope of the polarization curve, revealing an 84% greater internal resistance for MFC-C in comparison to MFC-A (41.0 and 22.3 ohms, respectively). This suggests a higher efficiency of the AEM in comparison to the CEM, which is consistent with earlier findings (Kim *et al.*, 2007; Rozendal *et al.*, 2008; Scott, 2016).

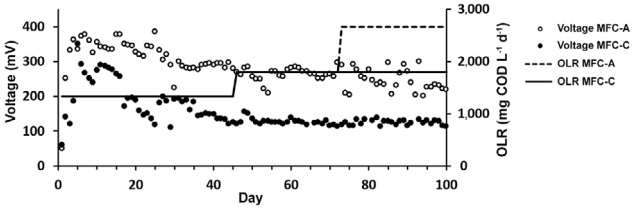


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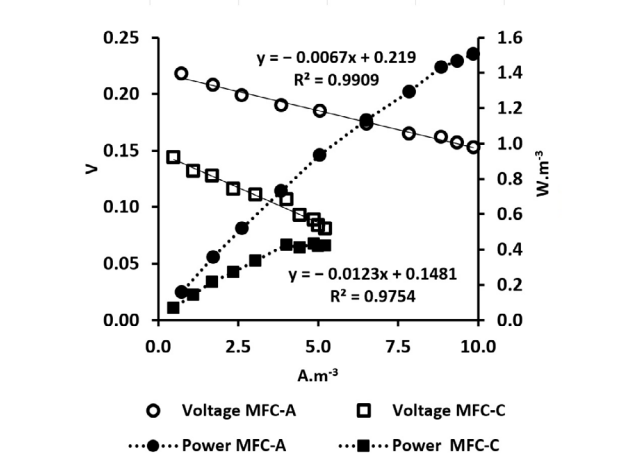
Figure 3 - Variation in voltage and organic loading rate (OLR) in the MFCs over the course of operation. The increase in nitrate loading rate at the cathode chambers was proportional to the organic loading rate increments.



Source: author (2024).

Figure 4 - Polarization and volumetric power density curves obtained from the MFCs.

Table 2 - Summary of key findings regarding energy generation and simultaneous carbon and nitrogen removal.[a]

	Volumetric power density[b] ($W \cdot m^{-3}$)		Anodic chamber		Cathodic chamber	
			pH	COD (mg $COD \cdot L^{-1}$)	pH	NO_3^- (mg NO_3^--$N \cdot L^{-1}$)
MFC-C	0.05	Influent	7.0	10,000	7.0	973
		Effluent	7.40 (±0.16)	192 (±26)	8.34 (±0.14)	21 (±1.6)
MFC-A	0.20	Influent	7.0	10,000	7.0	973
		Effluent	7.47 (±0.17)	132 (±47)	7.56 (±0.25)	4 (±2.4)

[a]To avoid distortions in the average resulting from the acclimation period of the reactor to each new condition applied, the effluent values and power data for system characterization were obtained considering only the last 20 days of operation; [b]volumetric power density was measured under a 1-kΩ resistor.
Source: author (2024).

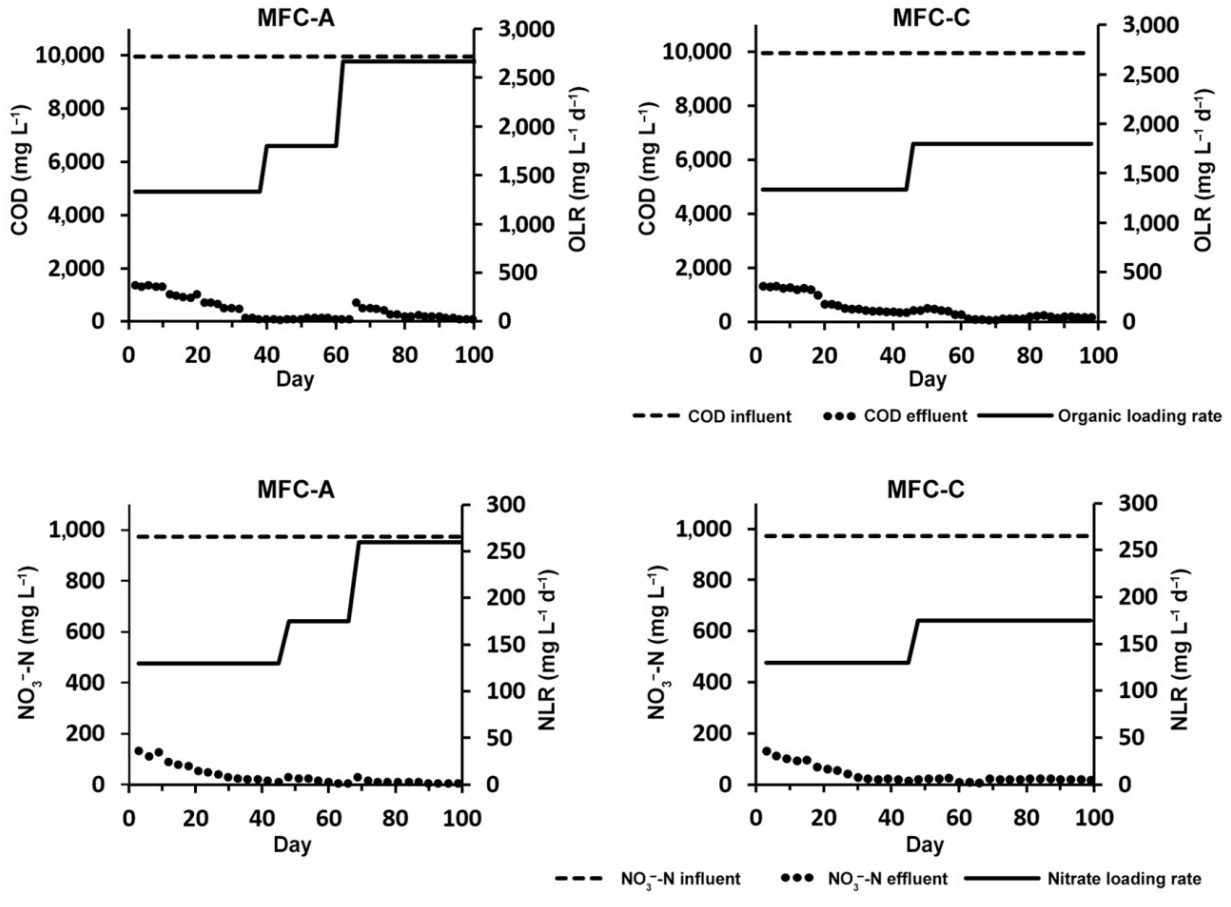
Chemical analysis

Following an initial stabilization phase of approximately 30 days, both reactors exhibited significant levels of COD and nitrate reduction (Figure 5). In MFC-A, COD reduction levels above 99% were achieved after the 35th day of operation, and this efficiency was not impacted by the increment in organic loading rates from 1,333 to 1,800 mg·L^{-1}·d^{-1}. After the second change in organic loading rate, which was increased to 2,666 mg·L^{-1}·d^{-1}, a slight reduction in efficiency was observed, but this was gradually restored throughout the experiment, with values again exceeding 99% after approximately 15 days. In terms of nitrate removal, around 99% removal was observed after initial stabilization. Minor disruptions were observed after increases in nitrogenous loading rate, but these were quickly recovered and did not significantly affect the high levels of removal observed.

The MFC-C showed slightly lower performance concerning the COD reduction, reaching a decrease of 96.5% before the increase in the organic loading rate to 1,800 mg·L^{-1}·d^{-1}. After that, it presented a slight decrease in efficiency that was subsequently recovered and reached, at the end of the experiment, a reduction efficiency between 98% and 99%. Nitrate removal levels in the MFC-C were also satisfactory, remaining above 90% from the 30th day of operation and reaching an average removal after that of 98.1% (± 0.6%).

Despite the high depletion of nitrate and organic matter, the estimated coulombic efficiency based on the removed nitrate was very low. In MFC-A, which performed slightly better, values around 5% were observed only in the first days of operation, gradually dropping to 1.5% by the end of the operation period. Low coulombic efficiency values are widely reported in the literature (Passos *et al*., 2016; Solomon *et al*., 2022) and remain a critical aspect to be optimized in MFCs, especially for wastewater treatment applications. Several factors can contribute to low efficiency, including MFC design and materials used, substrate diffusion across the membrane, inadequate microbial inoculum composition, type of organic substrates, and pH, among others (Logan; Rabaey, 2012; Santoro *et al*., 2017; Ramirez-Nava *et al*., 2021).

Optimizing the reactor operation mode is also a crucial factor in enhancing MFC efficiency. Decreasing the feed load could lead to increased efficiency by reducing the surplus of the substrate, which in turn minimizes the enrichment of competing microbiota, such as methanogens, sulfate-reducing bacteria, and heterotrophic denitrifying bacteria. Additionally, simply reducing the external resistance could promote an increase in coulombic efficiency since it only considers the current and not the generated power. The polarization curve obtained (Figure 4) shows that the reduction of external resistance increased the current to values up to 10 times greater than those observed at 1,000 ohms.



Source: author (2024).

Figure 5 – Profile of influent and effluent concentrations of COD and NO_3^--N, as well as the corresponding nitrogenous and organic loading rates applied to the MFCs over the course of operation.

When compared to AD, a well-established technology for coupling energy generation to wastewater treatment, MFCs and other bioelectrochemical devices are currently not competitive in terms of energy harvesting capacity. However, considering the unique features of both technologies, it has been suggested that they are not in direct competition but rather complementary to each other. AD is more efficient for treating high-strength wastewaters, while MFCs have a more limited application niche, primarily for low-strength wastewaters (PHAM *et al.*, 2006).

In this study, it is possible that the high levels of COD and nitrogen removal were a result of complementary processes, such as electrogenic autotrophic denitrification, methanogenesis, and heterotrophic denitrification, along with other less relevant processes. These processes likely worked together to produce a high-quality effluent. Currently, various strategies are being explored to associate both MFCs and AD technologies. These strategies include placing MFCs before the AD at the acidogenesis step, inserting them in parallel in a recycle way with the anaerobic reactor, using them as a final polishing step, and using them as a submerged MFC, among others. A review by Wang, Chang, and Lee (2022) provides further details on the different ways of combining MFCs and other bioelectrochemical devices with AD.

pH splitting

According to He *et al.* (2008), an appropriate pH range for optimal MFC performance falls between 7 and 8 for the anode chamber, a condition observed in both MFCs tested in this study. However, there was a notable discrepancy between the MFCs with respect to pH splitting observed between the cathode and anode chambers (as shown in Figure 6). MFC-A presented superior results in minimizing pH splitting, thus partially justifying the better electric power performance observed for MFC-A.

There is ample evidence in the scientific literature indicating that minimizing pH splitting is a crucial aspect for optimal performance in MFCs, with membrane material playing a significant role (Dhar; Lee, 2013). Our findings are consistent with previous studies indicating that the use of AEM results in lower pH splitting compared to CEM (Leong *et al.*, 2013; Dharmalingam; Kugarajah; Sugumar, 2019; Ramirez-Nava *et al.*, 2021).

CONCLUSIONS

The MFCs tested exhibited an upper limit on energy generation that was not influenced by the applied feed loading rate, suggesting that this limitation may be attributed to the inherent structural and biological characteristics of the MFCs rather than the physicochemical properties of the CPWW. Improvements in MFC configuration and optimization of operational parameters may lead to enhanced energy recovery.

The MFCs tested in this study showed that the MFC equipped with an AEM had better performance, as evidenced by a higher volumetric power density, likely due to a lower pH gradient between the chambers and lower internal resistance. On the contrary, the MFC-C had lower power generation efficiency but demonstrated a more stable performance after stabilization. It is possible to speculate that with an extension in the operating time, the performance of the MFC-A could become lower than that of the MFC-C due to its slow but constant decline in power generation over time.

Remarkably, the limited power generation efficiency of MFCs did not compromise their capacity to treat wastewater effectively, and, conversely, increasing carbon and nitrogen loading rates did not have a negative impact on electricity generation.

In this way, despite the low energy generation efficiency of MFCs when harvesting energy from CPWW, they have proven to be a promising alternative to complement other technologies, particularly AD. However, there are still relatively few technologies that integrate these biological processes in a complementary manner, necessitating further research to consolidate this approach.

AUTHORS' CONTRIBUTIONS

Silva, A.O.: Formal analysis, Investigation, Writing – original draft. Perazzoli, S.: Writing – review & editing. Soares, H.M.: Writing – review & editing. Azevedo, M.M.R.: Methodology, Writing – review & editing. Bressan, C.R.: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – review & editing.

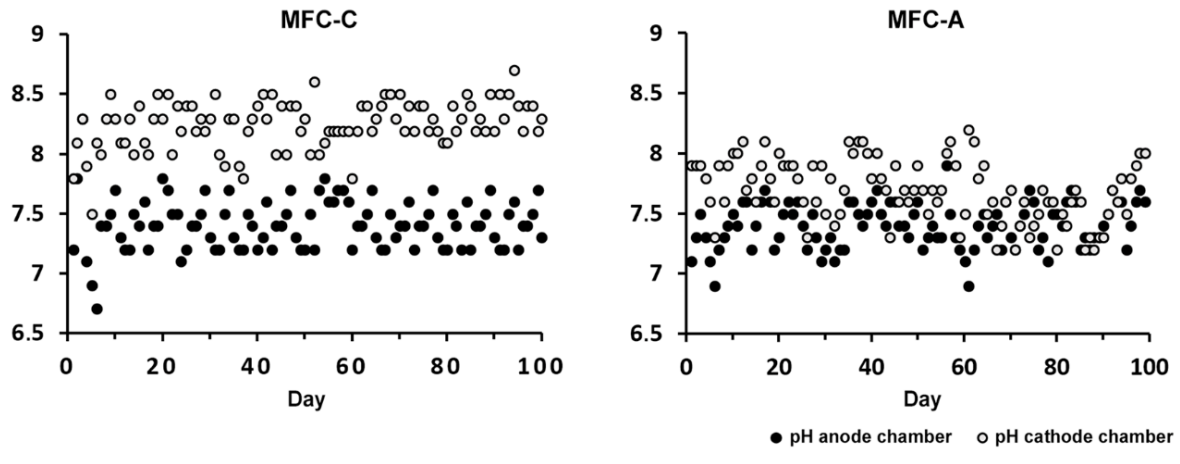


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Figure 6 – Variation in pH in the effluent of the anode and cathode chambers of the MFCs.

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