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Floating treatment wetlands integrated with microbial fuel cell for the treatment of urban wastewaters and bioenergy generation

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HIGHLIGHTS
- Integration of FTW-MFC for simultaneously wastewater treatment and bioelectricity.
- Different plant species were evaluated regarding uptake and bioenergy generation.
- Reductions of COD, TP and TN were 71.4%, 11.4% and 8.4%, respectively.
- Voltage peaks of up to 225 mV were obtained, and mainly decaying over time.
- Bioenergy generation was influenced by root lengths, weather conditions and HRT.

GRAPHICAL ABSTRACT
- 71% for COD.
- 91% for turbidity.
- 99.9% for total coliforms.
- Voltage peak: 225 mV
- Root size ↑ bioenergy.
- HRT ↓ bioenergy.
- TN: 9.2 g/m²/year.
- TP: 1.1 g/m²/year.
- K, Ca, Mg, S, B, Cu, Fe, Mg, Zn, Al...

ABSTRACT
The objective of the present study was to develop a combined system composed of anaerobic biofilter (AF) and floating treatment wetlands (FTW) coupled with microbial fuel cells (MFC) in the buoyant support for treating wastewater from a university campus and generate bioelectricity. The raw wastewater was pumped to a 1450 L tank, operated in batch flow and filled with plastic conduits. The second treatment stage was composed of a 1000 L FTW box with a 200 L plastic drum inside (acting as settler in the entrance) and vegetated with mixed ornamental plants species floating in a polyurethane support fed once a week with 700 L of wastewater.

In the plant roots, graphite rods were placed to act as cathodes, while on the bottom of the box 40 graphite sticks inside a plastic hose with a stainless-steel cable acting as the anode chamber. Open circuit voltages were daily measured for 6 weeks, and later as closed circuit with the connection of 1000 Ω resistors. Plant harvestings were conducted, in which biomass production and plant uptake from each of the species were measured. On average, system was efficient in reducing BOD5 (55.1%), COD (71.4%), turbidity (90.9%) and total coliforms (99.9%), but presented low efficiencies regarding total N (8.4%) and total P (11.4%). Concerning bioenergy generation, voltage peaks and maximum power density were observed on the feeding day, reaching 225 mV and 0.93 mW/m², respectively, and in general decaying over the 7 days. In addition, plant species with larger root development presented higher voltage values than plants with the smaller root systems, possible because of oxygen...


1. Introduction

The reduction in water availability is currently promoting the development of clean technologies and the integration of different processes towards the concept of circular economy, aiming for sustainable alternatives that allow for resource recovery and energy generation. Concerning wastewater treatments, the potential environmental impacts, technical issues and economic costs should help decision makings in the selection of the best treatment technology, or in reducing the impacts of the activities related to the wastewaters treatment (Resende et al., 2019).

In this sense, constructed wetlands (CWs) are engineered systems that use processes present in natural environments and plants for treating several types of polluted waters (Vymazal, 2010). A recent variation of the CWs are the so-called floating treatment wetlands (FTWs), in which naturally emergent macrophytes are placed in buoyant supports, leaving their roots permanently in contact with the treating water (Lucke et al., 2019). These systems present some advantages over traditional CWs, such as the absence of requirements for substrate materials (such as sand and gravel), which reduces construction costs and also removes clogging risks, and the larger and permanent contact area between roots and the water (Colares et al., 2020). Besides urban wastewater, FTW systems can be used for several applications, such as treating industrial wastewaters (Tara et al., 2019) and for biodegradation of oil contaminated water (Afzal et al., 2019a).

Depending on the design and operation, CWs can develop stratified redox conditions, with more aerobic zones near the surface and more anaerobic zones at the bottom, which are the same requirements and principles of cathodes and anodes in a MFC (Xu et al., 2016). Therefore, in the order to utilize the redox gradient present in the CWs, the addition of electrodes in the anaerobic zone (anode) and aerobic zone (cathode) has merged this technology with the name of constructed wetland - microbial fuel cell (CW-MFC). Over the last years, most studies have focused on design and operational conditions that may maximize the redox gradient and thus the energy generation from these systems (Srivastava et al., 2019).

Microbial fuel cells (MFCs) consist in bioelectrochemical device that generates electricity from organic matter oxidation by electrochemically active bacteria (Oodally et al., 2019). In these systems, organic matter is oxidized in the anodic compartment and the resulting are transferred to the electrode (anode), and due to redox gradient between cathode and anode, the electrons flow through a conductive material from the anode to reduce a higher electron acceptor (usually oxygen) at the cathode (Corbella and Puigagut, 2018). Another set-up consists on anodes buried in the sediment (anoxic zone) where microorganisms produce power through oxidation of organic matter, while cathodes are placed near the water surface. These open systems are called sediment microbial fuel cell or SMFC (Zhao et al., 2017; Song et al., 2019).

The integration of CWs and MFCs (CW-MFC) is a recently emerged technology for recovering bioelectricity from wastewater treatment, by using the combination of plants, bacteria and other processes to treat wastewater and harvest renewable chemical energy in order to produce electricity (Yakar et al., 2018). In general, most studies integrating CWs and MFCs in lab scale applied a non-proton exchange membrane (PEM), which usually consisted of glass wool, in order to isolate anodic and cathodic compartments to avoid negative influences from both sides. However, in order to maximize redox conditions, most studies have applied unrealistic operation modes, for example up-flow batch feed loading regimes (Dotro et al., 2017).

When up scaling these systems to pilot or real scale units, the feasibility of adopting membrane separators is compromised by costs and architecture issues, and membrane less systems are considered the most viable configuration for large scale applications (Wang et al., 2017). Additionally, membrane separators increase internal resistance of the systems, directly affecting the potential of energy generation (Xu et al., 2018). Thus, the application of CW + MFC without PEMs has recently received increasing attention from the scientific community (Araneda et al., 2018; Kadam et al., 2018).

Besides harvesting energy from wastewater in form of bioelectricity, integrating CWs and MFCs may also improve treatment performance compared to traditional CW systems. The improved COD efficiency removal can be observed mainly due to the anode acting as an insoluble terminal electron acceptor, increasing metabolic rate of anaerobic bacteria and accelerating the degradation of organic matter (Doherty et al., 2015). In this context, Tao et al. (2020) also verified improvements in denitrification and power generation of wastewater treatment plants (WWTPs) by integrating MFCs and CWs with the insertion of low-cost biomass (cellulose and xylan) as carbon sources, and successfully achieved enhancements of nitrogen removal.

In CW-MFC systems, plants directly affect the treatment performance and energy generation through photosynthesis, organic compounds and oxygen released by the roots, which also provide surface area for the microorganisms (biofilm) growth. Yet, there have been few studies investigating the effects of different plant species on CW-MFC systems (Liu et al., 2020). Given that each plant species has its own morphology and physiology characteristics, the selection of the plant types for CW-MFC systems is fundamental for its performance. Although several studies were developed considering one plant species, studies investigating and comparing different plants in CW-MFC systems are still rare in literature (Oodally et al., 2019).

The objective of the present study was to develop a treatment system integrating anaerobic biofilter (AF), FTW and MFC for urban wastewater treatment and bioelectricity generation. In the FTW-MFC system, five different macrophyte species were simultaneously and monitored and compared in relation to bioenergy generation and plant uptake. The mixed plant species configuration was applied in order to promote improved landscape conditions (with flowering plants), increase microorganism diversity in the root systems and to compare how each of the plant species would behave under hydropnic and MFC conditions for treating wastewater.

2. Material and methodology

2.1. AF + FTW-MFC system’s setup

The research was developed at the University of Santa Cruz do Sul WWTP, in Brazil. The performance of the integrated system was monitored over 6 months (from July 2019 to December 2019), with feeding performed once a week. The first treatment unit consisted of a 1450 L anaerobic biofilter (AF) that was fed with raw wastewater after passing through preliminary system (screening and grit removal), pumped directly from a flow equalization tank from the university wastewater using a centrifugal pump (1 HP). The AF was fed under 3 days batches while the FTW + MFCs system had fill-and-drain feeding, so that when this unit was fed the treated wastewater would overflow and be drained while the box would be filled with a new batch of wastewater. The AF had a filtering compartment filled with plastic conduits to provide a superficial area for biofilm growth (Fig. 1).
When feeding the AF, the treating wastewater was overflowed and drained through 40 mm PVC pipes to the second treatment unit, a CW designed as a FTW system. This stage was composed of a 1000 L (1.9 × 1.2 × 0.7 m) rectangular fiberglass plastic box with no substrate and a 200 L high-density polyethylene (HDPE) drum acting as a primary settler tank (Fig. 2). The draining device consisted of a 40 mm PVC pipe with several 8 mm holes located 30 cm from the bottom, so that floated solids were not drained with other and in order to reduce water resuspension of solids from benthic layer. Water level was kept to 50 cm by an adjustable standpipe outside the box.

The hydraulic retention time (HRT) of the FTW stage was of 7 days. The selection of the HRT was based on recommendations of the specialized literature, such as Olguín et al. (2017), who recommended at least 5 days for FTWs. It was also based on previous studies investigating FTW systems for the wastewater treatment from university campus, such as Colares et al. (2019) and Benvenuti et al. (2018), which used 7 and 11.5 days, respectively. According to Abed et al. (2017), the HRT and water velocity should be selected in order to ensure the sedimentation and trapping of suspended solids in the plant roots. In general, HRT in FTW is positively correlated with overall pollutants removal (Chen et al., 2016). However, oversized systems demand more area and may also compromise the feasibility of the treatment system (Colares et al., 2020).

In the FTW unit, 5 ornamental plant species were tested in the floating support: Canna generalis, Chrysopogon zizanioides, Cyperus papyrus Nanus, Hymenachne grumosa and Equisetum hyemale. These plants were placed in one tank with 3 lines of 5 baskets. Each of the three lines had 1 basket of each of the plant species, totaling 15 plants in the tank, and a density of approximately 7.5 plants/m², similar to recommendations of Olguín et al. (2017), who suggest plant densities in FTW systems of approximately 10 plants/m². Other plants were initially tested (Xanthosoma sagittifolium, Impatiens parviflora and Zantedeschia aethiopica). However, these plants did not adapt to the hydroponic conditions and were replaced.

Initially, the FTW system was composed of plastic pots filled with gravel n. 1 (9.5–20 mm) and approx. 2 cm lightweight expanded clay aggregate (LECA) with holes tied to circular expanded polyurethane foams, with roots permanently submerged in water. However, because of the proliferation of mosquito larvae, 100% of the water surface was covered with foam carpet and insects screen in order to prevent mosquitoes proliferation. Plants were initially exposed to diluted wastewater for acclimatization over 4 weeks.

### 2.2. Analytical methods and application rates

Samples were collected weekly after each treatment unit and thereupon analyzed. The characterization parameters of wastewater included analysis of chemical oxygen demand (COD) - titrated method, biochemical oxygen demand (BOD₅) - BOD after 5 days at 20 °C, total phosphorous (TP) - spectrophotometric-colorimetric method, total nitrogen (TN) - Shimadzu TOC-L, turbidity (optical), electrical conductivity (EC), pH, sedimentable solids (Imhoff cones), total coliforms and Escherichia coli (3 M pretri film plates). In addition, electrical
conductivity (EC) and dissolved oxygen (DO) were measured in different depths of the FTW system (5 and 60 cm - near in the water surface and deep on the bottom of the box, respectively) using a portable DO (Instrutherm MO-920) and an EC (Instrutherm CD-880) analyzers.

The control of the pollutant loads was carried out considering recommendations of the literature for CW systems (Table 2). Loading rates were calculated considering inflow pollutants concentrations, hydraulic loading rate (700 L/week) and FTW area (2.3 m²) of application. Analytical control of nitrites and nitrates were conducted through ionic chromatography (Metrohm 844 UV/VIS Compact IC). However, since all concentrations obtained were below the detection limits, these results are not presented in Table 1.

All the samples preservation and analyzes were conducted according to APHA/AWWA (2012). Three samples points were defined at the outlet of each treatment unit, and weekly collected during the system feeding and always at the same day and time. These points were: raw wastewater (from the WWTP equalization tank), after the anaerobic biofilter (AF), and after the FTWs unit. This last one was divided in two phases, the first 3 months, which included only the FTW without the graphite electrodes, and the last 3 months, when this unit was integrated with the MFC system.

2.3. Phenology and climate monitoring

Given that plants can directly influence treatment performance and energy generation, several phenological aspects were investigated over the monitoring period for each of the plant species, such as flowering, root development (length), biomass generation and plant uptake (macro and micro nutrients and metals). Characterization of the biomass was carried out using samples of all plant tissues 20 cm above water level (biomass resulted from pruning), similarly to Horn et al. (2014), who left 20 cm height of biomass to prevent plant mortality and increase its recovery and growth after pruning. For the determination of the dry biomass, tissue samples of each plant species were kept under 70 °C until constant weight. Biomass compositions were performed through microwave digestion and Inductively Coupled Plasma Optical Emission Spectrometry (ICP/OES) using argon in the University of Santa Cruz do Sul Analytical Center Laboratory, from tissue samples collected from one of plant harvestings (August 2019).

Over the 6 months of monitoring, 2 plant harvesting were performed, in the beginning and in the end of the monitoring, August and December 2019, respectively and another in the end of Summer, in March 2020. Therefore, plant harvesting was conducted every 4 months for one year. The macrophytes were pruned 20 cm above the water level (biomass resulted from pruning), similarly to Horn et al. (2014), who left 20 cm height of biomass to prevent plant mortality and increase its recovery and growth after pruning. For the determination of the dry biomass, tissue samples of each plant species were kept under 70 °C until constant weight. Biomass compositions were performed through microwave digestion and Inductively Coupled Plasma Optical Emission Spectrometry (ICP/OES) using argon in the University of Santa Cruz do Sul Analytical Center Laboratory, from tissue samples collected from one of plant harvestings (August 2019).

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Climate conditions monitoring was conducted through a meteorological station located at the campus of the University of Santa Cruz do Sul. Data on temperature (minimum, maximum and average), precipitation (mm) and average relative humidity (%) were recorded daily during the study period. Mean temperatures (minimum and maximum) ranged from 9.0 to 23 °C during winter and from 16.3 to 32.7 °C during spring. Mean month precipitations ranged from 56 mm (December) to 405.2 mm (October), with an average of 156.58 mm/month.

2.4. Microbial fuel cell configuration and monitoring

In the last trimester of monitoring, MFC systems were integrated with the FTW unit. For the cathodes, graphite rods (1 × 5 cm) were placed below the plant roots (about 10 cm below water surface), tied on the support pots with plastic clamps (Fig. 3A), so that the root systems could provide more oxygen to the cathodic reactions. One cathode chamber was placed for each of the plant species, resulting in 5 cathodes. The graphite rods were connected with copper wires liquid isolating tape (liquid electrical tape). Fig. 3 present photographic records of cathodes (A) and anodes (B).

For the anodic chamber, a punctured PVC hose (Fig. 3A) was filled with 40 graphite sticks (0.5 × 2 cm each) permanently in contact with a 40 cm stainless steel cable (3.2 mm) in a circular shape, similar to Xu et al. (2017), who wrapped the electrodes (activated carbon and graphite gravel) in stainless steel mesh. The anodic electrodes were placed at the bottom of the FTW, in contact with the benthic layer, where most sediment solids and organic matter are deposited. The end of the stainless steel was connected to a copper wire (covered with plastic) that went outside the box, while all connections were also isolated from water with liquid isolating tape (liquid electrical tape). The average spacing between cathodes and the anodic chamber was 40 cm.

The anode was placed inside a plastic hose mainly to protect the graphite sticks from physical impacts and also to facilitate the electrodes manipulation (for example for changing its position or switching the electrode material). Although non-conducting, this material (PVC transparent pipe) was choose due to its inert conditions (resistance to oxidation), availability, transparency, low cost and flexibility. Some other studies have also wrapped the electrodes in plastics materials, such as a plastic grid (Schievano et al., 2017), PVC pipes (Kadem et al., 2018) and plastic film (Wen et al., 2020).

Regarding bioelectricity generation, daily measurements of open circuit voltages were performed for each of 5 macrophyte species for 6 weeks. Later, 1000 Ω resistances were inserted to each of the cathode external circuits, and the FTW-MFC unit was also monitored as a closed system for 3 weeks (3 cycles of 7 days each). The cell potential between the electrodes was measured once every day (on the same time) using a

### Table 1

Mean concentrations (±SD) of raw and treated wastewater pollutants in relation to wastewater standard emissions.

| Parameter                  | Raw wastewater | After AF unit | After FTW system | CONSEMA 355/17 Resolution⁴ | UWTD 91/271/EEC⁵ |
|----------------------------|----------------|--------------|------------------|-----------------------------|-------------------|
| COD (mg L⁻¹)               | 525.0 ± 268.5  | 178.8 ± 8.8  | 137.8 ± 61.9     | 466.3 ± 295.4              | 1.0 × 10⁶         |
| TN (mg L⁻¹)                | 95.2 ± 35.8    | 81.8 ± 10.3  | 80.6 ± 24.1      | 7.2 ± 22.4                 | 1.0 × 10⁶         |
| TP (mg L⁻¹)                | 68.2 ± 17.3    | 69.0 ± 19.4  | 67.1 ± 10.2      | 3.57 ± 2.98                | 1.0 × 10⁶         |
| pH                         | 6.71 ± 0.50    | 6.76 ± 0.35  | 6.78 ± 0.34      | 15                          | 6 to 9            |
| EC (μS cm⁻¹)               | 1081 ± 308.67  | 1064 ± 171.27 | 997.9 ± 96.3       | 466.3 ± 295.4              | 1.0 × 10⁶         |
| Total coliforms (CFU/100 mL)| 1.0 × 10⁹     | 2.5 × 10⁹    | 466.3 ± 295.4    | 1.0 × 10⁶                   | 1.0 × 10⁶         |
| Absorbiometric color (420 nm) | 1.034 ± 0.44 | 0.347 ± 0.109 | 0.177 ± 0.058     | 48.09 ± 24.9               | 1.0 × 10⁶         |
| Turbidity (NTU)            | 589.2 ± 278.5  | 111.6 ± 71.8 | 48.09 ± 24.9     | 3.57 ± 2.98                | 1.0 × 10⁶         |
| Settleable solids (mL L⁻¹) | 30.2 ± 17.0    | 7.2 ± 22.4   | 3.57 ± 2.98      | 18.4 ± 5.6                 | 40                |
| Temperature (°C)           | 192 ± 5.3      | 18.4 ± 5.4   | 18.4 ± 5.6       | 18.4 ± 5.6                 | 18.4 ± 5.6        |

- Urban wastewater discharge standards for daily flow lower than 200 m³ day⁻¹ in Brazil.
- European emission standards for urban wastewater considering generation from 10,000-100,000 p.e.
digital multi-meter (Hikari HM-1000) for each of the cathodes until voltage stabilization. Voltage outputs were only started measured after the acclimation of the plants with diluted wastewater and 1 week after adding the graphite electrodes to the system, in order to ensure the formation of the biofilm on the surface of the electrodes, similar to Kamel et al. (2020).

Power densities were calculated and normalized to the projected superficial area of the anode (0.01256 m²) by using Eq. (1), based on the measured voltage values, on the external resistor and the anode superficial area:

\[ P = \frac{E^2}{Aan \cdot R_{ext}} \]

where: \( P \) = power density (W/m²); \( E \) = measured closed circuit voltage (V); \( Aan \) = superficial area of the anode (m²) and \( R_{ext} \) = external resistance (Ω).

The power density is often normalized to the projected anode superficial area since the anode is the compartment where most biological reactions are present (Logan et al., 2006).

3. Results and discussions

3.1. Raw wastewater characterization and climate conditions

The wastewater treated in the present study can be classified as urban wastewater since it is generated in a university campus and of black and yellow waters from urinals and toilets. Table 1 presents raw wastewater characterization and concentrations after each treatment stage for the first three months of the monitoring (without the MFC integration) in relation to discharge values defined by the Brazilian Resolution CONSEMA 355/2017, established by the State of Rio Grande do Sul Council on Environment (CONSEMA), and the European guideline for urban wastewater treatment (Directive 91/271/EEC) for WWTPs from 10,000 to 100,000 p.e.

As shown in Table 1, it was possible verify that most of the raw wastewater parameters were much higher than the maximum values allowed by the cited national and international guidelines. TN mean values, for example, were approximately 4 times higher than the emission standards, while TP was nearly twice the allowed emission values by the resolutions, even after the applied treatment. Therefore, it possible to identify the strong eutrophication loads of the university wastewater due to its high contents of TN and TP. Additionally, both COD and BOD₅, which are also important indicators of pollutant potentials of untreated effluents, presented mean values were higher than the maximum emission values before treatment, and although the Brazilian standards (CONSEMA 355/17) were met after treatment, for the international guideline (UWTD 91/271/EEC) the necessary COD and BOD₅ reductions were not achieved even after treatment. Concerning nitrites and nitrates, since all concentrations obtained were below the detection limits, these results are not presented in Table 1.

Pollutant and hydraulic loading rates directly affect both treatment performance in treatment wetlands (Dotro et al., 2017) and energy generation in microbial fuel cells (Corbella and Puigagut, 2016). Therefore, Table 2 presents pollutant application rates in the FTW unit and recommendations for free water surface (FWS) and free-floating CWs (CWs).
with naturally floating plants and without substrate) when treating wastewater, since information concerning design and operation recommendations for FTW systems treating wastewater were not found. Therefore, hydraulic and pollutant loading rates still demand more investigations (Shahid et al., 2018).

As shown in Table 2, it is important to highlight that although the organic loading rate was lower, in terms of BOD₅, than the recommended, the mean application rate of TN was higher than the values suggested by the literature. This is due the high content of nutrients compared to the content of organic matter, which indicated that the urban wastewater treated in the present study resembles more a commercial unit wastewater than a common domestic wastewater. A high nitrogen load was also verified in previous studies results that investigated the treatment of wastewaters generated at a university campus, such as De Souza Celente et al. (2020) and Silveira et al. (2017).

Regarding weather conditions, October was the rainiest month, presenting nearly 4 times the mean precipitation from other months (405.2 mm). December, on the other hand, presented besides the lowest precipitation volumes and the lowest number of rainy days, the highest temperatures from monitoring period, with mean maximum temperatures exceeding 32°C. According to Guadarrama-Pérez et al. (2019), temperature, humidity, conductivity and pH are important physical parameters that may affect the performance of CW-MFC systems, since temperature directly affects biological metabolism and thus biological activities. Zhao et al. (2017) verified a significant decrease in energy generation in SMFC systems when temperature reached values below 20 °C.

3.2. Treatment performance and influence of MFC integration

The performance of the integrated system was monitored and evaluated through analysis of the raw and treated wastewaters after each of the units. Results of the performance of the integrated system under open and closed circuit (with 1000 Ω) considering the possible reductions of the load parameters are depicted in Fig. 4. It must be pointed out that the raw wastewater characteristics presented high variability since real urban wastewater was used in the present study, which is directly affected by university activities.

Overall, the treatment system was efficient in reducing BOD₅ (55.1%), COD (71.4%) and suspended solids, which reduced from 30.2 ± 17.0 to 3.57 ± 2.98 mg L⁻¹, representing a reduction of 88.2%. In addition, the integrated system was also efficiently in the wastewater discoloration (74.0% in 420 nm) and in reducing turbidity (90.9%). On the other hand, the treatment system was not effective in reducing TN and TP, reaching concentrations still higher than the maximum emission, and presenting efficiencies of only 8.4% and 11.4%, respectively. The latter is expected since most P removal pathways in CWs are related to adsorption and precipitation in the substrate (Dell’Osbel et al., 2020),
and without a filtering material FTW systems depend more on sedimentation and plant uptake processes.

The low nitrogen removal rates, however, may be related to low oxygen levels in both AF and FTW systems, with mean DO concentrations of 1.57 ± 0.74 and 1.35 ± 0.46, respectively. Different results were obtained by Ijaz et al. (2016), who also investigated the application FTW for wastewater treatment, but observed a drastically increase in DO with the FTW, and justified it by the presence of the T. domingensis plants and endophytes. Since oxygen plays an important role in nitrification and consequently in N removal, (Vymazal, 2010), and that most TN in the wastewater was in the form of N-NH3 (Table 1), the anoxic conditions found in these systems may have compromised nitrification and thus nitrogen removal.

The treatment system also demonstrated a good disinfection potential, since mean reductions of total coliforms and E. coli (UFC) were of 99.99 and 100%, respectively. Afzal et al. (2019b) who also observed significant fecal coliforms when treating wastewater via FTW system (decreased by 200-fold), indicated that removal mechanisms for coliforms might adsorption in plant roots, protozoa predation and oxidation processes.

Benvenuti et al. (2018), who monitored a real scale FTW treatment system vegetated with Typha domingensis for urban wastewater, with an average feed flow rate of 67.4 m³ day⁻¹ (considering also rain fall) and for 12 months. Slightly lower COD and BOD₅ removal efficiencies were verified compared to the present study (55 and 56%, respectively). Although better efficiencies were obtained for both NH₄-N and TP (38 and 37%, respectively), the treatment also did not accomplish the Brazilian regulation (20 mg L⁻¹, respectively). Additionally, Benvenuti et al. (2018) also justified that the low N and P removal efficiencies may be related to the anaerobic conditions verified in the treatment and recommend adjustments to the system in order to increase DO concentration and improve its performance.

In another study, Colares et al. (2019) assessed the efficiency of a combined system for the treatment of urban wastewaters composed of anaerobic reactors (ARs) + hybrid CW systems and with 7 days HRT for each unit. For the first two treatment units, an ARs and the CW1, a FTW vegetated with H. grumosa, the results obtained were very similar to the present study, since Colares et al. (2019) also observed high efficiencies for organic matter (total organic carbon - TOC), color (420 nm) and turbidity (>90% for all) reductions, as well as low P removal rates (<10% for soluble P). However, higher efficiencies were verified for TN removal (43%), which may be related to the lower water depth (40 cm) and some of the water surface uncovered (about 50%), possible increasing DO concentrations in the wastewaters.

Abed et al. (2017), who also monitored a FTW system for the wastewater treatment, indicated that an increase in BOD₅ in the FTW unit may be justified by an increase of the biodegradability of the organic compounds present in the wastewater due to the presence of macrophytes, since BOD₅ concentrations increased while COD concentrations were reduced during the FTW treatment stage.

Therefore, according to the system performance and final concentrations verified (Table 1), in order to achieve the emission standards from the CONSEMA 355 Resolution, it becomes necessary the addition of at least one more treatment unit, such as CW or a biofilter, preferable filled with a phosphorous adsorbent material, or to drastically reduce the loading rates (g m⁻² day⁻¹) so P could be removed through plant uptake. Also, further investigation on the integration of mechanisms or additional units to increase DO concentrations is still required, aiming to promote nitrification and improve nitrogen removal. This can be obtained via artificial aeration or gravityators, such as cascades. Besides, higher DO levels can be obtained through semi-continuous regime (pulse feeding), instead of weekly batch feedings.

The integration of the FTW system with MFCs did not seem greatly affect the treatment performance. The exception were COD and BOD₅, since the removal efficiency of the FTW system for COD slightly increased from 25.2% to 32.8% and 34.4% (open and closed circuit, respectively), whereas BOD₅ removal efficiencies increased from −29% to 0.1% (open circuit) and to 24.9% (closed circuit) after the integration of the MFC system. In Fig. 4, one can observe that some of the concentrations increased after the AF unit, which is like due to the accumulation of sludge after nearly 2 years of operation.

This is consistent with literature, since the anodes can act as insoluble terminals for electron acceptor, increasing the metabolic rate of anaerobic bacteria, accelerating organic matter degradation and so improve COD reduction (Doherty et al., 2015). When comparing traditional horizontal flow CW systems with CW-MFC systems, the latter presented approximately 10% more COD removal than the first (Fang et al., 2013).

According to Dotro et al. (2017), integrating MFC to traditional CW systems may also benefit phosphorous removal. This improvement is possible related to the formation of phosphorous precipitates at the cathodic chamber, resulted from higher pH condition in these locals. On the other hand, this removal mechanism is likely to be short-term in nature, and may depend and be influenced directly by scale and duration of experiment. Therefore, this effect of cathodes may become less significant when upscaling CW-MFC systems. Yet, this is an aspect that should be further investigated.

Besides samples after each treatment unit, measurements were carried out considering different depths (deep and shallow) in the FTW unit, including water temperature, electrical conductivity and dissolved oxygen (DO), i.e., parameters that directly influence the REDOX gradients and the potential bioenergy generation.

As seen in Table 3, temperature and EC did not vary in relation to the water depth. DO, on the other hand, presented higher values closer to the surface (1.98 mg L⁻¹) than in higher depth (1.35 mg L⁻¹). Van de Moortel et al. (2010), when evaluating the redox potentials (mV) of a FTW system, also verified influences of water depth and presence of macrophytes. Redox potentials were monitored under different depths (5 and 60 cm) for planted and control systems. The latter one presented mean redox potentials of 68 ± 225 and −93 ± 226 mV for the 5 and 60 cm depths, respectively. In the planted CW system, Van de Moortel et al. (2010) found redox potentials of −24 ± 145 and −122 ± 111 mV for 5 and 60 cm depths, respectively. In addition, the redox potential in the floating mat was measured, and presented mean values of 72 ± 478 mV. The higher redox potential in the floating mat was justified by the oxygen released by the plant roots. They concluded that the redox potential in these systems is affected not only by oxygen diffusion from air, but also by vegetation roots.

3.3. Phenology and plant uptake

Phenology monitoring included measurements of roots length of the 15 plants every 2 months, biomass weight after harvesting (20 cm above water level) and plant tissues compositions. Table 4 and Fig. 5 summarize these aspects and the plant uptake and incorporation in tissues, respectively. Plant uptake was estimated based on each of the plant species biomass composition and total biomass produced between harvestings.

Garcia Chance and White (2018), monitored pilot scale FTW systems initially vegetated with Juncus effusus (year 1) and later with Canna flaccida (year 2) treating simulated runoff, with and without artificial aeration. High differences between the two plant species concerning

| Parameter | Deep water (50 cm) | Water surface (5 cm) |
|-----------|--------------------|---------------------|
| DO (mg L⁻¹) | 1.35 ± 0.44 | 1.98 ± 0.85 |
| Temperature (°C) | 20.28 ± 4.4 | 20.33 ± 4.0 |
| EC (μS cm⁻¹) | 1170 ± 450 | 1230 ± 123 |
plant uptake were also verified, since *J. effusus* presented mean values (standard error) for N uptake of 24.1 (1.93) and 20.4 (1.40) g m\(^{-2}\) experiment\(^{-1}\) for the aerated and non-aerated respectively, and P uptake of 4.49 (0.35) and 3.21 (0.25) g m\(^{-2}\) experiment\(^{-1}\). The *C. flaccum* species presented higher for both N and P uptakes: 50.7 (6.71) and 40.1 (4.36) g TN m\(^{-2}\) experiment\(^{-1}\) for the aerated and non-aerated systems, respectively. The macronutrients that were mostly incorporated in the biomass were TN and K, whereas Fe and Mn were the most incorporated micronutrients. In relation to TN and TP removals, plant uptake did not seem to play an important role on the performance of incorporated micronutrients.

In relation to TN and TP removals, plant uptake was evaluated 7 different plant species in FTW systems besides plant uptake. For example, biofilm growth in the plant roots and the buoyant support, suspended solids filtration/adsorption, release of organic compounds and oxygen by the roots, sedimentation, reducing in water turbulence, metal adsorption and biofilm growth in the benthic layer (Colares et al., 2020).

**Table 4**

| Plant       | C. generalis | C. papyrus Nanus | H. grumosa | C. zizanioides | E. hyemale |
|-------------|--------------|------------------|------------|----------------|------------|
| Root length (cm) | 13.8 ± 4.6 | 22.3 ± 3        | 28.7 ± 12.6 | 13.5 ± 2.9    | 9.8 ± 1.2  |
| Total wet biomass (kg/m\(^2\)year) | 0.879 | 1.15      | 0.386       | 0.067          | 0.015      |
| Dry biomass (kg/m\(^2\)year) | 0.138 | 0.123     | 0.055       | 0.018          | 0.003      |
| Macro nutrients (g kg\(^{-1}\)) | N 24.32 | 29.54 | 29.78 | 23.94 | 34.33 |
|          | P   2.77  | 3.99    | 2.54       | 3.25           | 2.98       |
|          | K   17.34 | 17.69   | 11.07      | 13.73          | 28.62      |
|          | Ca  2.59  | 4.32    | 2.2        | 2.53           | 7.03       |
|          | Mg  1.44  | 2.37    | 1.69       | 1.08           | 2.04       |
|          | S   1.88   | 3.08    | 3.28       | 2.79           | 5.97       |
|          | B   23.04  | 25.11   | 12.24      | 22.2           | 22.48      |
|          | Cu  23.12  | 5.27    | 9.64       | 7.84           | 6.65       |
|          | Fe  115.16 | 65.6    | 142.97     | 138.13         | 92.06      |
|          | Mn  73.37  | 261.99  | 107.54     | 73.9           | 148.28     |
|          | Zn  16.81  | 43.06   | 28.91      | 14.1           | 49.73      |
| Micro nutrients (mg kg\(^{-1}\)) | Al  0.04   | 0.03    | 0.05       | 0.06           | 0.04       |

* Considering biomass harvested 20 cm above water surface.

As shown in Fig. 5, *C. generalis* and *C. papyrus Nanus* were the most important plant species regarding plant uptake and incorporation, followed by *H. grumosa*. The macronutrients that were mostly incorporated to the biomass were TN and K, whereas Fe and Mn were the most incorporated micronutrients. In relation to TN and TP removals, plant uptake did not seem to play an important role on the performance of the integrated system. Considering average loading rates from Table 2 during the biomass produced from 3 harvestings in one year, plant uptake and incorporation were estimated for being responsible for removing only 0.82% and 1.05% of inflow load rates of TN and TP, respectively. According to Garcia Chance and White (2018) plant uptake in the FTW systems accounted for less than 25% of phosphorous removal in the system.

The hydroponic conditions in the FTW unit may have influenced plant growth and uptake in the treatment, since the obtained results are lower than those presented by Vymazal (2007), who indicated that N uptake and storage in tissue in CWs depends on the plant species used, and that it usually ranges from 0.2 to 0.8 g N m\(^{-2}\) day\(^{-1}\). The obtained mean values for uptake, however, were below this range, estimated in 0.0251 g N m\(^{-2}\) day\(^{-1}\) (9.16 g N m\(^{-2}\) year\(^{-1}\)). According to Benvenuti et al. (2018), nitrogen uptake capacity of emergent plants in CWs can vary from 20 to 250 g m\(^{-2}\) year\(^{-1}\). However, Benvenuti et al. (2018) also indicated that the main mechanisms to N removal in CW is through bacteria conversion (nitrification) rather than plant uptake, and that the combination of aerobic and anoxic conditions can promote simultaneously nitrification and denitrification in treatment.

On the other hand, the plant uptakes seem to be more in accordance with other studies monitoring FTW systems. Zhu et al. (2011), for example, evaluated 7 different plant species in FTW systems treating a polluted river from receiving domestic wastewater and verified mean plant uptakes for each of the plant species ranging from approx. 1.43 to 12.5 g m\(^{-2}\) for N and from 0.174 to 0.927 g m\(^{-2}\) for P. Similarly, Garcia Chance and White (2018) verified plant uptakes of 22.3 ± 1.21 g m\(^{-2}\) for N and 3.85 g m\(^{-2}\) year\(^{-1}\) for P when evaluating a FTW system vegetated with *Juncus effusus*. However, when the FTW was vegetated with *Canna flaccum*, higher plant uptake values were obtained.

In this context, it is important to highlight that several important pollutant removal mechanisms are present and most related to the macrophytes in FTW systems besides plant uptake. For example, biofilm growth in the plant roots and the buoyant support, suspended solids filtration/adsorption, release of organic compounds and oxygen by the roots, sedimentation, reducing in water turbulence, metal adsorption and biofilm growth in the benthic layer (Colares et al., 2020).

### 3.4. Bioenergy generation

Concerning bioelectricity generation, the treatment reached peaks of up to 225 mV considering open circuit of voltage of only one plant (*C. generalis*). When adding all plant voltages, a peak of 749 mV was attained. It was possible to verify that the mean voltage values (mV) were related to the HRT of the wastewater treatment (hours) and to each of the plant species. Figs. 6 and 7 present the information obtained regarding potential bioelectricity generation for open circuit and closed circuit (1000 Ω) configurations, respectively.

Maximum open circuit voltages obtained for each of the plants (Fig. 6B) were 225 mV (*C. generalis*), 212 mV (*H. grumosa*), 144 mV
Fig. 6. Open circuit voltages over time for each of plant species. A) Circuit mean voltages B) total open circuit voltage monitoring over the 6 weeks monitoring for each of plant species.

Fig. 7. Closed circuit voltages over time for each of plant species. A) Circuit mean voltages B) total open circuit voltage monitoring over the 3 weeks monitoring for each of plant species.
(C. zizanioides), 137.4 (C. papyrus Nanus) and 89.6 mV (E. hyemale). However, under closed circuit (with each cathode external circuit connected to 1000 Ω resistances in parallel) maximum voltage values obtained were considerable lower: 21.0 mV (C. generalis), 31.6 mV (H. grumosa), 31.7 mV (C. zizanioides), 40.4 (C. papyrus Nanus) and 43.7 mV (E. hyemale), reaching a maximum of 108 mV when considering all plants voltages. In this context, from Eq. (1), maximum power density obtained was only 0.93 mW/m² when considering the summation of all external circuit voltages (108 mV) and based on the anode surface area. Although this value is considerable low when compared to other studies in literature (Zhao et al., 2017), Tao et al. (2020), who also used real wastewater for MFC and MFC-CW systems, verified similar power densities, with mean values ranging from 0.90 and 1.82 mW/m² (MFC) to 6.09 and 2.91 mW/m² (MFC-CW).

By comparing Table 4 and Fig. 6, it is possible to verify that the two plant species with the largest root length (H. grumosa - 28.7 cm, and C. papyrus Nanus - 22.3 cm) were also the two species which presented the highest mean voltage values (56.6 and 69.7 mV, respectively). Additionally, E. hyemale, showed the smallest root development (only 9.8 cm) as well as the lowest mean voltage values (37 mV). The influence of the roots in the system performance is consistent with the literature information (Liu et al., 2020; Oodally et al., 2019). The rhizosphere provided by macrophytes play an important role regarding the bioelectricity production in CW-MFCs systems. Plants not only transport and release oxygen from the roots through their aerenchyma, but also excrete exudates specifically in the roots. These exudates can be degraded by electroactive bacteria present in the anode biofilm and be used as a source of electron donors for bioenergy generation (Quadarrama-Pérez et al., 2019).

As depicted in Fig. 6A, mean open voltage values for all the macrophytes presented a tendency of decrease over time, from on average 85.5 mV on the fed day to an average of 46.6 mV at the end of cycle (7 days). This can be explained due to organic matter reduction over time and therefore less organic substrate to microorganisms oxidize and generate power, since bioelectricity production can be limited by the amount of organics or COD concentrations (Oodally et al., 2019). Given that most biodegradable organic matter is first removed in wastewater treatment (Merino-Solís et al., 2015), oxidation or organic matter speed is expected to decrease after a while. Under the closed circuit condition (Fig. 7), on the other hand, mean voltages values did not present a decrease tendency over time, but a more variable behaviour, since for some plants voltage values even increased over time.

Oodally et al. (2019), who obtained similar results, justified that the voltage gradually increased over time due to the high initial N-NH₃ concentrations, which may have harmed the plants and therefore compromise their metabolic and photosynthetic activities. As the N-NH₃ concentrations dropped and plants acclimated to the wastewater conditions, it is likely that the plants increased cell voltages through the release of oxygen and exudates by the roots.

From Fig. 6, it is possible to verify that the overall mean voltage values in open circuit decreased over time for most plant species, from 77.5 mV in the 3 first cycles to only 29.9 mV in the last three. Additionally, one can observe that although the mean voltage values were lower in the closed circuit configuration than in the open circuit, the values obtained in the latter configuration followed the same tendency as well as similar values than the results obtained in the last cycle (week 6) of the open circuit monitoring. This overall reduction in voltage is likely to be related to differences in the wastewater characteristics (such as in COD concentrations), and may also be related to a growth in the biofilm thickness in the floating electrodes, which can result in less contact of the graphite surface with oxygen and other electron acceptors, thus reducing the cathode redox potential.

It is important to highlight that the closed-circuit monitoring (Fig. 7) was conducted after the COVID-19 pandemic started, and since real wastewater was used for feeding the treatment system, the characteristics of the raw wastewater were considerable affected due to the reduced number of people in the university campus. This was reflected for example in the BOD and COD values, what may also have impacted in the voltage values, since as demonstrated by Xu et al. (2017), COD concentrations can considerable influence bioenergy generation.

Although COD directly affects bioelectricity generation, exoelectrogenic bacteria are only capable of oxidizing simple carbohydrates. Consequently, complex organic substrates such as particulate organic found in real wastewater must be converted to fatty acids before used (Corbella and Puigagut, 2018). In addition, CWs operated in batch mode and fed with high COD loads tend to present a decrease in DO concentrations due to oxygen consumption and to develop a more anoxic or anaerobic conditions over time (Srivastava et al., 2020). Therefore, if no further oxygen supply is provided to the cathodes, redox gradient may decrease and thus the bioelectricity potential.

Similarly to the HRT, the treatment performance may also present a negative relation with the bioenergy generation. Given that the HRT in general has a positively relation with COD and BOD removals in FTWs (Chen et al., 2016), the potential bioenergy generation may have reduced due to lower concentrations of COD and BOD over time and thus the reduced availability of organic compounds for microorganisms to oxidize and convert into bioelectricity. In order to maximize bioenergy generation, some studies investigated the application of raw wastewater (without pre treatments) in MFC systems, as well as feeding the system in up-flow in order to increase organic matter on the bottom (anode). Although this operations modes may maximize bioenergy generation, these configurations may be unrealistic in large systems and therefore difficult the feasibility of these systems in large scale (Drotto et al., 2017).

In this context, during the present study slightly increases in voltages were observed in rainy days. Furthermore, October, which was the rainiest month, presented the highest overall voltage mean values (87.4 mV). This can be explained by an increase in DO concentrations on the shallow zones due to drop aeration during precipitation events, since when air makes sufficiently contact with water while water drops down, air-oxygen can be effectively transferred to water. Besides, dropping water can also promote waves in water surface, which can also promote higher air diffusion to water (Liu et al., 2016). Therefore, rain events may have increased redox gradient between shallow and deep zones in the water column, improving bioenergy generation.

Water surface coverage is another important aspect that may have impacted both treatment performance and bioenergy generation of FTW-MFC unit, since it can directly influence DO concentrations in FTW systems. Garcia Chance and White (2018), who evaluated the effects of plant density and surface coverage in DO levels in FTW systems, monitored three FTW configurations, one with 100% surface coverage and 100% plant density (20 plants), 100% coverage and 50% plant density (10 plants) and 50% surface coverage and 100% plant density. Similar to the present studies, DO never declined to less than 1 mg L⁻¹. However, using units planted with Canna flaccida, Garcia Chance and White (2018) obtained DO concentrations of approximately 6.1 mg L⁻¹ for the 50% coverage and plant density system, 4.5 mg L⁻¹ for the 100% plant density and coverage, and 4.3 mg L⁻¹ for the 100% coverage and 50% plant density. On the other hand, with Juncus effusus, the obtained DO concentrations did not vary much, 3.4, 2.7 and 2.9 mg L⁻¹, respectively.

Given that the presence of a buoyant support in a FTW system may act as a barrier for oxygen diffusion into wastewater (Borne et al., 2015), since they can reduce gas exchange between water and air, algae photosynthesis (through shading) and wind activity (Headley and Tanner, 2012), some authors recommend leaving some open areas in the water surface. Samal et al. (2019), for example, suggest a 1:1 open water surface/ floating mats ratio, or the water coverage of nearly 100% only when denitrification of nitrates is the main objective, since more anoxic conditions should be present.

The exception to the voltage decreases tendency for both open and closed circuit configurations was the cathode placed on the C. papyrus
Nanus plants, which was kept nearly constant and presented low variation over the seven days of monitoring. These plants showed the second longest length averages (22.3 cm), and visually the most developed rhizosphere zone. Therefore, one explanation to justify the stability in the voltage values is that even with the decrease in organic matter content in wastewater, the cathode biofilm was fed with oxygen and dissolved organic carbon released from the plant roots. According to Liu et al. (2020), plant species can greatly vary in terms of oxygen and organic carbon released by the roots and in the presence of heterotrophic aerobic bacteria in the rhizosphere. Nevertheless, Liu et al. (2020) highlight that if the roots reach the anodes, it may compromise the anaerobic conditions, hence reducing redox gradient and bioelectricity generation. In addition, increasing the diversity and the number of plant species in a FTW systems should also increase microorganisms diversity in root zones, possible improving both treatment performance (Colares et al., 2020) and bioelectricity generation (Kadam et al., 2018).

Some studies have adopted artificial aeration in FTW systems in order to improve both TN and COD removals as well as promote larger root development and consequently increase the surface area for biofilm growth, decreasing the required area for treatment (Colares et al., 2020). Furthermore, artificial aeration of the cathode area may also improve redox potential and bioelectricity generation (Doherty et al., 2015). However, artificial aeration usually implies in high increases in energy demand and additional costs to the treatment (Corbella and Puigagut, 2016).

Oodally et al. (2019) investigated the efficiency of different macrophyte species in laboratory scale batch-fed CW-MFC systems filled with LECA. The anode materials were granular activated carbon (grain size 1.7 × 3.3 mm, much higher superficial area than the present study) mixed with sludge from anaerobic reactor, placed on the bottom of the box below the root zone and separator (composed of permeable plastic sheet). The cathode consisted of platinum-coated carbon placed on the top of the stone layer. For the 5 first days, the systems were monitored and operated as open circuit and later as a closed system for 28 days and with 1000 Ω external resistors connected to the cathodes and anodes.

Oodally et al. (2019) verified higher open circuit voltages than the ones found in present study (240 mV for Cyperus prolifer, 170 mV for Wachendorfia thyrsiflora and 170 mV for Phragmites australis). In the closed-circuit, Phragmites australis initially produced 0.26 V and slowed increased to a maximum of 0.4 V, and then stabilized. The authors stated that the increasing voltage over time and the higher values when compared to the control system (without plants) should be related to the photosynthesis activity, since the presence of plants likely increased the cathode potential through the release of oxygen and exudates in its roots. Therefore, Oodally et al. (2019) concluded that plant species can significantly affect bioelectricity generation. Hence, when selecting the macrophyte it is desirable to use plants that can adapt quickly to wastewater and that can develop higher root specific area and biomass, since these aspects positively impact bioelectricity generation.

Yang et al. (2020), who investigated four different laboratory scale CW-MFC systems for municipal wastewater treatment (synthetic), aimed to evaluate the influence of macrophyte presence and plant species in the treatment efficiency and bioenergy generation in CW-MFCs. Authors monitored a control system (unplanted) and compared to three CW-MFC systems with different macrophytes: Iris pseudacorus, Hyacinth pink, and Phragmites australis, operating in open circuit and closed-circuit (connected to 1000 Ω resistances). Yang et al. (2020) verified that plant uptake was similar in open and closed-circuit systems, and that the presence of macrophytes considerable enhanced bioelectricity generation (88–97%), and the obtained maximum power densities ranged from 8.74 to 25.14 mW/m². Similar to the present study, authors also stated that the plant species can influence bioenergy generation in CW-MFC systems, and associated it to the release of oxygen by the plant roots.

In addition, Yang et al. (2020) indicated that the presence of dead plant tissues have negatively impacted bioenergy generation in CW-MFC systems, since part of the oxygen can be consumed in the cathodic zone in order to biodegrade dead plants and thus limiting voltage production. In this context, authors stated that periods of plants experiencing non-growing and withering seasons are an important challenge when up scaling CW-MFCs systems. Therefore, plant species selection in these systems should be addressed considering local climate and harvestings availability.

An up flow CW-MFC system was developed by Oon et al. (2016) for treating synthetic wastewaters (organic carbon, nutrient and buffer solution). The single chamber unit was filled with gravel and glass beads on the bottom of the box to promote better distribution of the wastewater. Electrodes were made of activated carbon (each layer estimated in 2544.69 cm²), and while the cathode was place near the surface in the plant roots zone, anodic chambers were placed in different depths and connected through an external resistance (1000 Ω) and later as an open circuit. Below the cathode, authors also integrated an optional supplementary aeration was also integrated. After 228 days of monitoring, it was verified that DO presented the same tendency as voltage values for both open and closed circuits, reaching similar voltage values to the present study. COD removed obtained (influent concentration 624 ml L⁻¹) reached 99% and NO₃⁻, 46%.

Regarding open circuit voltages, Oon et al. (2016) verified that maximum open circuit voltage values were found in 30 cm electrode spacing 1 M (314 mg L⁻¹ of COD) and 2 M (624 mg L⁻¹ of COD) were 286 ± 13 mV and 421 ± 16 mV, respectively. However, for the 20 cm spacing (smallest distance) mean voltages were only 20 ± 7 and 116 ± 12 for 1 M and 2 M, respectively, while maximum power density was 0.035 mW/m² for 1 M. On the other hand, higher voltage values were obtained with closed-circuit operation, with averages of 283 ± 11 (30 cm - 1 M) and 435 ± 18 (30 cm - 2 M), and power densities (mW/m²) of 36.48 and 92.99, respectively. Oon et al. (2016) justified the lowest values due to consumption of carbon on the bottom of the reactor and due oxygen diffusion from supplementary aeration into this anodic chamber, promoting an aerobic condition in the anodes, since no membrane separator was present. It was concluded that the system was overall more towards aerobic condition, and found DO concentrations of up to 3.5 mg L⁻¹ near the surface, probably due to supplementary aeration.

In another study, Kadam et al. (2018) developed a bench scale system integrating a floating bed with macrophytes and microbial fuel cell (cathodes in the rhizosphere region and anodes in a deeper region) for textile effluent treatment and energy generation. The systems were monitored after 4 days of treatment, and the unit with the MFC was augmented with a bacterium consortium for effluent treatment. Kadam et al. (2018) also investigated the performance of the system with and without the integration with the MFC unit and got similar results regarding BOD and COD. For BOD, the removal efficiency went from 70 to 75% after the integration, whereas for COD a slight improvement, from 66% to 75%, was obtained. Through the polarization curve, internal resistance of the system was calculated as 25 Ω, while the maximum produced power and current densities were of 0.0769 W m⁻² and 0.3846 A m⁻², respectively. Although Kadam et al. (2018) evaluated the treatment of real textile wastewater, the COD and BOD, contents were much higher than in the present study, of 1734 ± 1.8 and 1478 ± 1.5, respectively, which may also have directly affected the bioenergy generation.

The potential bioelectricity produced can be used for several applications. Different research groups have investigated practical usages of the produced energy in bioelectrical systems, for example for biohydrogen and biofuel production, charge and discharge of ultracapacitors, onsite BOD sensors, wireless temperature sensors, smartphone charging and also light emitting diode (LEDs) for internal lighting (Guadarrama-Pérez et al., 2019).

In addition, the generated bioenergy may also be used for improving the treatment performance through the addition of another treatment unit, such as advanced oxidation processes (AOPs). Jayasree et al.
(2019), for example, who monitored a combined system for treating different synthetic and real wastewaters (municipal, dairy and cassava), developed a dual-chamber H type MFC unit and applied the biocurrent generated from this stage in another unit for promoting a peroxicoagulation process. The best results using real wastewater were obtained with the dairy effluent (rich in organic matter and carbohydrates), reaching maximum current output of 5.23 mA and removal efficiencies up to 94% for COD. Furthermore, the current generated and supplied to peroxicoagulation obtained 98% decolorization for rhodamine B (Rhb). Therefore, the application of MFC for providing power to peroxicoagulation process may be an efficient method for improving pollutant degradation and treatment performance.

It is important to highlight that in the present study real wastewater was used in the treatment system, which contains a complex composition and several aspects that can inhibit electrogentic bacteria and compromise bioenergy generation, differently from several studies in literature that used only synthetic wastewater and verified higher voltage values. Besides, if the FTW-MFC system was fed directly with raw wastewater (without the AF), better voltage values may be obtained due to higher concentrations of COD and BOD₅ in the effluent.

In accordance to other studies, root size positively impacted energy generation, due to oxygen release, exudates excretion and because it provides higher surface area to biofilm growth. Therefore, plant selection in a FTW-MFC system should aim for macrophytes with large root systems. On the other hand, HRT and sparse batch feeding negatively impacted bioenergy generation under open circuit, since mean voltages values tended to decrease over time, whereas in closed circuit configuration (with cathodes connected to 1000 Ω resistances) mean voltages in general did not present the same decreasing tendency. In this context, different HRTs and feeding interval may be investigated in order to improve bioelectricity generation. Therefore, further research should be developed to better understand and evaluate the influence of different design and operational conditions in order to maximize FTW-MFC treatment performance, for example inoculation of bacterium in the biofilm, and bioelectricity generation by increasing the stratification in the FTW systems and thus maximizing the redox differences between cathodes and the anodic compartment, for example through the application of zones with uncovered water surface, application of intermittent artificial aeration, HRT as well as the flow regime.

**CRediT authorship contribution statement**

Gustavo Stolzenberg Colares: Methodology - development of methodology, Investigation - conducting research, Writing - original draft.
Naira Dell'Osbel: Design of methodology, Investigation - performing experiments. Carolina Vieira Barbosa: Investigation - performing experiments and data collection. Carlos Alexandre Lutterbeck: Investigation - performing experiments, Writing - reviewing and editing. Gislayne Alves Oliveira: Investigation - performing experiments, Writing - reviewing and editing. Lucia Ribeiro Rodrigues: Conceptualization – ideas - provision of resources. Carlos Perez Bergmann: Conceptualization - ideas - research goal and aims. Diosnel Antonio Rodriguez López: Conception of the treatment system, Provision of resources - analysis tools. Adriane Lawisch Rodriguez: Conception of the treatment system, Provision of resources - reagents - materials. Jan Vymazal: Writing - review & editing. Enio Leandro Machado: Conception of the treatment system, Writing - review and editing. Supervision.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Acknowledgments**

Gustavo Stolzenberg Colares and Gislayne Alves Oliveira thank the CAPES “Comissão de Aperfeiçoamento de Pessoal de Nível Superior – Funding Support Code - 001”. Carlos Alexandre Lutterbeck thanks the Brazilian “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) for their financial support (Grant N. 157933/2018-0). Enio Leandro Machado acknowledges the financial support provided by FAPERGS “Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul”, PgG2017, CNPq-9 Project 307257/2015-0 and CNPq-Project 307599/2018-3.

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