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Combined microalgal photobioreactor/microbial fuel cell system: performance analysis under different process conditions

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Abstract

Increasing energy demands and greenhouse gases emission from wastewater treatment processes prompted the investigation of alternatives capable to achieve effective treatment, energy and materials recovery, and reduce environmental footprint. Combination of microbial fuel cell (MFC) technology with microalgal-based process in MFC-PBR (photobioreactor) systems could reduce GHG emissions from wastewater treatment facilities, capturing CO₂ emitted from industrial facilities or directly from the atmosphere. Microalgae production could enhance recovery of wastewater-embedded resources.

Two system MFC-PBR configurations were tested and compared with a control MFC, under different operating conditions, using both synthetic and agro-industrial wastewater as anolytes. COD removal efficiency (ηCOD) and energy production were monitored during every condition tested, reaching ηCOD values up to 99%. Energy recovery efficiency and energy losses were also evaluated. The system equipped with microalgal biocathode proved to be capable to efficiently treat real wastewater, surpassing the effectiveness of the control unit under specific conditions. Oxygen provided by the algae improves the overall energy balance of this system, which could be further enhanced by many possible resources recovery opportunities presented by post-processing of the cathodic effluent.

Keywords

Chlorella; Bioelectrochemical Systems; CO₂ recovery; Renewable energy; Wastewater treatment; Biorefinery
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1. Introduction

Fossil fuels combustion and CO$_2$ emissions from anthropic activities contribute to ongoing climate change effects, with the first decade of the new millennium registered as the warmest ever (Arndt et al., 2010). At the same time, water systems, including wastewater treatment facilities, have been indicated among major energy consumers at municipal level worldwide (Rosso and Stenstrom, 2008). It was estimated that they alone may require 1-3% of the total electrical energy output of a country (US DOE, 2014). Current wastewater treatment state-of-the-art technology requires energy consumption between 20 and 45 kWh/PE-year (population equivalent), consequently, it is not only highly energy intensive, but also a significant sources of greenhouse gas (GHG) emissions (Sabba et al. 2018), whose reduction has been recently mandated by European Union and other countries’ policies. Energy savings and wastewater valorization by exploitation of its residual resources content, may provide significant contribution to Circular Economy and GHGs reduction (Capodaglio and Olsson, 2020). Based on current knowledge, novel concepts of biorefinery could be developed to satisfy the need of more sustainable environmental protection technology and, at the same time, recover necessary energy and resources (Cherubini, 2010).

Microbial fuel cells (MFCs) are a promising technology for wastewater treatment, characterized by electrical energy recovery coupled with low greenhouse gases (GHG) emissions and reduced sludge production (Capodaglio et al., 2013). MFCs convert the chemical energy of organic pollutants (substrate) directly into electrical energy, catalytic activity of electrochemically active bacteria (EAB) (Logan et al., 2006; Molognoni et al., 2018), potentially achieving higher conversion efficiency (44%) than a conventional anaerobic treatment (28%) (Luo et al., 2017; McCarty et al., 2011).

In MFCs, bacteria at the anode oxidize organic substrates producing CO$_2$, protons and electrons; electrons flow through an external circuit to the cathode, producing electrical energy and closing the redox reaction (Capodaglio et al. 2015). Energy production is limited, among many factors, by the availability of a terminal electron acceptor (TEA) at the cathode, most commonly, oxygen (Bolognesi et al., 2020). CO$_2$ is released by oxidative treatment of organic matter and is also
contained in MFC anodic effluents; its sequestration could be helpful to decrease global CO₂ emissions, even at low emission rates, whenever feasible. Recently, the use of microalgae for co-treatment of wastewater was proposed, being an effective process for both resources recovery and CO₂ sequestration (Gabriel et al., 2018; Wang et al., 2010).

Microalgae, on the other hand, are well known as potential candidates as feedstock in biorefineries (third generation feedstocks) for biofuels and biomaterial production. They can in fact generate recovered raw materials more sustainably than first and second generation feedstocks, with lower land footprint and no food crops competition issues (Callegari et al., 2020; Chew et al., 2017). They contain lipids, minerals, carbohydrates and proteins that could be elaborated into valuable products, such as biofuels, primary chemicals, food, livestock and aquaculture feed and other value-added products (Kothari et al., 2017). Microalgae also contribute to CO₂ emissions reduction, due to their photosynthetic nature. Microalgae can be grown under different conditions: open ponds, closed reactors (such as photobioreactors, PBR) and in different types of water, including nutrient-rich wastewater (Richmond, 2004).

Combining MFC technology with algal metabolism, e.g. by coupling a PBR to a MFC cathode, could be an advantageous process improvement: (i) achieve sustainable wastewater treatment (carbon and nutrients removal) by an emerging green technology, MFCs, with low gaseous emission and low solids production, and consequently reduced sludge production (Logan and Rabaey, 2013); (ii) energy recovery by direct conversion of chemical energy in electrical energy (Capodaglio et al., 2016); (iii) CO₂ capture by microalgae with conversion into process-required TEAs and oxygen (Jiang et al., 2013); (iv) production of biofuels or valuable recovered materials from algal process residuals (Brennan and Owende, 2010; Goglio et al., 2019). It is important to point out that in such scheme, microalgae could equally capture anode-produced CO₂, or alternatively utilize gaseous effluents originated from an industrial facility, or atmospheric CO₂, converting it into oxygen (Wang et al., 2019). Some authors explored the possibility of enhancing nutrients removal by using algal biocathodes (Nguyen and Min, 2020). Based on these premises, interest on MFC-PBR systems has
increased in the latter years amongst the research and professional communities (Cui et al., 2014; Do et al., 2018; Gouveia et al., 2014; Khazraee Zamanpour et al., 2017).

Light/dark cycles influence O₂ production, growth rate and algal stress of these processes, and consequently may affect both bioelectricity production and possible recovery products from the effluent, affecting the global energy and economic balance of the system (León-Vaz et al., 2019).

This study evaluates the influence of lighting conditions and electron acceptor supply in an MFC-PBR unit operated both on synthetic substrate (acetate) and real agro-industrial wastewater as anodic feed, in long-term operation (4 months). Energy losses were evaluated under two different aeration conditions in the second part of the study, to highlight how TEA availability affects system performance.

2. Materials and methods

2.1 Experimental setup and operation

Two identical double-chamber MFCs (MFC1 and MFC2, respectively) were built and operated as described by Cecconet et al. (2018). Each MFC consisted of an anodic and a cathodic chamber, both filled with 800 g of granular graphite (diameter 1.5-5 mm, model 00514, EnViroCell, Germany), decreasing the free volume to 430 mL net anodic (NAC) and cathodic (NCC) compartment. The two chambers are separated by a cationic exchange membrane (CEM, CMI-7000, Membranes International Inc., USA). A graphite rod (250 x 4 mm, Sofacel, Spain) was used as electron collector.

The external electrical circuit was closed by using a 33 Ω resistance as a load. This value was assumed to be as close as possible to the static internal resistance of the system, as confirmed by polarization curves operated on the system and reported in previous works (Molognoni et al., 2018, 2014).

The study herein reported was divided into two phases, according to variations of system configuration. In the first phase, two different anolytes were tested: acetate solution (1 g L⁻¹) during period I, then dairy wastewater (DW, collected periodically from a nearby cheese factory) in period II. These were fed continuously, at flow rate of 1 L d⁻¹. In the second phase, only DW was fed to the
units. Characteristics of DW varied throughout the study following the production schedule at the factory. DW was stored at 4°C until use to limit bacteria activity, and then fed to the system using collapsible 10 L jerry cans to limit contact with the atmosphere. Table 1 summarizes anolyte characteristics during the study.

Table 1 – Summary of the characteristics of the influents used throughout the study.

<table>
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<th>Substrate</th>
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<th>Conductivity [mS cm⁻¹]</th>
<th>COD_IN [mgCOD L⁻¹]</th>
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</table>

A similar feeding mode was adopted for the cathodic chambers, fed with a phosphate buffer solution (PBS, 10 mM, pH 7) containing macroelements and an inorganic source of carbon (507 mg L⁻¹ NaH₂PO₄, 819 mg L⁻¹ Na₂HPO₄, 1000 mg L⁻¹ NaHCO₃, 130 mg L⁻¹ KCl, 310 mg L⁻¹ NH₄Cl, modified...
from Xia et al., (2013)). In MFC2 recirculation of the effluent from the PBR was also returned to the cathode, as explained in Section 2.2. An internal recirculation loop was activated in each chamber to achieve well mixed conditions within.

2.2 First phase

During the first phase of the study, both MFCs were operated for 60 days, 32 using acetate as substrate, 28 using undiluted raw DW; MFC2 was coupled with the PBR, while MFC1 was individually operated as control. This phase was sub-divided in two separate periods, each corresponding to a different substrate used as anolyte for the MFCs: synthetic wastewater (acetate) in period I, DW in period II. Oxygen (from air) was selected as electron acceptor, introduced according to two different methods in the two MFCs. In the MFC2 setup, a PBR unit consisting of two methacrylate tubular reactors (d=0.03 m, h=0.3 m) and containing a mixed culture of microalgae (Chlorella) was operated in the cathode recirculation line. Microalgae Chlorella converted CO₂ (captured from the atmosphere or from the gaseous effluent produced by the anode) into oxygen during daytime. Two different configurations for MFC2’s TEA supply were tested during the study: PBR-aerated (PBR-air) configuration, with a fish tank air pump connected to the PBR via an aeration buffer unit to introduce ambient air (CO₂ for conversion, and O₂); and CO₂-capture configuration, in which the PBR was attached to a gas phase separator receiving both liquid and gaseous effluent from the anodic chamber, exploiting the anodic bacterial produced biogas containing CO₂. Gas phase was pushed to the cathodic chamber by the increasing volume of liquid effluent in the methacrylate tube. PBR light source consisted of a conventional led bulb (40 W). Cathode effluent was collected from an overflow device in the topmost section of the PBR. MFC1, acting as experimental control, was equipped with an aeration buffer in in the cathodic recirculation loop, to obtain an oxygen-saturated catholyte. The exhausted catholyte was expelled from the system via an overflow in the same buffer. In either case, the ensemble of cathode plus aeration buffer/PBR will be referred to, from now on, as
“cathode system”. *Chlorella* was cultivated into an external reactor, and changed in the PBR every 9-10 days (two feeding cycles). The complete experimental setup is illustrated in Figure 1.

![Figure 1](image_url)

**Figure 1** – Experimental setup configuration in the first phase. (a) MFC1 with aeration buffer. (b): MFC2 setup with PBR. A: Anode. C: Cathode. R<sub>ext</sub>: External resistor. (1) Feeding pump; (2) recirculation pumps; (3) aeration buffer; (4) photobioreactor (PBR); (5) CO<sub>2</sub> separator. Orange lines: anodic chamber feeding and recirculation line. Blue lines: cathodic chamber feeding and recirculation line. Black dotted lines: air supply. Dashed lines: effluent discharge.

PBR performance in MFC2 was evaluated under six different conditions for each period, by varying lighting conditions (light/dark ratio 16/8, 12/12, 24/0) and CO<sub>2</sub> supply conditions (PBR-air and CO<sub>2</sub>-capture), while MFC1 was operated as a control throughout the experiment with the same substrate. Each test lasted 4-5 days, and all tests were executed in succession. A summary of the operational conditions operated during the first phase is reported in Table 2.

**Table 2** - Operational conditions throughout the first phase of the experimentation for MFC2. DW: dairy wastewater

<table>
<thead>
<tr>
<th>Test</th>
<th>Substrate</th>
<th>CO&lt;sub&gt;2&lt;/sub&gt; source</th>
<th>Light/dark ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Acetate</td>
<td>PBR-Air</td>
<td>16/8</td>
</tr>
</tbody>
</table>
During the second phase of the study the two systems were configured as shown in Figure 2: raw DW was fed as anolyte, as in the first phase of the study; both systems cathodes were coupled to a PBR, containing microalgae, applying the best dark/light condition (16/8) determined in the first phase, but with different TEA supply conditions. MFC1 was operated under PBR-air mode, while MFC2 was equipped with the CO₂-capture system. Each test cycle lasted 5 days (except for two cycles lasting only 4 days), for a total duration of 58 days (12 cycles). The aim of the second phase was to highlight the different performance of the two systems under the same conditions and characteristics, except for the TEA-supply method. During this phase, energy losses of the two MFC-PBR systems were evaluated to determine advantages and drawbacks of each configuration.
Figure 2 – Experimental setup configuration in the second phase. (a) MFC1 under PBR-air configuration. (b): MFC2 under CO₂-capture configuration. A: Anode. C: Cathode. R<sub>ext</sub>: External resistor. (1) Feeding pump; (2) recirculation pumps; (3) aeration buffer; (4) photobioreactor (PBR); (5) CO₂ separator. Orange lines: anodic chamber feeding and recirculation line. Blue lines: cathodic chamber feeding and recirculation line. Black dotted lines: air supply. Dashed lines: effluent discharge.

2.4 Data analysis and evaluation

Anodic potentials were monitored with an Ag/AgCl reference electrode (+197 mV vs Standard Hydrogen Electrode, Xi’an Yima Opto-electrical Technology Co., China) and recorded at 1-min intervals by an automatic data acquisition system (NI USB-6008, National Instruments Italy, Milan) connected to a PC. Overall MFC potentials were recorded with the same time interval, power (P) was determined from continuous current (I) and voltage measurement (V). Current (dI) and power (dP) densities were then calculated dividing the respective value of I and P by the NAC volume of each compartment. Anodic coulombic efficiency (CE) was computed as described in Cecconet et al. (2018). Determination of effluent COD (one sample per MFC per test) and acetate/wastewater influent COD (one common sample for every feed bag refill) was performed using a spectrophotometer (HI83224 Wastewater Treatment Photometer, Hanna Instruments, Italy). Organic
matter removal efficiency ($\eta_{\text{COD}}$ - %) was determined as described in Molognoni et al. (2014). Conductivity and pH were measured at least once during every test for both anode and cathode influents and effluents (IntelliCAL™ probes + HQd™ Digital Meter, Hach Lange, Italy).

The normalized energy recovery (NER) of the MFCs, a parameter that expresses the amount of energy recovered per removed mass of COD (NER$_S$, kWh kg$^{-1}_{\text{COD removed}}$) and per volume of treated wastewater (NER$_V$, kWh m$^{-3}_{\text{treated}}$) was calculated for each period and for the total experiment with the following equations, as proposed in Ge et al. (2014):

$$NER_V = \frac{P \cdot t}{V_{treated}}$$  \hspace{1cm} (1)

$$NER_S = \frac{P \cdot t}{kg_{\text{COD removed}}}$$  \hspace{1cm} (2)

Energy loss factors were calculated, corresponding to each available polarization curve, using the energy balance equation with the methodology reported by Molognoni et al. (2014). In particular, anode and cathode overpotentials ($\eta_{\text{An}}$ and $\eta_{\text{Cat}}$), ionic (Eionic), pH gradient (E$\Delta$pH) and membrane transport losses (Et) were evaluated. Ohmic losses other than ionic were not directly measured, but included in the terms $\eta_{\text{An}}$ and $\eta_{\text{Cat}}$ (Sleutels and Hamelers, 2009).

3. Results and discussion

Results for the first and second phases are presented separately, since each one focused on a different specific aspect. The aim of the first phase was to evaluate the system’s energy recovery performance and substrate conversion efficiency, by using both synthetic and real wastewater, under different conditions. During the second phase, where MFCs were fed only with DW, evaluation of PBR CO$_2$ conversion efficiency was the main focus.

3.1 Electrical production
The first operational period was characterized by the use of a synthetic substrate as anodic feed. Organic loading rate (OLR) was nearly constant during this phase (1.25 ± 0.06 kgCOD m$^{-3}$ d$^{-1}$), lasting 32 days. Figure 3a shows voltage generated by the two MFCs during tests I-VI.

MFC1 showed constant electricity production throughout this phase, due to the simple characteristics of the treated substrate, achieving an average voltage of 409.71 ± 46.10 mV (corresponding to a current density of 28.28 ± 2.57 A m$^{-3}$). MFC2 performance overall was less stable, and more susceptible to variability in different feeding periods due to changes of cathodic conditions. It can be noted that alternation of light and darkness influenced electric production, due to varying availability of oxygen as cathodic TEA. Generally, direct atmospheric O$_2$ supply led to better performances (as shown in tests 1, 3, 5) than supply of captured anode-produced CO$_2$ and subsequent conversion into O$_2$ by algae: in the former case, difference between day/night conditions were detectable, but not inducing large variations in electricity production, with electrical performance presenting an overall increasing trend. During test I and V, electricity production of MFC2 overtook MFC1, achieving the highest voltage of the whole experimentation (573.92 mV). Test under CO$_2$-capture conditions (2, 4, 6) instead, were more likely influenced by the activity of algae at the biocathode, and presented high voltage drops during night-time, and an overall lower energy production. Light/dark alternation periods seems to influence both availability of TEA and algal stress, resulting in optimal oxygen production with the 16/8 sequence in the atmospheric-aerated test. As for the CO$_2$-capture configuration, the best electric production was achieved with the 24/0 sequence, although increased algal stress by constant lighting caused a big voltage drop in day 31. Stress conditions for algae entail metabolic changes, affecting metabolic rates. In this case, stress limited photosynthetic activity efficiency in the long run.
Figure 3 - Voltage from MFC1 and MFC2 in the first phase of the experimentation a) with acetate feed; b) with dairy wastewater. Light/dark ratios for MFC2 are reported in the graph. Odd numbers: PBR-air configuration; even numbers: CO₂-capture configuration.

As for period II, during which the MFCs were fed with DW, results are more difficult to interpret, due to the variability of the influent itself, and hydrodynamic issues related to the nature of DW, frequently causing obstructions in the feeding line, which required extra maintenance (Cecconet et al., 2018). Figure 3b shows the voltage profile observed in period II. Although the absolute value of current produced was lower, the voltage gap observed between MFC2 and control MFC1 decreased; in tests 7, 9 and 11 (PBR-air configuration) the two profiles are very close. In CO₂-capture
configuration tests (8, 10, 12) the gap is still high, especially in test 12, with algal stress causing voltage drop earlier than in the corresponding test with acetate. Comparing average voltage measured during periods I and II in the two MFCs, a voltage drop due to the change from synthetic to real wastewater in the control experiment is obvious: MFC1 accounted for 387.60 ± 85.65 mV in period I, against 290.24 ± 130.46 mV, when using DW as anolyte, with difference of about 100 mV. A different behavior is observed for MFC2, with average voltage of 286.77 ± 102.53 measured in period I, and of 236.77 ± 97.53 mV in period II. Comparing performance in terms of generated voltage for the two systems in each period, it is evident that MFC1 energy production in period I was significantly higher, while the difference with DW as anolyte between the two systems is not that relevant. When using an easily biodegradable substrate, such as acetate, electron transfer efficiency is limited by cathode TEA availability only. This is obviously lower in MFC2 since it depends on light availability, and algae respiration during night-time. It is encouraging, however, the gap reduction when using real wastewater as substrate: substrate complexity in fact slows down the anodic reactions, limiting the amount of electrons released by substrate degradation, and consequently reducing the limiting influence of microalgal metabolism on cathodic activity.

In the second phase, microalgae were applied at both cathode systems, under a 16/8 light/dark sequence and DW feed. Under PBR-air configuration, the performance of MFC1 showed higher variability in generated voltage and, overall, lower current productions were observed in both MFCs. Average MFC1 voltage throughout the whole phase was 299.34 ± 133.91 mV (corresponding to an average current density of 20.61±9.27 A m⁻³). The difference with MFC2 (in CO₂-capture configuration) was lower, because the main factor that affected electricity production was the nature of the substrate. MFC2 achieved an average voltage of 231.42 ± 98.70 mV, corresponding to a current density of 15.91 ± 6.83 A m⁻³. Voltage monitored in this phase of the Study is reported in Figure S1 (supplementary information).
3.1 Organic matter removal efficiency and energy efficiency

Organic matter removal (ηCOD) was evaluated throughout the study. MFC1 and MFC2 showed similar behavior in terms of organic matter removal efficiency, with slightly better performance by MFC1, achieving COD removal of 91 ± 8% against 85 ± 14% of MFC2 (Figure 4). With acetate as influent, COD removal efficiency of MFC1 overcame the one obtained by MFC2 (Table 3), while the opposite happened with DW as a feed, where MFC2 achieved the best results, except for test 11, in which the lowest organic matter removal efficiency (56 %) was observed. CE varied throughout the study, depending on the influent feed, and on the TEA supply method, with slightly better results for MFC2. CE of both systems was higher when using acetate as a substrate rather than in the case of DW anolyte: its highest values (17.2–17.7% for MFC1, 23.2–23.8% for MFC2) were obtained with this substrate. The best results in MFC2 operation were achieved in tests under PBR-air mode, due to greater TEA availability at the cathode (Test III and V). The same trend was seen also in test with DW as influent, where PBR-aeration tests overcame CO2-capture tests in terms of CE values. In PBR-air configuration MFC2’s CE was even higher than MFC1’s. The lowest CEs (5.3% for MFC1 in Test 11, 3.2% for MFC2 in Test 12) were observed with DW as feed, for both MFCs. For MFC2, this value confirmed the low efficiency of continue lighting. While OLR in acetate-feed tests was constant, in DW tests OLR variability was dependent on variable substrate characteristics. Tests under DW feed were generally characterized by higher OLR (average: 2.09 kgCOD d⁻¹); observed results confirmed reports from previous studies: in presence of high OLR, MFCs tend to develop methanogenic biomass, competitive to EABs, which leads to higher COD removal efficiency, while decreasing MFC’s electric efficiency (Molognoni et al., 2016). Results concerning the second phase have been reported extensively in SI, figure S3. Methanogens also consume organic substrate, increasing the overall COD removal of the system. The present study achieved comparable results in terms of CE and better results in terms of ηCOD (up to 10% more) than previous experiences of the group on similar substrate, operating in the same configuration as MFC1 in the first phase, meaning that the addition of microalgae improved systems’ efficiency (Ceconnet et al., 2018).
Figure 4 – OLR, COD removal and CE for MFC1 and MFC2 throughout the first phase of the study: a) acetate; b) DW. MFC acted as a control with no microalgae in the system.

Table 3 - Values of $\eta_{\text{COD}}$, CE, NER$_V$ and NER$_S$ under different substrate and TEA supply conditions in period I.
Net energy recovery (NER) was evaluated for both systems, which achieved comparable NERV and NERS values (Figure 5). The best performance in terms of NER in the MFC-PBR system was achieved in Test 3 (0.131 kWh m⁻³ and 0.285 kWh kgCOD⁻¹ removed, respectively), while the lowest performance was obtained in the 24/0 light sequence, CO₂-capture configuration. While NERV does not highlight any coherent pattern in the data, an analysis of NERS data shows that, in tests under PBR-air configuration, MFC2 overcame MFC1 (except for the very first test). Comparing NERS plot with ηCOD’s in the first phase with synthetic wastewater, both COD removal efficiency and power production were higher for MFC1, explaining why this specific indicator value is lower. In the second phase with DW as anolyte, ηCOD is higher for MFC2 under two out of three conditions tested (16/8 and 12/12 light sequence), meaning that under these conditions energy recovery is more efficient in the microalgal cathodic configuration. This information is further confirmed by the volumetric normalized indicator, higher than that reported for MFC1, proving that a PBR-biocathode could be beneficial for energy production when using raw DW wastewater as anolyte.
Figure 5 - NER$_s$ (a) and NER$_v$ (b) throughout the first phase of the study.

In the second phase, with DW as feed, energy recovery decreased significantly, achieving values lower than in the second period of phase one (figure S2, supplementary info). The maximum NER$_v$ value reached in CO$_2$-capture configuration (MFC2) was 0.041 kWh m$^{-3}$, while in PBR-air configuration (MFC1) was 0.061 kWh m$^{-3}$. As for NER$_s$, MFC2 maximum value reached 0.092 kWh kgCOD$^{-1}$, while MFC1’s was 0.086 kWh kgCOD$^{-1}$.

3.2 Light/dark ratio and CO$_2$ availability influence on MFC performance
Light/dark sequence affects electricity production, as shown in Figure 6. PBR-air configurations show more stable current productions, even at night-time when algae activity is limited to respiration, consuming oxygen produced during the day. It can be noticed that the 16/8 PBR-aerated operation (Figure 6.a) is the best in terms of current density production (maximum density 39.18 Am⁻³), with an overall growing trend and low reduction in dark conditions. Under CO₂-capture configuration and 12/12 light/dark sequence (Figure 6.d) more stable current output conditions are reached, with current production up to 24.13 Am⁻³, decreasing in dark conditions. Under 24/0 sequence (Figure 6.e, f), current production is quite stable under light, due to consistent availability of TEA; however, after four days of operation the CO₂-capture configuration (Figure 6.f) shows decreasing energy production, due to excessive algal stress, causing inhibition of algal activity.

Unfortunately, in tests with DW these differences were less consistently detectable due to variable nature of the substrate, leading to some unpredictability in results (voltage drops were sometimes linked to obstructions in feeding/recirculation lines, in addition to the varying quality of the substrate).

Day/night behavior with DW is represented in Figure S4 [SI].
Figure 6 – MFC2 performance under different light/dark sequence with acetate as feeding substrate:

a) 16/8 with PBR-air; b) 16/8 with CO₂-capture; c) 12/12 with PBR-air; d) 12/12 with CO₂-capture;

e) 24/0 with PBR-air; f) 24/0 with CO₂-capture.

4. Energy losses: differences in PBR-air and CO₂-capture setups

Energy losses represent the difference between MFC electromotive force (i.e. theoretical maximum voltage) and measured voltage at the electrodes. Losses depend on several factors: anode and cathode overpotentials, membrane overpotentials, pH and conductivity (ionic) gradients are easily detectable by performing polarization and power curves. Drawing a polarization curve is an important diagnostic method through which MFC performance efficiency can be assessed, determining also the best external resistance ($R_{ext}$) value to achieve a MFC’s maximum performance, for example applying the maximum power point tracking (MPPT) technique (Molognoni et al., 2014). Different strategies can be used to overcome or mitigate the problem of energy losses, maximizing energy recovery.
An example of polarization curve performed during the experimentation is shown in Figure 7.

**Figure 7** – a) Example of polarization and power curve (day 28). Orange: MFC1, green: MFC2. Triangles highlight power curves, dots polarization curves. b) Distribution of energy losses at day 28.

In the present study, it was determined that cathode overpotentials accounted on average for 45% of MFC1’s losses, 44% of MFC2’s, while membrane overpotentials for 22% in the PBR-air configuration, and 31% in the CO2-capture configuration. Anodic overpotential and pH gradient only moderately affected energy losses balance. Low pH gradients (between anode and cathode chambers) of maximum one pH unit granted lower losses (less than 10%) than in previous experiences, where significantly higher losses (23%, 2 pH-units) were detected (Molognoni et al., 2018). Anode overpotential accounted on average for 15% of total losses in both MFCs, while electrolyte overpotentials (E_{\text{ion}}) could be considered negligible, representing less than 1% of overall losses, due to low difference in conductivity between anode and cathode media (1.3 ± 0.4 mS cm^{-1} for anolyte, 2.6 ± 0.5 mS cm^{-1} for catholyte). Anodic overpotential may be caused by increased methanogenic community activity. Comparing the first and the second phases’ anodic influents, it can be noticed that pH values increased in the latter, reaching pH up to 8, a value suitable for development of a methanogenic biomass, although no microbial analysis were performed to confirm this hypothesis.

Feeding an influent with lower pH, pH-gradient related losses would increase; these could be reduced
by modifying the system’s hydraulic retention time, or by varying its design. Data collected in this phase for MFC1 and MFC2 are reported in Figure 8.

As reported in literature, cathode overpotentials may be reduced by: (i) introducing new, more efficient electrode and catalyzer materials; (ii) improving oxygen transfer kinetics at the cathode; (iii) developing a biocathode. Algal biocathodes, as shown from experimental data of this study, seem to reduce electron transfer efficiency, due to increase in membrane and electrode fouling. However, no significant difference in cathode overpotential was detected between the unit purged with air and the one relying only on anodic CO₂ conversion. Membrane overpotentials could be reduced by introducing different materials characterized by lower internal resistance, or less subject to biofouling.

![Figure 8](image)

**Figure 8** – Energy losses in MFC1 (a) and MFC2 (b), respectively.

### 5. Energy and circular economy considerations

Few authors explored the possibility of coupling MFC and microalgae. Table 4 reports a summary of studies found in literature, allowing a comparison between the present work and other experiences. It is possible to notice that the system configuration used in this study overcame other architectures’ power productions.
Table 4 – Reported studies of MFC with microalgae.

<table>
<thead>
<tr>
<th>MFC type</th>
<th>Influent type</th>
<th>Power production</th>
<th>CE [%]</th>
<th>ηCOD [%]</th>
<th>Microalgal species</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two chambers</td>
<td>LL + MW</td>
<td>0.517 W m⁻³</td>
<td>-</td>
<td>96.8 (A)</td>
<td>Not specified</td>
<td>(Nguyen et al., 2017)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.050 W m⁻²</td>
<td>-</td>
<td>0÷56.8 (C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular, external PBR</td>
<td>MW (diluted)</td>
<td>0.006 W m⁻²</td>
<td>-</td>
<td>80.8</td>
<td>Chlorella</td>
<td>(Kakarla and Min, 2019)</td>
</tr>
<tr>
<td>Two chambers</td>
<td>SUW</td>
<td>0.031 W m⁻²</td>
<td>&lt;1</td>
<td>40.0÷90.0</td>
<td>C. vulgaris</td>
<td>(Gonzalez et al., 2015)</td>
</tr>
<tr>
<td>Tubular</td>
<td>MW</td>
<td>0.124 W m⁻³</td>
<td>57÷78</td>
<td>4.1÷5.5</td>
<td>C. vulgaris</td>
<td>(Bazdar et al., 2018)</td>
</tr>
<tr>
<td>Two chambers + PBR</td>
<td>AC</td>
<td>2.8 ± 0.9 W m⁻³</td>
<td>16 ± 5</td>
<td>65.3÷97.2</td>
<td>Chlorella</td>
<td>Present study, first phase (AC)</td>
</tr>
<tr>
<td>Two chambers + PBR</td>
<td>DW</td>
<td>1.9 ± 0.5 W m⁻³</td>
<td>9 ± 4</td>
<td>56.1÷98.1</td>
<td>Chlorella</td>
<td>Present study, first phase (DW)</td>
</tr>
<tr>
<td>Two chambers + PBR</td>
<td>DW</td>
<td>2.5 ± 0.4 W m⁻³</td>
<td>7 ± 3</td>
<td>85.5÷99.9</td>
<td>Chlorella</td>
<td>Present study, second phase</td>
</tr>
</tbody>
</table>

AC: acetate; DW: dairy wastewater; LL: landfill leachate; MW: municipal wastewater; SUW: synthetic urban wastewater.

Using microalgae as oxygen providers in a MFC system can improve its overall energy balance by decreasing the cost of aeration for TEA supply. The presence of microalgae can also improve the overall energy and economic balance of waste substrate treatment, by exploiting different materials and biofuels precursors potentially recoverable from conversion of algal biomass. Liquid biofuels, e.g. biodiesel, bioethanol, biobutanol and jet fuels, are the most likely outcomes of algal biorefining (Dasan et al., 2019; Liang et al., 2015). Biodiesel may be obtained from oil extraction and following transesterification, with properties complying with EU specifications, bioethanol and biobutanol may be derived from algae fermentation processes (Callegari et al., 2020), while biochar may be obtained by thermal treatment (Yu et al., 2017). One of the major challenges with microalgae is to achieve efficient and inexpensive oil extraction (Chiew and Shimada, 2013). International regulations and shrinking of fossil fuels reserves will expand the renewable energy market in the next decades. Algal biomass has been indicated as a major component of the future eco-fuel panorama (Callegari et al., 2020), even though, considering current market prices of liquid biofuels, they are still not an
economically appealing solution per se, with production costs higher than traditional fossil fuels. Lundquist et al., in fact estimated the cost of large scale production of algae-derived oil from wastewater at 332 $ per barrel when focusing on oil production alone; however, when considering wastewater treatment as the main focus, with algal biomass recovered as a by-product precursor of oil, the calculated cost of algae-derived oil would drop to 28 $ per barrel (lower than the average cost of crude oil) (Lundquist et al., 2010).

Microalgae can also be considered a feedstock for chemicals and materials recovery, such as slow-release fertilizers, since they are capable of accumulating surplus quantities of nutrients, recoverable as dried microalgal biomass or biochar from pyrolysis (Bolognesi et al., 2019). Biofertilizers and biostimulants appear to be one of the most economically appealing fields in algal technology, with market prices in the range of 9-23 € kg-1 for biostimulants, and 0.2-0.5 € kg-1 for biofertilizers (Voort et al., 2015). Anticipated climatic changes and increasing costs of fertilizers due to reserve shortages (Daneshgar et al., 2018) will open the agronomy field to new green biostimulants development.

Finally, the nutritional value of microalgae could open the possibility for their use in the food and feed (aquaculture or livestock) market, however, food, feed and pharmaceutical reuse of algae grown in wastewater treatment processes still present issues of social acceptance; so far, the most favorable market outlets for microalgal recovery consist of biofuels production, biofertilizers and soil amendment products.

6. Conclusions

This study aimed at evaluating the performance of an MFC-PBR system treating synthetic (acetate and real (dairy wastewater) substrates with energy biorecovery under different operational conditions, and to establish optimal process configuration. Two systems of identical base configuration were operated continuously for up to 60 days at a time, using the same substrate as feed, but using different TEA supply methods. Both systems proved to be effective for wastewater treatment (COD removal), and showed higher power density generation than similar systems described in literature studies.
However, concerning bioelectricity production, a traditional system proved to be more stable and better performing than the MFC-PBR under almost every condition tested, when using synthetic substrate. Systems’ performance gap reduced when passing from synthetic substrate to real wastewater feed, showing increasing performance of the MFC-PBR unit, as confirmed by the relative increase of NER$_S$ and NER$_V$, compared to the same parameters in the conventional unit. This fact was attributed to greater substrate complexity slowing down the anodic reactions in the better performing system, reducing the limiting influence of microalgal metabolism on cathodic activity. This indicates that MFC-PBR combination systems with microalgae may become a feasible option for sustainable wastewater treatment, when the key limitations of MFC will be solved.

Despite many efforts to increase these systems efficiency, in fact, the major issue in MFC technology is linked to internal energy losses, impairing net energy production and recovery, which unfortunately was not sufficiently improved by the introduction of algae as oxygen (TEA) providers. Several existing and envisioned possibilities of recovery and valorization of algal effluent, however, could help improve the overall economic and energetic balance of these system, at the same time reducing their atmospheric CO$_2$ impact.

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