ENERGY RECOVERY FROM WINERY WASTEWATER USING A DUAL CHAMBER MICROBIAL FUEL CELL

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Abstract

BACKGROUND: Microbial Fuel Cell (MFC) can treat agro-industrial wastewater, but only a few studies have reported the treatment of winery waste and much work is needed in order to develop this interesting application of MFC technology, in particular in evaluating how the unfavorable COD/N and COD/P ratios may affect the performance of MFC. In this work, a dual chamber MFC was used to treat actual effluents of wine processing factories.

RESULTS: MFC was not efficient in terms of COD removal, even when nutrients concentration was increased and daily removals which oscillate around 1000 mg L^{-1} d^{-1} are observed during the complete experimental period, with COD removals around 17%. Increases in the phosphorus and nitrogen concentrations influenced positively on the production of electricity. By increasing the concentration of phosphorus and nitrogen, Coulombic efficiency increased from 2% to almost 15% and maximum power density from 105 to 465 mW m^{-2}.

CONCLUSIONS: Results demonstrate that electricity can be efficiently produced and that the unbalanced nutrients/COD ratio is a major challenge in the treatment winery wastewater, in spite of the very high organic load contained in this type of wastewater.

Keywords

Bioelectricity, wastewater treatment, energy recovery, winery wastewater, microbial fuel cell

Highlights

- MFC fed with winery wastewater generate power with startup period lower than 1 week.
- maximum power density of 465 mW m^{-2} and removals of COD of 17% was achieved in MFC.
- unbalanced nutrients/COD ratio is a challenge in winery wastewater treatment in MFC.
- Increasing the concentration of nutrients, CE increased from 2% to almost 15%.

Introduction

Wineries are one of the most important agricultural industries in the European Mediterranean countries[1]. The annual wine production in Spain is around of 3.6 million tons, generating 8.0 million.m^3 of winery wastewater[2]. Winery wastewater is produced from washing operations during the crushing and pressing of grapes, as well as washing of fermentation tanks, barrels, machines, and production rooms[3]. These effluents contain huge amounts of biodegradable organics (up to 15 g BOD L^{-1}) and low concentrations of nitrogen and phosphorous[3, 4]. Therefore, the treatment and disposal of winery wastewaters is one of the main environmental problems in wine making industries.

In the last two decades, several methods have been proposed for the treatment of winery wastewater, being particularly interesting for this work the biological and the electrochemical technologies[5-10]. The use of these systems is effective for removing organic matter, but they require a high energy input, which increased the cost associated to the manufacturing of wine[1, 3, 4]. Nowadays, there is a tremendous need to develop cost-effective and less energy intensive technologies for the treatment of wastewater. In this context, novel systems for the simultaneous recovery of energy and treatment of wastewater have gained interested.

In recent years, microbial fuel cells (MFC) have attracted researchers' attention because they are a new system for directly generating electricity from organic matter in wastewater. MFCs have been used to convert the chemical energy contained in organic matter into electrical energy using microorganisms that oxidize the soluble organics and produce electrons in the anode chamber (for example when glucose is oxidized according to Eq 1). The electrons flow through an electrical circuit towards the cathode, where they reduce a high redox potential electron acceptor, for example when oxygen is used water is generated (Eq. 2). Charge neutrality is kept by transport of ions through the electrolyte, being much extended the use of ion selective membranes in order to improve efficiency in dual chamber MFC[11, 12]. In fact, the MFC can be considered as an environmental friendly alternative to treat wastewaters and generate electricity[12].

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

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O \qquad \text{(Eq. 2)}$$

MFC uses successfully several types of wastewater, including synthetic wastewater based on pure compounds and complex wastewater, such as beer brewery[13], domestic[14], starch processing[15], paper recycling plants[16], cassava mill[17] and olive oil[18] wastewater. The power generated in MFC treating wastewater varied several orders of magnitude (1-1000 mW m^{-2}) depending on inoculums, substrate and reactor design used[13-21]. Several studies have stated that MFC can treat agro-industrial wastewater, but only a few studies have reported the treatment of winery waste in this bio-electrochemical system[22, 23] and much work is needed. One of the bottlenecks to develop this interesting application of MFC technology is the unfavorable COD/N and COD/P ratios (characteristic of winery wastewater) which may affect the performance of the bio-electrochemical system. Therefore, this paper focuses on the startup and the assessment of the influence of the nutrients/COD ratio in the performance of this type of systems, concentrating on the study of COD removal of winery wastewater and the energy recovery using a dual chamber MFC.

Materials and methods

MFC configurations.

The MFC (Fig. 1) was made of acrylic tubes, each with an inner diameter of 40 mm and length of 180 mm. The volume of anode and cathode chambers: with 70 and 100 mL, respectively which separated with Sterion® membrane.

The cationic membrane was preconditioned using a 3% (v/v) hydrogen peroxide solution, 0.5 mol L^{-1} sulfuric acid and ultrapure water. The carbon felt (KFA10, SGL Carbon Group®) functioned as electrodes in cathode and anode chamber. In order to minimize the internal resistance (related with ohmic loses), both electrodes were placed in direct contact with the exchange membrane. An external resistance of 120 Ω was connected to the electrodes.

Inoculum and wastewater.

The inoculum used in anode compartment was obtained from the activated sludge reactor at the municipal Wastewater Treatment Plant of Ciudad Real (Spain) and concentrated by sedimentation. The amounts of the total suspended solids and total volatile solids were 15.8 and 11.1 g L^{-1}, respectively. To inoculate the MFC, the medium containing microorganisms (90% v/v) was prepared and added into the MFC.

Two winery wastewater samples were collected from the regulating reservoir of the industrial wastewater treatment plant of the winery Bodegas Crisve (Socuéllamos, Spain), and stored at 4°C before being used. The characteristics of both samples of wastewater are shown in Table 1.

$NaHCO_3$ (6000 mg L^{-1}) was used as buffer to adjust the pH to 6.5. Dibasic sodium phosphate ($Na_2HPO_4 \cdot 2H_2O$) and ammonium sulfate ($(NH_4)_2SO_4$) were added to increase the phosphorous and nitrogen concentrations.

MFC operation.

The MFC was operated in semi-continuous mode and at room temperature (25 ± 2 °C). Every day, 20.0 mL of liquid was removed from anode chamber and replaced by 20.0 mL of fresh winery wastewater. HCl solution (pH 3.5) was used as catholyte and was circulated from the reservoir of 250 mL to the cathode chamber of the MFC at 1.66 mL s^{-1} using a peristaltic pump. In the reservoir, oxygen was supplied by an aquarium compressor and porous stone diffusers.

Analytical methods.

The pH, conductivity and dissolved oxygen were measured using a GLP22 Crison® pH-meter, a GLP 31 Crison® conductivity meter and an Oxi538 WTW® oxy-meter, respectively. The total suspended (TSS) and volatile suspended (VSS) solids were measured gravimetrically according to standard methods previously used in this type of studies[24]. The COD and concentration of phosphorous were measured using a spectrophotometer (DR2000, HACH®). The total nitrogen was monitored using a Multi N/C 3100 Analytik Jena analyzer.

A digital multimeter (Keithley® 2000) was connected to the system to continuously monitor the value of the cell potential and the data were recorded in a personal computer. The polarization curves from the MFC were obtained by varying the resistance in the circuit and measuring the voltage. Power density (mW m^{-2}) and current density (mA m^{-2}) were based on the surface area of anode (7.0 cm^2). The current (I) was calculated using Ohm's Law (I = U/R), and the output power of the cell using P= I U, where I (A) is the current, U (V) is the voltage, R (Ω) is the external resistance and P (W) is the power. Coulombic efficiency (CE) was based on total current generation and the maximum current that can be produced from COD oxidation and it was calculated according to Rodrigo *et al.* [25].

Results & Discussion

Winery Wastewater treatment

Fig. 2 shows the changes in the COD concentration in the anode chamber before and after the daily feeding cycle and the COD consuming rate calculated by mass balance during the startup and operation of the MFC.

Throughout the experimental time, it can be observed that the COD in the anode chamber increased until the ninth day of operation. In this first period, wastewater type 1 with an influent COD of 19640 mg L^{-1} was fed daily and the increase in the COD can be understood as a normal consequence of the MFC semi-continuous operation, because the removal achieved by the biological system is much lower than the organic load fed and this difference in the supplying and consumption rate explains the accumulation of COD. During a second period (from the 9th to the 16th day of operation), the COD in the anodic tank decreases because the influent winery wastewater was changed to type 2 (with an influent COD of 6850 mg L^{-1}). This change was kept till the end of the experiment (day 41) and it was due to experimental requirements because composition of winery wastewater changed as a consequence of seasonality of wine making (and, unfortunately, this factor was not considered in the preparation of the tests). However, and in spite of this large change in the characteristic of the influent, no significant differences are observed in the COD consumption rate, which fluctuates almost randomly in between 500 and 4000 mg L^{-1}: the MFC removed only a very small part of organic matter contained in the winery wastewater (around 1000 mg L^{-1} d^{-1}). This low efficiency can be explained taking into account that the winery wastewater contains important concentrations of recalcitrant species which are difficult to be biodegraded[23]. However, the COD removal efficiency was around 17%, being this value much lower than those observed by Cusick *et al.*[22] and Pepe-Sciarria *et al.*[23] who reported yields of about 67 % and 27 %, respectively, for the same type of wastewater. Several biological processes different of MFC and based on aerobic or anaerobic systems have also been proposed to treat winery wastewater. The COD removal efficiency attained which such systems was around 70–95% (remaining COD is due to the un-biodegradable soluble fraction) [26] which is much higher than that obtained with MFC. However, the more complex mechanisms in the MFC can help to explain this difference.

One of the key characteristics of the winery wastewater is the unbalanced COD/nutrients ratio for biological processes, which make important to look for a co-substrate waste in actual full-scale treatments and becomes a major challenge for the application of the technology. Obviously the MFC is a biological process and its performance can be greatly affected by this poor content in nutrients. To evaluate this issue, synthetic co-substrates consisting of solutions of phosphates (co-substrate 1) and ammonium (co-substrate 2) were used. Fig. 3 shows the temporal profiles of phosphorous and nitrogen concentration before and after each daily feeding cycle and the resulting consuming rates during the complete period studied.

As it can be observed in Fig. 3A, in between the 7th day and 13rd day of operation, the phosphorous content in the anode chamber was completely depleted, becoming the phosphorus content a limiting factor for the performance of the MFC. This depletion can be explained in terms of the low concentration of P in the influent winery wastewater (<1 mg L^{-1}), which does not compensate the higher rate of assimilation caused by the high COD degradation. It is worth considering that a removal of 1000 mg L^{-1} d^{-1} of COD requires at least a supply of 10 - 20 mg P L^{-1} d^{-1} taking into account the typical ratio in which COD and P are assimilated in biomass (1-2% P)[24, 25, 27]. Mixed liquor of the activated sludge seeded in the MFC during the startup had a good COD/P ratio but this appropriate initial phosphorus concentration was continuously decreased after each daily feeding cycle and after 6 days, phosphorus became a limiting factor in the performance of the MFC.

In order to solve this problem, co-substrate 1 consisting of an $Na_2HPO_4 \cdot 2H_2O$ solution was added to the anode chamber to increase the phosphorous concentrations up to 10 mg $P\text{-}PO_4^{3-}$ L^{-1} from day 14th until the end of operation. As a consequence of the availability of more phosphorus, the phosphorus consuming rate increased four times (from 0.5 to 2.1 mg $P\text{-}PO_4^{3-}$ L^{-1}) and the phosphorus in the anode chamber at the end of each daily feeding cycle was not depleted, meaning that it did not become a limiting factor any longer during the test.

Then, once increased the phosphorous concentration, the nitrogen became the new limiting factor on MFC performance. As it can be seen in Fig. 3B, from the 21st day to the 26th day of operation of the MFC, this nutrient was exhausted after each daily cycle. Nitrogen contained in the winery wastewater (20 mg L^{-1}) was clearly insufficient for the removal of COD and it was completely depleted. In the case of nitrogen, the typical ratio for the assimilation of nitrogen is 10% of the COD assimilated[24, 25, 27]. To solve this problem, the nitrogen concentration in winery wastewater was supplemented with co-substrate 2 (ammonium sulfate) up to 100 mg TN L^{-1} from the 26th day until the end of operation. As it can be observed, with this new co-substrate the nitrogen consuming rate increased five times from 5.0 to 25.0 mg TN d^{-1} L^{-1} and concentration of nitrogen was no longer a limiting factor, because an important concentration remains in the anode chamber at the end of the daily feeding cycle. A very interesting observation is that the modification in the COD/N and COD /P ratios does not influence significantly on the removal of COD, which is kept around 1000 mg L^{-1} d^{-1}. This means that COD removal was only slightly affected by the nutrients, in spite a great change was observed in the consumption of both nutrients. Another important observation is that with the addition of co-substrates 1 and 2, COD, N and P are not limiting the processes because a great excess is obtained after the daily feeding cycles. There are three different ways to explain this observation: 1) another nutrient (micronutrient) became the limiting factor of the performance of the MFC; 2) the bio-refractory content in the COD is very high and the removal of organic pollution is not limited by nutrients but by the nature of the species contained in the waste and 3) microorganisms with a low requirement of nitrogen and phosphorus are playing an important role in the process.

Electrochemical Characterization of the system

Differences in electricity generation during all the experimental period were confirmed by monitoring the voltage in a resistance connected to the anode and cathode of the MFC and polarization curves.

Fig. 4A shows the average daily changes in the electricity produced. When winery wastewater was added into the anode chamber of MFC, an initial voltage of 6 mV was immediately produced, which should be explained in terms of chemical factors. Then, the voltage increased reaching an average value of 23.6 mV. It is important to observe that the startup of a MFC with winery wastewater as fuel is very fast and after one week almost stationary conditions are met. With adding phosphorous, the voltage increased and the average value doubled with respect that observed in the condition without adding nutrients (51.7 and 23.6 mV, respectively). This is important, because it clearly shows the limiting behavior of phosphorus in the bioelectricity generating organisms. Thus, although it was not observed an improvement in the COD removal, the fraction of COD that is used as fuel to produce electricity is clearly increased.

The addition of extra nitrogen from the 26th day showed a significant influence on voltage of MFC, increasing the average value in about 30%, up to 67.8 mV. It is worth mentioning that the voltage was more stable when nitrogen and phosphorus were added in winery wastewater, due to better nutritional conditions of winery wastewater. Again, it is worth pointing out that although no significant changes in the COD depletion rate were observed, the activity of electrogenic microorganisms improved greatly and a higher ratio of the COD was processed by them, improving their prevalence with respect to non bioelectrogenic microorganisms. This prevalence can be clearly seen in Fig. 4B, where it is shown the changes in the Coulombic Efficiency calculated using the power generated and the COD consumed.

When the phosphate extra-supply was added in winery wastewater, the Coulombic Efficiency increased three times from 2.1% to 6.7%, showing that the addition of phosphorous favored the exoelectrogen microorganism. The addition of the nitrogen extra supply from the 26th day showed a significant influence on Coulombic Efficiency, doubling the average value in 6.7%, to 14.7%. These values of the Coulombic Efficiency can be considered as high, in particular if it is taken into account that maximum expected efficiency (for a pure culture of bioelectrogenic microorganisms) cannot exceed 40%[25, 27, 28], because this is the typical ratio of catabolic consumption of COD (the remaining 60% is used in biological assimilation reactions). This observation also suggested that other competing microorganisms such as methanogens and sulfate reducers are playing an important role and that for these microorganisms the COD/N and COD/P ratios are not as important as for bioelectrogenic microorganisms. At this point, it is interesting to observe that the reported Coulombic Efficiencies were similar to those of other studies carried out by Cusick *et al.*[22] and Pepe-Sciarria *et al.*[23] who used a single chamber air-cathode MFC (18 % and 15 %, respectively) for which ohmic loses are lower.

At this point, it is interesting to observe the transient response of the MFC, which was operated in semi-continuous mode, feeding the anode compartment with fresh wastewater every day. In Fig. 5, the daily voltage profile in different days and conditions are shown. When the MFC was fed with fresh winery wastewater around 9:00 and 10:00 am, the voltage instantaneously increased but later it dropped. Such unstable voltage profile was observed by Lee et al.[29], who credited the rise to sulfate reduction and sulfite oxidation.

The sulfate present in winery wastewater (810 mg L^{-1}) was used as the final electron acceptor and converted to sulfide which was electrochemically active on anode surfaces. When the fresh winery wastewater was added, the disturbance in reactor caused the oxidation of sulfide to elemental sulfur or sulfate, increasing the electrons generation. Therefore, these processes increased the electricity generation in MFC in this moments and sulfur species acted as electro mediators. Other cause for this raise is the additional hydrochloric acid to cathode to control the pH at 3.5 which increased the concentration of protons reacting with oxygen and electrons and it generated more energy. As the time passes, the concentration of protons in cathode decreased, consequently the voltage diminished and the pH raised.

Polarization curves were recorded during experimental period (Fig. 6) and they showed interesting profiles.

When nutrients were not added in winery wastewater, 5th and 13rd day of operation, the MFC showed a maximum of power density of 105 mW m^{-2} at 830 Ω (248 mV). Adding 10 mg P-PO_4^{-3} in winery wastewater, 18th and 25th day of operation, the maximum power densities were three times higher than without adding nutrients, reaching a value of 353 mW m^{-2} (287 mV). The addition of nitrogen in the 26th day showed an effect on power density, increasing it up to 465 mW m^{-2} (269 mV) at 220 Ω. It is interesting to observe that the main differences in the polarization curves are obtained in the zones where the ohmic loses (first zone with a constant slope) and the mass transfer (second zone of the curve) control the performance of MFC. The higher slope observed in polarization curves without supplementation of nutrient in winery wastewater can be related to the observed positive effect on the ionic conductivity when nutrient solutions was added. The addition of nitrogen and phosphorus increased the conductivity of the anolyte, decreasing the slope of the first zone of polarization. Fig. 7 shows the changes in the conductivity during the complete experimental period. As it can be observed, there is an important initial increase which can be explained by the higher conductivity of the winery wastewater as compared to the sludge seeded. Then, the addition of phosphorus and nitrogen clearly reflects on a smaller increase in the ionic conductivity which may help to explain the lower slope of the ohmic loss zone in the polarization curves as time goes by. Moreover, another effect of supplementation of nutrient can be observed in the mass transfer control zone (Fig. 6B), which clearly becomes less limiting (less abrupt change in the voltage vs. intensity) with the addition of nutrients, indicating the necessity of coexistence of microorganisms, substrate and nutrients in order to prevent mass transfer control in the production of electricity. Hence, the addition of these nutrients increased the solution conductivity and improved the generation of electrons by microorganisms and the transfer of electrons to electrode decreasing ohmic and mass resistances[25].

It seems that winery wastewater with a relation C:N:P of 700:10:1 has a great potential to generate power in MFC comparing with other agro-industrial wastewater as substrate (Table 2). The winery wastewater with phosphorous and nitrogen added generated a considerable of power density of 465 mW m^{-2} due to the high COD concentration and high conductivity present in the wastewater. Nevertheless, it should be considered that it is difficult to compare the power generation, because it is a consequence of the substrate, electrode, inoculum, reactor design, temperature and pH under study and all these conditions are very different in the different experimental works shown in Table 2. Anyway, they reflected the promising behavior of this type of fuel for MFC technology.

Conclusions

From this work, we can conclude that the dual chamber MFC was able to generate electrical energy using winery wastewater as fuel. However, this technology was not effective in organic matter removal, because only a small part of COD was removed even when extra nitrogen and phosphorus are added. Increase in the nitrogen and phosphorous concentrations does not influence the total removal of COD but it has a very important influence on the production of electricity, indicating that performance of bioelectrogenic microorganisms is limited by these nutrients in a more important way than in the competing non bioelectrogenic microorganisms. The supplementation of phosphorous and nitrogen up to concentrations in which both species remained in significant concentrations after the daily feeding cycle attain an increase in the coulombic efficiency from 2% to almost 15% and in the maximum power density from 105 to 465 mW m^{-2}. The startup of the MFC is very rapid and in less than 1 week it is obtained a stationary response in the production of electricity. This time has to be also suitable to have a stationary response when changes are made.

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Table 1: Characteristics of winery wastewaters used.

Parameter	Sample 1	Sample 2
pH	4.25	4.11
Conductivity ($mS\ cm^{-2}$)	2650	2030
COD ($mg\ L^{-1}$)	19640	6850
BOD ($mg\ L^{-1}$)	14500	5000
TOC ($mg\ L^{-1}$)	2600	1030
Total Nitrogen ($mg\ L^{-1}$)	39.2	18.3
Total Phosphorous ($mg\ L^{-1}$)	0.8	0.95
SO_4^{2-} ($mg\ L^{-1}$)	-	810
Cl^- ($mg\ L^{-1}$)	-	39.90

-: not measured.

Table 2: Comparison between present study and other results found in the literature.

Substrate	Power Density (mW m^{-2})	CE (%)	COD (mg L^{-1})	Type of MFC (electrodes)	Ref.
Winery wastewater	465	15	6850	Dual chamber (carbon felt)	This study
White and red wine lees wastewater	263 - 111	9-15	10100 - 6400	Single chamber air cathode (graphite fiber)	[23]
Winery wastewater	278	18	2200	Single chamber (anode: graphite fiber brushes; cathode: applied platinum in carbon cloth)	[22]
Cassava Mill wastewater	1800	20	16000	Single chamber (graphite plates)	[17]
Starch processing wastewater	239	8	4852	Single chamber air cathode (anode: carbon paper; cathode: applied platinum in carbon paper)	[15]
Swine wastewater	45	-	8320	Dual chamber (carbon paper)	[14]
	261	8		Single chamber (carbon paper)	
Olive mill and domestic wastewater	124	29	4300	Single chamber air cathode (anode: graphite fiber brushes; cathode: applied platinum in carbon cloth)	[18]

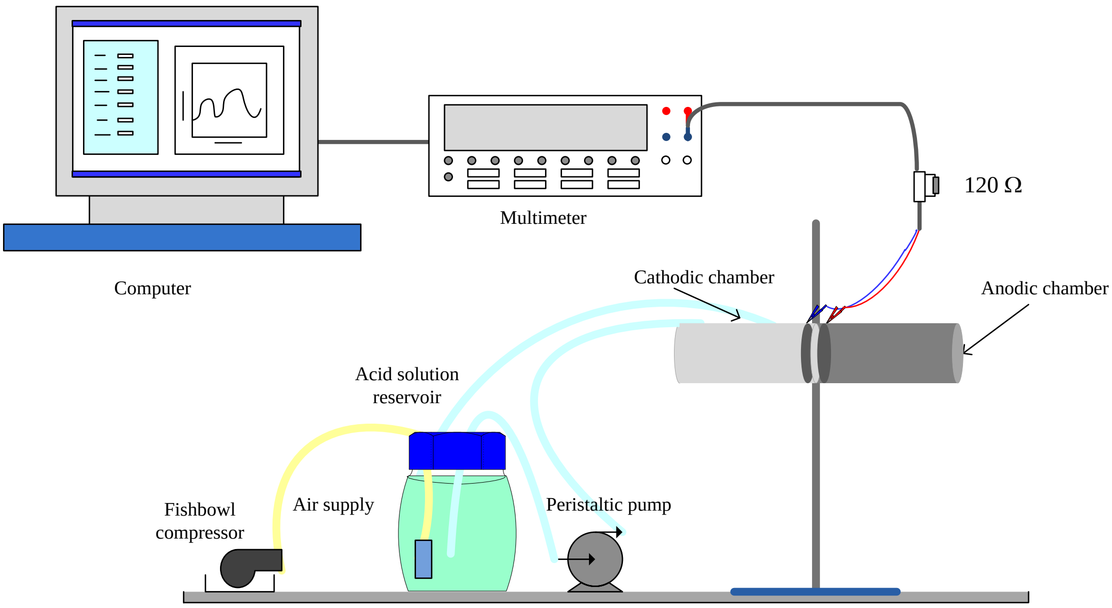


Figure 1. Experimental setup used in this study. The acid ultrapure water was continuously pumped to cathode chamber and the 20 mL of anode chamber was removed everyday and replace with fresh winery wastewater, operating the MFC in semi continuous system.

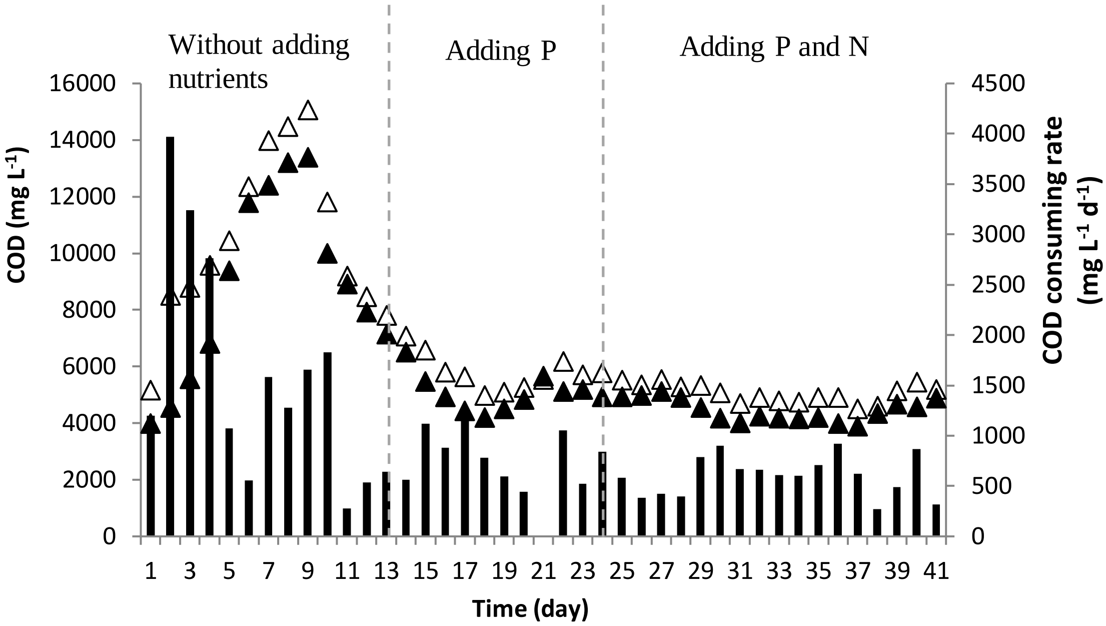


Figure 2: Changes in the anodic chamber COD before (△) and after (▲) the feeding stage and in the COD consuming rate (■) during the performance of the MFC.

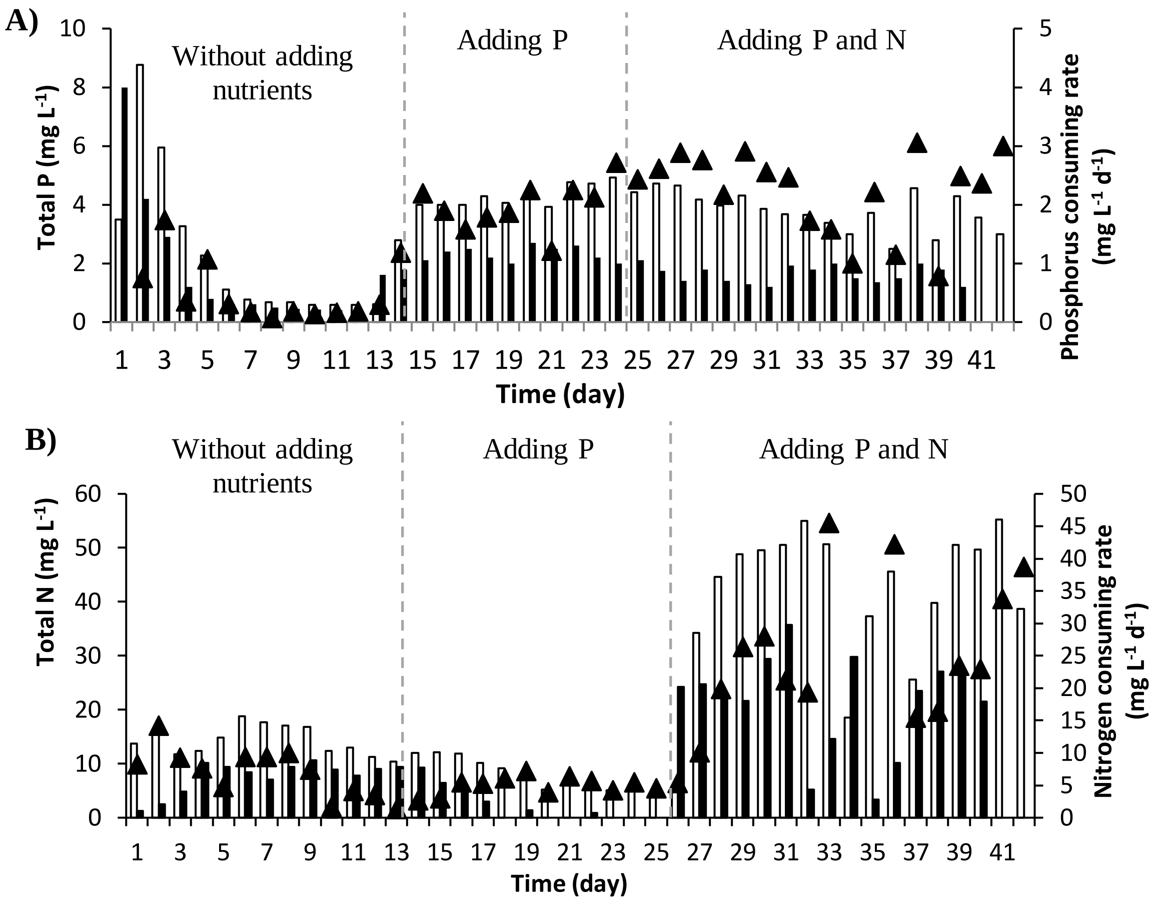
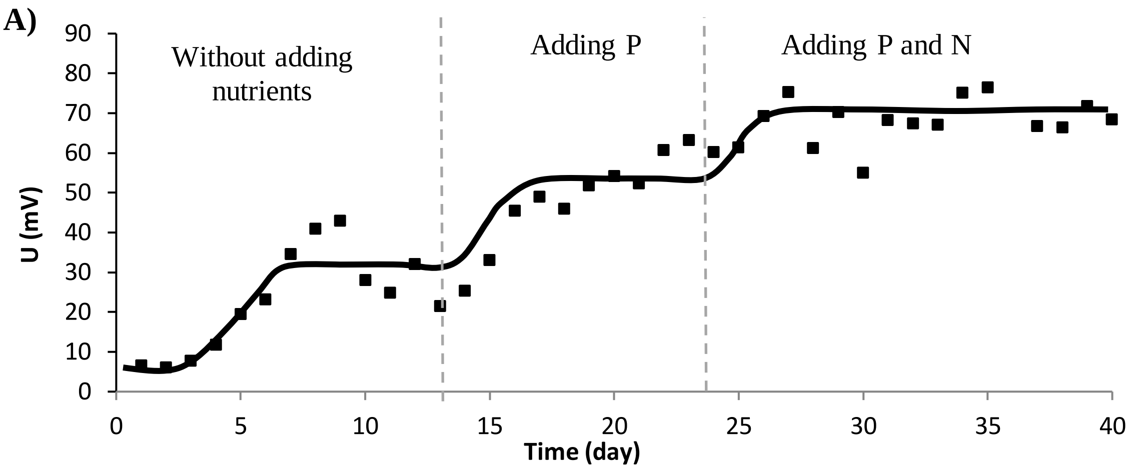


Figure 3: Changes in the anodic chamber phosphorus (A) and nitrogen (B) before (□) and after (■) the feeding stage and the consuming rate (▲) during the performance of the MFC.



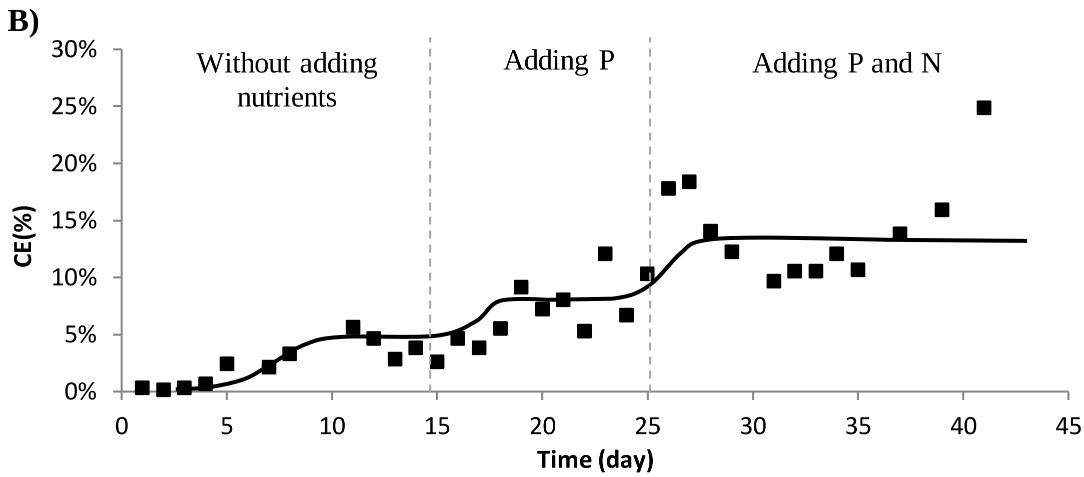


Figure 4: Electricity produced (A) during the lifetest of the MFC fed with winery wastewater: the daily average voltage (■) and the tendency (▬).The temporal profile of Coulombic Efficiency (B,CE) obtained in the MFC fed with winery wastewater during the experimental period: the daily average Coulombic Efficiency (■) and the tendency (▬).

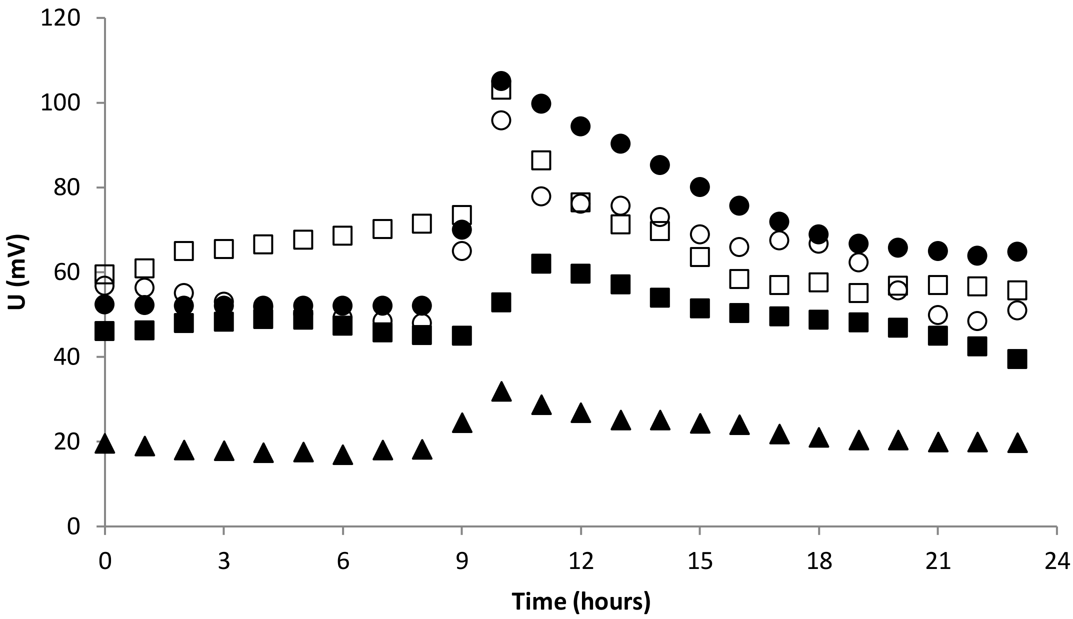


Figure 5: Daily voltage profile in different days and conditions: the winery wastewater without supplementation of nutrient at 12nd day of operation (▲), the winery wastewater supplanted with 10 mg P-PO_4^{3-} L^{-1} at 16th day (■) of operation and winery wastewater supplanted with 10 mg P-PO_4^{3-} L^{-1} and 100 mg NT L^{-1} at 27th , 30th and 37th day of operation (○, ● and □, respectively).

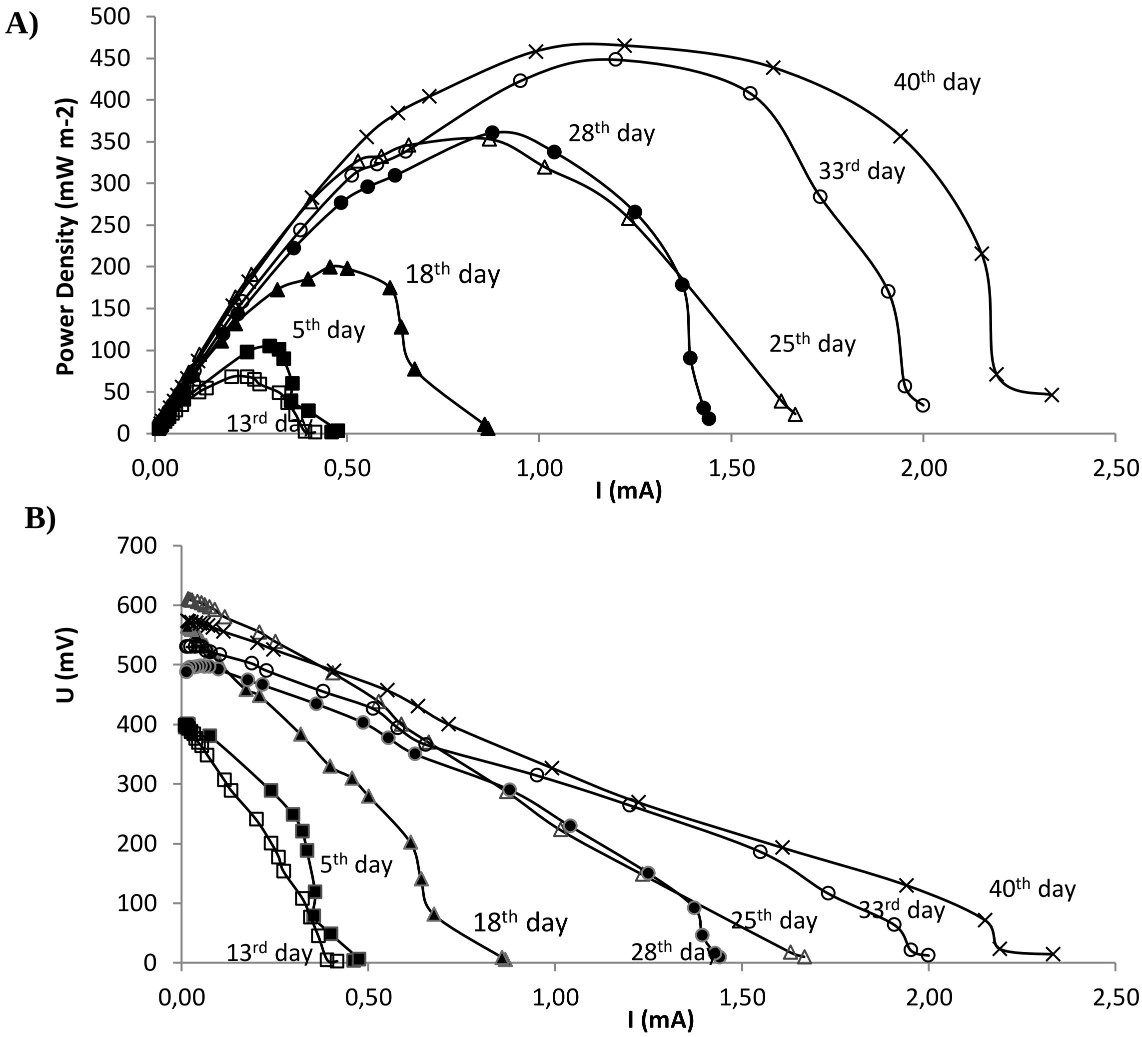


Figure 6: Polarization curves (A and B) obtained in the MFC fed with winery wastewater. The polarization 5th (■) and 13th (□) were done with winery wastewater without supplementation of nutrient, the 18th (▲) and 25th (△) were done with winery wastewater supplemented with 10 mg $P\text{-}PO_4^{3-}$ L^{-1} and the 28th, 33rd and 40th (●,○, ×,respectively) were done with winery wastewater supplemented with 10 mg $P\text{-}PO_4^{3-}$ L^{-1} and 100 mg NT L^{-1}.

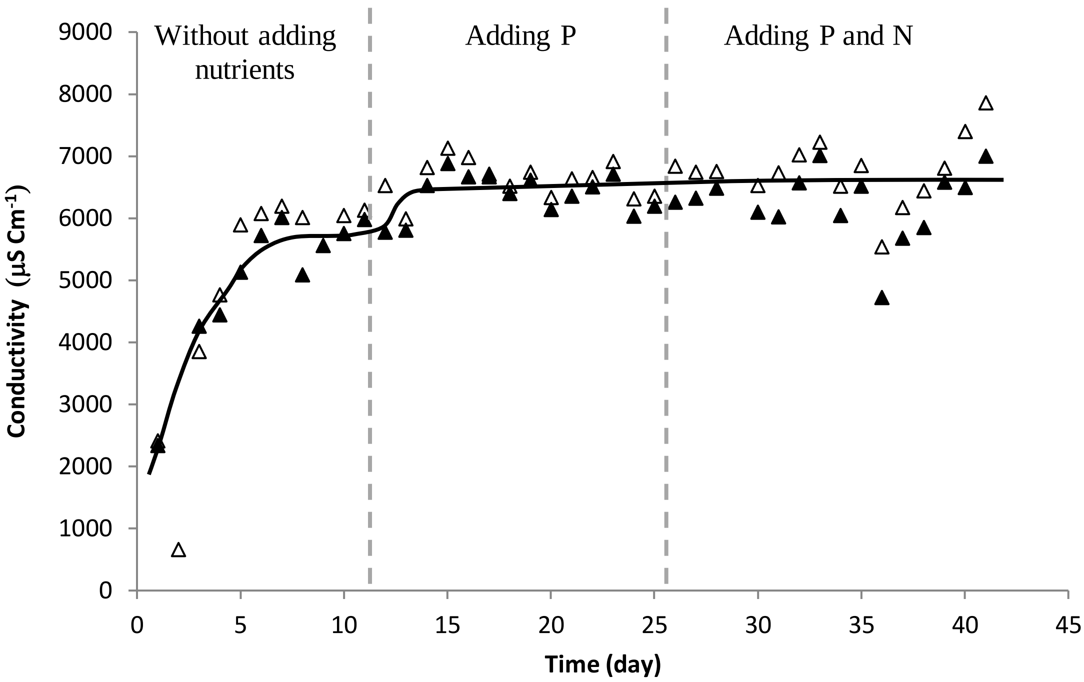


Figure 7: The temporal profile of conductivity monitored in the anodic chamber during the experimental period: before feeding (▲) after feeding (△) and the tendency (▬).