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Voltage production and simultaneous municipal wastewater treatment in microbial fuel cells performed with *Clostridium* strains

Producción de voltaje y tratamiento simultáneo de agua residual municipal en celdas de combustible microbianas operadas con cepas *Clostridium*

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Abstract

Clostridium strains are known due to their capability to produce hydrogen and free electrons during their metabolism; however, voltage production and electric current generation by Microbial Fuel Cell (MFC) have not been deeply explored yet employing these microorganisms. In this paper we evaluated both voltage generation and Chemical Oxygen Demand (COD) reduction in municipal wastewater by one chamber MFC performed with graphite cloth electrodes and inoculated with *Clostridium bifermentans*, *Clostridium sordellii* and native bacteria. *Clostridium* strains grew properly on both Ravot medium and municipal wastewater reaching the maximum develops at 96 and 120 hours for *bifermentans* and *sordellii* respectively. Microbial growth kinetics determined by Gompertz modified model showed low lag times for all tests. *Clostridium sordellii* showed not only the maximum voltages but also the highest potential to remove organic matter from wastewater since COD diminished around 60% at the end of MFC's operation. The addition of phosphate salts solution increased the removal of organic matter but was not efficient to generate voltage, moreover, the microorganisms present in wastewater were able to produce voltage but the amount of organic matter removed by them were low. The maximum generated voltage, Power Density (PD), Volumetric Power Density (VPD) and Current Density (CD) values were observed in MFC inoculated with *Clostridium sordelli* and performed with wastewater with no buffer solution, they were; 0.372 V, 153.43 mW m^{-2}, 1.73 W m^{-3} and 0.413 A m^{-2} respectively. *Clostridium* strains showed a high potential to reduce COD in wastewater generating green energy as by-product.

Keywords: bioelectrochemistry, *Clostridium* bacteria, graphite cloth, microbial fuel cell, municipal wastewater.

Resumen

Las bacterias del género *Clostridium* son conocidas por su capacidad para producir hidrógeno y electrones libres durante su metabolismo, sin embargo, la generación de voltaje y de corriente eléctrica a través de sistemas bio-electroquímicos aún no ha sido del todo explorada utilizando dichas bacterias. En el presente trabajo se evaluó la producción de voltaje y la reducción de la Demanda Química de Oxígeno (DQO) en agua residual municipal utilizando Celdas de Combustible Microbianas (CCM´s) de una cámara con electrodos de tela de grafito, inoculadas con *Clostridium bifermentans*, *Clostridium* sordelli y bacterias nativas. Las bacterias *Clostridium* crecieron adecuadamente tanto en el medio Ravot como en el agua residual municipal alcanzado el máximo crecimiento a las 96 y 120 horas para *bifermentans* y *sordellii* respectivamente. La cinética de crecimiento de ambas bacterias, obtenida con el modelo de Gompertz modificado mostró tiempos de adaptación bajos. La máxima generación de voltaje y el potencial más alto para degradar la materia orgánica presente en el agua residual se obtuvo con la bacteria *Clostridium sordellii*, con dicha bacteria la DQO disminuyó aproximadamente un 60% con respecto al valor inicial. La adición de una solución de sales de fosfatos al agua residual incrementó la remoción de materia orgánica, sin embargo, la generación de voltaje disminuyó; las bacterias nativas mostraron un alto potencial para la producción de voltaje más no para la remoción de materia orgánica. Los valores más altos del voltaje, densidad de potencia, densidad volumétrica de potencia y la densidad de corriente se observaron en la CCM operada con agua residual con solución de fosfatos inoculada con la bacteria *Clostridium sordellii*, los cuales fueron: 0.372 V, 153.43 mW m^{-2}, 1.73 W m^{-3} and 0.413 A m^{-2} respectivamente. Las bacterias *Clostridium* mostraron un alto potencial para disminuir la DQO en el agua residual generando energía como subproducto.

Palabras clave: agua residual municipal, bacteria *Clostridium*, bioelectroquímica, celda de combustible microbiana, tela de grafito.

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1 Introduction

The generation of wastewater has increased in the last decades mainly caused by the population growing and constant urbanization. In developing countries more than 90% of generated wastewater is discharged into rivers, oceans and lakes contributing to environmental detriment and propagation of human diseases (Kumar *et al*., 2019). In Mexico, the generation of municipal wastewater reaches 250 m^3/seg, of which only 25% is properly treated (Buitrón & Pérez, 2011). Municipal wastewater is usually treated by activated sludge processes, which use mechanical aeration to facilitate the oxidation of organic matter in wastewater, but the consumption of energy is rather high. Other disadvantages of this technology are the high quantities of residual materials obtained at the end of the treatment and the impossibility to recover the chemical energy present in organic substrates (He *et al*., 2017; Tatinclaux *et al*., 2018). Dark fermentation is another method capable to reduce the amount of organic compounds in wastewater; this method employs anaerobic bacterial metabolism to transform organic substrates into low molecular weight organic acids and gases such as methane, hydrogen and carbon dioxide. Furthermore, there are reports about the use of dark fermentation technology to partially treat not only municipal wastewater (He *et al*., 2017) but also textile wastewater (Ya-Chieh *et al*., 2012) and municipal solid waste leachate (Liu *et al*., 2011), however, the organic matter removal yields were low, suggesting that dark fermentation technology is inefficient to recover energy from wastewater.

Bioelectrochemical Systems (BES) has been explored in the last decades as a promising technology to produce bioenergy from a wide range of wastewater types (Domínguez-Maldonado *et al*., 2014). BES's are defined as electrochemical systems in which at least one of the electrodes involves electrochemical reactions in the presence of microorganisms. The main advantages of BES's are the capability to operate at ambient temperature, the use of different raw materials (from simple sugars to complex carbohydrates) and precious metal catalyzers such as platinum and gold are not needed (Guadarrama-Pérez *et al*., 2018; Pérez-Rodríguez *et al*., 2018). Microbial Fuel Cells (MFC) are the most important and studied of all the BES. In this kind of cells organic matter is oxidized in anodic chamber by anaerobic bacteria producing carbon dioxide, protons and electrons; these last ones are transferred through an electrical circuit from the anode to the cathode where combines with protons and oxygen to produce water (Gómora-Hernández *et al*., 2020; Valdez-Ojeda *et al*., 2014). In agreement to He *et al*., 2017, MFC are a sustainable technology for wastewater treatment due to the following advantages: high capability to transform organic matter into voltage or current, energy input is not necessary and can be applied in locations with insufficient electrical infrastructures (González-Paz *et al*., 2020). Microbial Electrolysis Cell (MEC) is another type of BES which has been extensively studied in the last years. The conformation of MEC is similar to that used in MFC; however, the main difference between them is the atmosphere in cathodic chamber, since anoxic environment is required for MEC in order to reduce protons and electrons into hydrogen gas by an external energy source (Wrana *et al*., 2010). The generation of energy from MFC has been evaluated with native bacteria employing different substrates such as aromatic molecules (Chang *et al*., 2017), fermentative leachates (Hernández-Flores *et al*., 2015) and wastewaters (Buitrón & Pérez, 2011; He *et al*., 2017; Valdez-Ojeda *et al*., 2014), however, only few reports have studied the potential of pure *Clostridium* bacteria to produce bioenergy by BES. The aim of this paper was to evaluate Chemical Oxygen Demand (COD) reduction in municipal wastewater by MFC employing *Clostridium* strains. Voltage generation and microbial growth were monitored throughout the experiments; while COD and pH were quantified before and after MFC operation. The effect of buffer solution composed by phosphate salts on voltage generation and COD reduction was also reported and discussed.

2 Materials and methods

2.1 *Municipal wastewater*

A 10 L municipal wastewater sample was taken from Wastewater Treatment Plant (WWTP) located at the community of Metepec, Mexico State. The sample was transported to Environmental Engineering Research Laboratory at Technological Institute of Toluca and characterized according to that described in Mexican regulations as shown in Table 1. After that, sample was stored at 4°C until used.

2.2 Exoelectrogenic Bacteria and culture medium

The microorganisms employed for voltage production by MFC were native bacteria isolated at ININ came from Lerma River sediments, which were previously characterized and identified by 16S DNA sequencing as microorganisms with capability to produce extracellular electrons. From GenBank sequences, these isolated bacteria presented 98% similarity to *Clostridium sordellii* ATCC9714 and *Clostridium bifermentans* ATCC638 (Serment *et al.*, 2017). Both strains were preserved and cultured every month in Ravot medium (Ravot *et al.*, 1995), as well as in thioglicolate medium (Gómora-Hernández *et al.*, 2016). Culture mediums were prepared by Hungate modified technique (Terry *et al.*, 1974) to reach anoxic atmosphere and sterilized in autoclave at 121 ºC for 15 minutes, after that, microorganisms were inoculated into each culture media and incubated at 32 ºC during 24 hours, finally they were stored at 4 ºC.

2.3 Microbial growth kinetics in wastewater and culture mediums

Microbial culture growth was monitored by direct count in a Petroff-Hausser counting chamber. Two samples were taken at different incubation times and the amount of bacteria was quantified in 5 big squares randomly selected. The total number of bacteria expressed as cell mL^{-1} was obtained employing Eq. 1. Additionally, Gompertz modified model (Eq. 2) has been reported as an adequate mathematical model to estimate kinetic parameters of microbial growth not only for fermentative purposes but also for bioelectrochemical (Mateo *et al.*, 2019). Microbial growth experimental data were fitted to this model by non-linear regression performed in Origin 8.6.

$$\frac{Cell}{mL} = \frac{Cell_{av}}{16} * 400 * 50 * 1000 \tag{1}$$

$$H(t) = P \exp\left[-\exp\left[\frac{R_m \cdot e}{P}(\gamma - t) + 1\right]\right] \tag{2}$$

Where Cell_{av} is the average number of bacteria counted in 5 squares randomly selected, 16 the number of small squares into a big square, 400 the total amount of small squares in Petroff-Hausser chamber, 1000 mm^3 to cm^3 conversion factor, 50 the inverse of distance between chamber and coverslips, P the maximum potential growth (cell mL^{-1}), R_m the maximum rate (cell mL^{-1} hr^{-1}), γ lag time (hr) and e the Euler number (2.712828).

2.4 MFC construction and operation

MFC's were constructed of acrylic material as previously described (Serment *et al.*, 2017). MFC's consisted of two acrylic cubes (chambers) each one with a total volume of 110 mL and a working volume of 80 mL, both cubes were interconnected to each other by stair rods and screws located in each corner of the cubes. Proton Exchange Membrane employed in this study was CMI-7000 obtained from Membranes International INC. The membrane was treated with 2%w sodium chloride solution during 2 hours at 37 °C in order to activate and expand it (Kim *et al.*, 2008); after that, membrane was sterilized in autoclave and a 4 cm diameter was placed in each MFC between both chambers. Electrodes employed for voltage production were made by graphite cloth, which were previously treated with 1 M hydrochloric acid solution during 1 hour followed by 1 hour contact with 1 M sodium hydroxide solution, after that, electrodes were washed with deionized water and sterilized in autoclave (Serment *et al.*, 2017). The electrodes pretreatment is aimed to increase roughness and current production and to reduce ohmic loses (Jung *et al.*, 2014). The assembled MFC's were washed with 70%v ethanol prior to operation. Anode was placed in direct contact with municipal wastewater inside the anodic chamber, while the air-cathode was installed attached to the membrane. Both electrodes were coupled to titanium wires.

For voltage generation in MFC's, the following tests were carried out: 1) Ravot medium inoculated with C. *bifermentans* (RMB), 2) Ravot medium inoculated with C. *sordellii* (RMS), 3)Sterilized municipal wastewater inoculated with C. *bifermentans* (SWB), 4) Sterilized municipal wastewater inoculated with C. *sordellii*(SWS), 5) Sterilized municipal wastewater supplemented with buffer solution and inoculated with C. *bifermentans* (SWBB), 6) Sterilized municipal wastewater supplemented with buffer solution and inoculated with C. *sordellii* (SWBS) and 7) non-sterilized wastewater (NSW). Each test was performed by duplicate, the inoculation volume was 100 μL and the incubation temperature was 32 °C. According to Ávila-Vera *et al.* (2015), buffer solution employed in this work is an efficient way to reduce pH gradients caused by mesophilic bacteria metabolism, it was composed by (g/L); ammonium sulfate, 1; monobasic potassium phosphate, 4.5; dibasic potassium phosphate, 10.5.

Table 1. Characterization of municipal wastewater.

Parameter	**Experimental data**	**Maximum allowed limit**	**Method or technique**
pH	7.4 ± 0.2	NR	NMX-AA-008-SCFI-2011
Conductivity	740 μS cm^{-1}	NR	NMX-AA-093-SCFI-2000
COD	345 ± 7 mg L^{-1}	200 mg L^{-1}	NMX-AA-030-SCFI-2012
BOD5	156 ± 5 mg L^{-1}	200 mg L^{-1}	NMX-AA-028-SCFI-2001
Total solids	547 ± 10 mg L^{-1}	200 mg L^{-1}	
Suspended solids	387 ± 5 mg L^{-1}	NR	NMX-AA-004-SCFI-2013
Suspended volatile solids	294± 8 mg L^{-1}	NR	
Total nitrogen	39 mg L^{-1}	60 mg L^{-1}	NMX-AA-026-SCFI-2010
Oils and fats	86 mg L^{-1}	15 mg L^{-1}	NMX-AA-005-SCFI-2013
Coliforms	390000 CFU mL^{-1}	NR	NMX-AA-042-SCFI-2015

NR: Not reported.

The open circuit voltage obtained in all MFC's was measured in a Keithley Tektronix 2000 multimeter, and current was calculated according to Ohm's law (Eq. 3) considering the external resistance of the potentiotast (1000 Ω), then Power Density (PD) given in mW/m^2 can be calculated according to Eq. 4.

$$I = \frac{V}{R} \tag{3}$$

$$PD = \frac{VI}{S} \tag{4}$$

Where I is the current given in A, V the voltage in V, R the external resistance of potentiotast in Ohms and S the total surface area of electrode (9 cm^2). In order to compare the efficiency of different sizes MFC's PD was calculated in terms of anodic chamber working volume (VPD) as shown in Eq. 5, moreover, Current Density (CD) was obtained with Eq. 6 as reported before by Martínez-Santacruz *et al*. (2016).

$$VPD = \frac{VI}{v} \tag{5}$$

$$CD = \frac{V}{SR} \tag{6}$$

The acronyms V, I, S and R have the same meaning to that described previously, and v is the working volume of anodic chamber. COD and pH were determined by triplicate before and after MFC's operation employing Mexican regulations, reported in Table 1.

3 Results and discussion

3.1 *Municipal wastewater characterization*

Municipal wastewater obtained from treatment plant was characterized before electrochemical treatment stage. Table 1 summarizes not only the characterization of municipal wastewater and their respective determination methods but also the maximum allowed limits in Mexico for municipal wastewater discharges. The municipal wastewater has a high content of COD and total solids. The amount of oils and fats determined by Soxhlet method was 85.57 mg L^{-1}, which was higher to that suggested by Mexican regulations. Biological Oxygen Demand (BOD) and total nitrogen had minor concentrations compared to that allowed by the same national regulations.

3.2 *Clostridium strains growth on wastewater and common culture media*

Ravot and thioglicolate media described in section 2.2 were used to evaluate *Clostridium* strains growth. As can be seen in Figure 1a, *Clostridium bifermentans* showed a greater growth on thioglicolate medium (TMB) than in Ravot medium (RMB), the highest bacteria growth were reached at 96 hours in both media. *Clostridium sordellii* showed an opposite behavior since it better development was achieved with Ravot medium (RMS) reaching it maximum growth at 120, this time was also observed in thioglicolate media.

Clostridium bifermentans and *Clostridium sordellii* inoculated on sterilized wastewater with or without buffer solution showed their maximum growth at 144 h (Fig. 1b), after this time the number of cells/mL diminished which can be related to bacterial death phase. Wastewater supplemented with buffer solution increased *Clostridium* strains growth suggesting that phosphate ions improve bacteria metabolism and the voltage generation in MFC's.

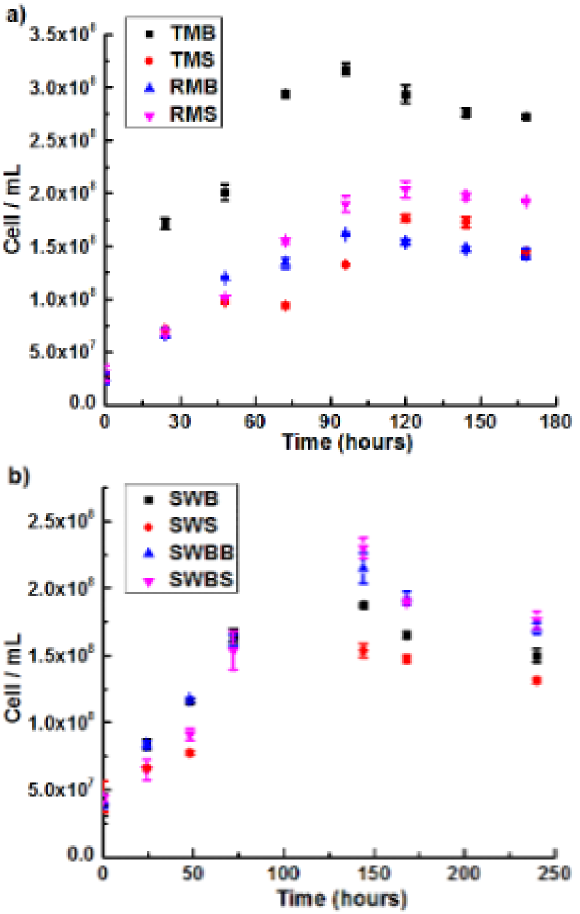

Figure 1. *Clostridium* strains growth employing as substrate a) common culture mediums and b) wastewater with or without buffer solution

The highest concentration of cells per mL was observed for *Clostridium bifermentans* on thioglicolate medium (TMB), excepting this test the rest of cultivations on culture mediums showed a minor growth in comparison to that observed on wastewater, this fact can be associated to the high amount of organic matter and complex carbohydrates in wastewater which improve *Clostridium* bacteria development.

Experimental data of *Clostridium* growth were fitted to Gompertz modified model by non-linear regression and the obtained kinetic and statistical parameters are summarized in Table 2. The highest potential growth was 2.78×10^8 cell mL^{-1} calculated for *Clostridium bifermentans* cultivated on thioglicolate medium and the lowest value was 1.34×10^8 cell mL^{-1}obtained for *Clostridium sordellii* in the same media. The lowest lag times were 1.16 and 1.99 hours calculated respectively for *Clostridium bifermentans* and *Clostridium sordellii* cultured on sterilized wastewater with no buffer solution. Employing sterilized wastewater supplemented with buffer solution, *Clostridium* cultures showed not only a major potential growth but also a higher lag times in comparison to that calculated for wastewater without buffer, showing that phosphate ions delay bacteria growth at initial times but led to its development after 20 hours incubation time. The highest lag time was around 11.5 h obtained for *Clostridium sordellii* cultured on sterilized wastewater with phosphate buffer solution (SWBS). With exception of RMS test, the rest of *Clostridium* cultivations on both Ravot and thioglicolate mediums showed the highest growth rates, despite this trend, the potential growths of TMS and RMB were lower to that observed for *Clostridium* strains cultured on wastewater with or without buffer solution.

3.3 *Voltage generation and wastewater treatment by MFC's*

Figure 2 shows the conformation of a MFC employed in this study, as seen in this Figure anodic chamber contains municipal wastewater and a graphite cloth electrode was submerged inside it. Two rubber plugs were used to seal anodic chamber and the air-cathode was placed next to membrane.

Table 2. Kinetic and statistical parameters of *Clostridium* strains growth on culture mediums and wastewater.

Test	P (cell mL^{-1})	(hr)	Rm (cell mL^{-1} hr^{-1})	R^2
TMB	2.78×10^8	5.23	1.35×10^7	0.998
TMS	1.34×10^8	3.63	4.04×10^6	0.974
RMB	1.53×10^8	6.66	4.19×10^6	0.997
RMS	1.93×10^8	5.68	3.18×10^6	0.993
SWB	1.67×10^8	1.16	3.51×10^6	0.956
SWS	1.45×10^8	1.99	1.67×10^6	0.952
SWBB	1.74×10^8	4.18	3.39×10^6	0.979
SWBS	1.84×10^8	11.49	2.67×10^6	0.995

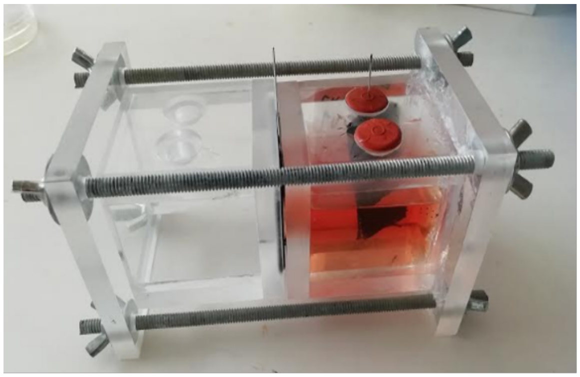

Figure 2. Conformation of a MFC employed for voltage production by COD reduction in municipal wastewater.

For voltage generation, a negative control MFC consisting of sterilized Ravot medium with no inoculum (RM) was performed and monitored through the experiment. The main idea of negative control is to show the importance of *Clostridium* bacteria on BES's, since in this MFC voltage generation was only appreciated at the initial operation times, and after 10 hours it diminished until 0 mV. This behavior can be associated to the presence of free ions in anodic chamber solution came from organic matter and salts in Ravot medium. Figures 3a and 3b shows voltage generation by MFC's inoculated with *Clostridium bifermentans* and *Clostridium sordellii* respectively, as well as the generated voltage with native bacteria in wastewater and the negative control MFC. The highest voltage obtained with *Clostridium bifermentans* was 203.6 mV obtained in Ravot medium (RMB) and the lowest voltage was observed in sterilized wastewater supplemented with buffer solution (SWBB); 125 mV. Employing sterilized wastewater without buffer solution (SWB), the maximum voltage was 130.25 mV observed around 95 hours; however this voltage was unstable and rapidly stabilized to a minor value; 100 mV, which was similar to that observed in SWBB test at the last operating times. Native microorganisms in wastewater showed a major potential to produce voltage than *Clostridium bifermentans* strain, since MFC operated with non-sterilized wastewater (NSW) reached a maximum voltage of 248 mV, this value was higher to that observed for any MFC inoculated with *bifermentans*.

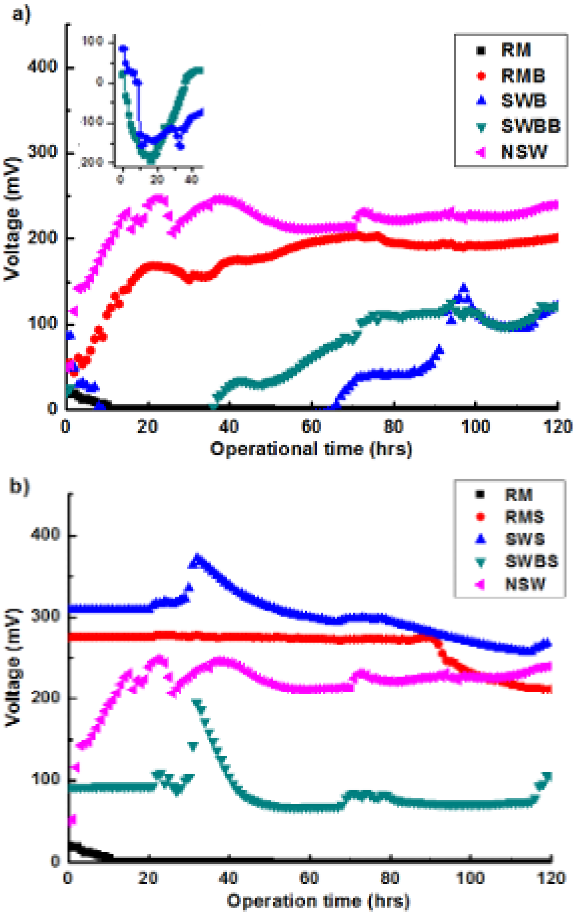

Figure 3. Voltage generation from different substrates by MFC's inoculated with a) *Clostridium bifermentans* and b) *Clostridium sordellii*.

Clostridium sordellii strain was a viable option to produce voltage from Ravot medium (RMS) and sterilized wastewater (SWS); the voltages obtained from these substrates were higher in comparison to that observed with NSW test, as can be seen in Figure 3b. The maximum voltages observed with RMS and SWS tests were 278 and 371.6 mV respectively. Sterilized wastewater with buffer solution inoculated with *Clostridium sordellii* (SWBS) reached a maximum voltage of 194 mV at 36 hours in the MFC, after this time the voltage diminished and stabilized around 100 mV. Similar voltages were observed in SWBB in the operating time range of 65 to 100 hours. MFC's inoculated with *Clostridium sordellii* showed a higher voltage generation to those performed with *Clostridium bifermentans*, this behavior can be associated to the capability of this last bacterium to produce hydrogen by hydrogenases activity (Wong *et al.*, 2018), which reduces its potential to produce voltage. In addition to this, buffer solution does not benefit the bioelectrochemical performance since it reduces the generated voltage.

The maximum voltage achieved in this paper was 0.372 V observed in SWS MFC. This voltage was higher in comparison to that obtained by Mateo *et al*. (2019), Naureen *et al*. (2016) and Pérez-Rodríguez *et al*. (2018), who used wastewater as substrate and native bacteria. The voltage generated by NSW MFC was also higher to that reported by the same researchers and according to Naureen*et al.* (2016) and Mora and Bravo *et al*. (2017) it could be attributed to the presence of *Enterobacter* sp. *Escherichia coli* and/or Geobacter sp bacteria. Table 3 summarizes the bioelectrochemical performance of all evaluated MFC's. The highest values of PD, VPD and CD were; 153.43 mW m^{-2}, 1.726 W m^{-3} and 0.413 A m^{-2} respectively, they were determined in SWS MFC. MFC operated with sterilized wastewater supplemented with buffer solution and inoculated with *Clostridium bifermentans* (SWBB) showed not only the lowest values of PD (17.36 mW m^{-2}), VPD (0.195 W m^{-3}) and CD (0.139 A m^{-2}) but also the minimum voltage (0.125 V), despite this trend, it PD was higher to that reported in some previous works (Martínez-Santacruz *et al*., 2016; Mora and Bravo, 2017). With exception of SWB, SWBB and SWBS tests, the rest of experiments showed a higher PD in comparison to the maximum values reported by Guadarrama-Pérez *et al.* (2018) and Tatinclaux *et al*. (2018), who respectively evaluated the potential of sucrose and wastewater to produce voltage in a single chamber MFC. The VPD values calculated in this work were higher to that obtained by Pérez-Rodríguez *et al*. (2018), but lower in comparison to the most efficient MFC's operated with glucose and anaerobic sludge-sediment mixture (Alzate-Gaviria *et al*., 2010; Guadarrama-Pérez *et al*., 2018). The differences in VPD are associated with the nature and complexity of substrates, inoculum, configuration and working volume of MFC's and electrodes material. For CD, the values obtained in this work were in the range reported by Tatinclaux *et al*. (2018) during wastewater treatment by air cathode MFC and lower than the highest CD's calculated by Mateo *et al*. (2019) in miniature MFC's performed with synthetic wastewater. Not only substrates, but also working volume, MFC's configuration and the obtained voltage are variables that can explain the differences in bioelectrochemical performances.

In all MFC's inoculated with pure strains the total amount of bacteria necessary to produce 1 mV was calculated and summarized in Table 4. As can be seen, SWS MFC needed the lowest amount of microorganisms showing a value of 3.90×10^5 cel mL^{-1} mV^{-1}. The highest amount of bacteria per mV was 1.62×10^6 observed in SWB MFC, this fact can be related with the acidogenic metabolism of *Clostridium bifermentans* which produces other metabolites such as hydrogen, organic acids and carbon dioxide diminishing its potential to generate voltage (Wong *et al*., 2018).

Table 3. Bioelectrochemical performances of MFC's operated with Ravot medium and municipal wastewater.

Test	**Maximum voltage (V)**	**Current (A)**	**PD (mW / m^2)**	**VPD (W / m^3)**	**CD (A /m^2)**
RMB	0.203	2.03×10^{-4}	46.08	0.518	0.226
RMS	0.278	2.78×10^{-4}	85.87	0.966	0.309
SWB	0.13	1.30×10^{-4}	18.85	0.212	0.145
SWS	0.372	3.72×10^{-4}	153.43	1.726	0.413
SWBB	0.125	1.25×10^{-4}	17.36	0.195	0.139
SWBS	0.194	1.94×10^{-4}	41.87	0.471	0.216
NSW	0.249	2.48×10^{-4}	68.54	0.771	0.276

Table 4. COD removal in wastewater by MFC's performed with *Clostridium* strains and native bacteria.

Test	**Cel mL^{-1} mV^{-1}**	**Final pH**	**Final COD (mg L^{-1})**	**COD removal (%)**
RMB	7.51×10^5	6.95 ± 0.05	ND	ND
RMS	6.94×10^5	6.6 ± 0.3	ND	ND
SWB	1.62×10^6	6.7 ± 0.1	239.77 ± 4.9	30.56
SWS	3.90×10^5	6.75 ± 0.05	139.22 ± 2.1	59.68
SWBB	1.39×10^6	6.9 ± 0.01	126.29 ± 5.8	63.43
SWBS	9.47×10^5	7.05 ± 0.05	109.59 ± 1.7	68.26
NSW	ND	7.25 ± 0.05	282.38 ± 3.4	18.23

ND: Not determined

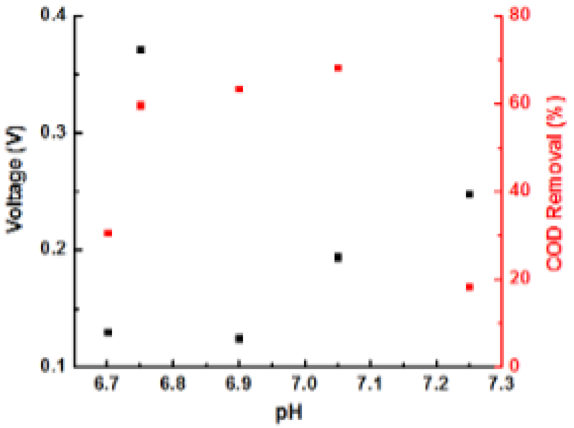

Figure 4. Behaviour of voltage generation and COD removal as a function of pH determined after MFC's operation.

At the end of the experiment MFC's operated with wastewater showed the lowest pH's values for *Clostridium bifermentans*, suggesting the production of organic acids from this strain. The addition of buffer solution decreased pH gradient for both *Clostridium* strains. NSW MFC showed not only the lowest change in pH but also the minimum COD removal (18.23%), this behaviour can be due to a low bacterial activity which suggests that some part of the generated voltage could be associated to free ions in wastewater. SWBB and SWBS tests reached the highest COD reductions showing removal percentages of 63.43 and 68.26% respectively. In Table 4 can appreciate that *Clostridium sordelli* degraded more organic matter in comparison with *Clostridium bifermentans*, furthermore, the addition of buffer solution increased COD removal but it was not efficient for bioelectrochemical purposes. The highest COD removals were observed in the pH range of 6.75 and 7.1; different values of pH correspond to low percentages of COD reduction. With exception of the maximum voltage obtained (0.372 V), voltage generation increased as pH rises showing the minimum values at 6.7 and 6.9 (Figure 4). This trend suggests that better performance of MFC's can be obtained maintaining the pH as close as possible to neutrality employing *Clostridium sordellii* strain.

The amount of COD degraded by MFC's inoculated with *Clostridium* strains and native bacteria is in the range reported by Mateo *et al.* (2019), who reached a maximum reduction of 160 mg L^{-1} in a serial process composed by 4 miniatures MFC's. Martínez-Santacruz *et al.* (2016) working with domestic wastewater (COD, 220 - 370 mg L^{-1}) reported a maximum COD removal of around 80%, which was slightly higher to that obtained in present work, however the PD's calculated by them were lower than the reported here. The percentages of COD removal obtained in all MFC's (Table 4) were lower in comparison with the 80% reported by Buitrón and Pérez (2011), the difference in removal efficiency can be attributed to the nature of substrate and inoculum source, since they enriched municipal wastewater with glucose from 430 to 2250 mg L^{-1} COD concentration and employed a native consortia. Moreover, COD removal efficiencies obtained in this study, are in the range reported previously by González-Paz *et al.* (2020), who operated MFC with sludge, acetate and butyrate.

MFC's were modified and used as MEC's in order to perform preliminary tests for bioelectrohydrogen production from municipal wastewater. For this, NSW, SWB and SWBB conditions were evaluated and a 1.2 V battery was employed as external energy source. Electrodes were made by graphite cloth and treated as described in section 2.4. The amounts of hydrogen determined by gas chromatography were 3.80, 3.46 and 3.50 mL for NSW, SWB and SWBB respectively. As MFC's, MEC's showed COD demand removal, however, to increase the performance of MEC's and to exploit *Clostridium* strains potential later researches are needed.

Conclusions

The conformation of bioelectrochemical system allows producing voltage and treat municipal wastewater in an economical and feasible way. MFC's inoculated with *Clostridium sordellii* showed not only a high potential to reduce COD in wastewater but also to produce voltage. Buffer solution increased COD reduction but was not efficient for voltage production. Native bacteria in NSW were able to produce voltage diminishing low amounts of COD. Organic matter present in municipal wastewater can be removed by MFC's operated with graphite cloth electrodes and inoculated with *Clostridium* strains generating green energy as by-product. The COD concentrations obtained at the end of the experiments with *Clostridium* strains are lower to that allowed by Mexican regulations showing the capability of these bacteria to treat municipal wastewater and to produce sustainable energy simultaneously.

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