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Influence of the cellulose and sulfate ratio on voltage generation in Winogradsky columns

Influencia de la proporción de celulosa y sulfato en la generación de voltaje en columnas de Winogradsky

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Abstract
The Winogradsky column is a nutrient-enriched sediment column for studying microbial diversity and the sulfur cycle. In this device, microorganisms generate a vertical chemical gradient, and voltage production can be measured with a graphite plate (buried in sediment) and a graphite plate (submerged in water). This system is similar to sediment microbial fuel cells (SMFC) used for generating electrical energy. Voltage generation in SMFCs has been established with different cellulose concentrations. However, cellulose and sulfate proportions have not been tested so far. Cellulose/sulfate ratios can change the microbial community, vertical chemical gradient, and potential difference. Therefore, this work aims to test the effect of different cellulose/sulfate ratios in Winogradsky columns to improve voltage generation. Voltage was generated in cellulose/sulfate ratios between 0.08 and 1.28. An optimal cellulose/sulfate ratio of 0.75, identified with the response surface model, produced a voltage between 300 to 400 mV for 20 days. These devices represent an alternative for renewable energy generation where cellulose waste could be used as a carbon source.
Keywords: sediment microbial fuel cell, Winogradsky column, voltage.

Resumen
La columna de Winogradsky es una columna de sedimento enriquecido con nutrientes para estudiar la diversidad microbiana y el ciclo del azufre. En este dispositivo, los microorganismos generan un gradiente químico vertical y la producción de voltaje se puede medir con una placa de grafito (enterrada en sedimento) y una placa de grafito (sumergida en agua). Este sistema es similar a las celdas de combustible microbianas de sedimentos (CCMS) que se utilizan para generar energía eléctrica. La generación de voltaje en las CCMS se ha establecido con diferentes concentraciones de celulosa. Hasta ahora no se ha probado la proporción de celulosa y sulfato. La relación celulosa/sulfato puede cambiar la comunidad microbiana, el gradiente químico vertical y la diferencia de potencial. Por tanto, este trabajo tiene como objetivo probar el efecto de diferentes proporciones de celulosa/sulfato en columnas de Winogradsky para mejorar la generación de voltaje. El voltaje se generó en relaciones de celulosa/sulfato entre 0.08 y 1.28. Una relación óptima de celulosa/sulfato igual a 0.75, se identificó con el modelo de superficie de respuesta, la cual produjo un voltaje entre 300 y 400 mV durante 20 días. Estos dispositivos representan una alternativa para la generación de energía renovable y se podrían utilizar residuos de celulosa como fuente de carbono.
Palabras clave: celdas de combustible microbianas de sedimento, columna de Winogradsky, voltaje.

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1 Introduction

The Winogradsky column consists of a cylindrical recipient with sediment and water, enriched with nutrients and exposed to light. This system favors the development of different microbial communities that participate in the sulfur cycle, such as sulfate-reducing bacteria, phototrophic sulfur bacteria, and sulfur-oxidizing bacteria (Rogan *et al*., 2005; Rundell *et al*., 2014; Abbasian *et al*., 2015). Bacterial metabolic activities generate over time a vertical gradient of chemical conditions that include redox potential and the concentration of oxygen and hydrogen sulfide (Rogan *et al*., 2005). Winogradsky columns equipped with an anode (buried in sediment) and a cathode (submerged in water) allow evaluating the voltage generated from the potential difference between the lower and upper zones of the column (Bacchetti-DeGregoris *et al*., 2015). Such Winogradsky columns with electrodes and an electrical circuit are similar to sediment microbial fuel cells (SMFC) for electric energy generation (Zhao *et al*., 2016; Zhu *et al*., 2016).

Sediment microbial fuel cells (SMFCs) transform the chemical energy of the compounds into electric power. This process begins with the bacterial degradation of chemical compounds, generating electrons and protons. Then, a charge transfer occurs to the anode; electrons travel from the anode to the cathode through an external circuit, and the protons and ionic species travel along the column (Sajana *et al*., 2014; Bhande *et al*., 2019). In SMFCs, anaerobic conditions predominate in the cell's lower zones, while aerobic conditions prevail in the upper zone (Wang *et al*., 2014).

These devices and microbial fuel cells represent an alternative for electricity generation during the removal of organic waste (Gómora-Hernández *et al*., 2020; González-Paz *et al*., 2020). Therefore, strategies have been sought to increase energy generation such as: greater oxygenation in the cathodic zone through the light that favors the activity of photosynthetic microorganisms (Commault *et al*., 2014; Wang *et al*., 2014); and type and concentration of the substrate (Ribot-Llobet *et al*., 2014; Sajana *et al*., 2014). The substrate serves as a nutrient for microorganisms and an electron donor. Therefore, the substrate affects the performance of SMFCs in terms of energy recovery (Bhande *et al*., 2019). Acetate (Ribot-Llobet *et al*., 2014), organic matter (Zhao *et al*., 2016), and cellulose (Sajana *et al*., 2014; Bhande *et al*., 2019) have been used as electron donors in SMFCs.

Voltage generation in SMFCs has been improved with different cellulose concentrations (Sajana *et al*., 2014; Bhande *et al*., 2019). However, the effect of the carbon/sulfate ratio on voltage generation has not been tested so far. In the Winogradsky column, carbon and sulfur cycles occur simultaneously through a source of cellulose and sulfate that encourages the development of sulfur microbial communities (Rogan *et al*., 2005; Rundell *et al*., 2014). The use of sulfate in microbial fuel cells has been suggested because sulfide generated by sulfate-reducing bacteria proved to be an efficient redox mediator to the anode (González-Paz *et al*., 2020) and capable of changing the anodic potential (An *et al*., 2013; Bratkova *et al*., 2019). Therefore, the sulfate concentration in the sediment could modify the microbial community and improve voltage generation.

The microbial community in anaerobic environments can change based on nutrient availability. The presence of sulfate is crucial in the competition of sulfate-reducing bacteria with other anaerobes. The degradation of organic matter by sulfate-reducing bacteria will occur in the presence of sulfate; in contrast, in the absence of sulfate, acetogenic and methanogenic bacteria will be present (Muyzer and Stams 2008). In an anaerobic reactor, nutrients with low carbon/sulfate ratios favor the sulfate-reducing bacteria, while high carbon/sulfate ratios promote the growth of acetogenic and methanogenic bacteria (Dar *et al*., 2008).

Different microbial communities in the Winogradsky column developed by changing the cellulose/sulfate ratio could also modify the anode potential and voltage generation. Therefore, this study aims to test the effect of different cellulose/sulfate ratios in Winogradsky columns on voltage generation.

The Winogradsky columns were prepared with sediment from the Tecolutla estuary and equipped with anode, cathode, and an electrical circuit to measure the voltage generation. Nine treatments (each in triplicate) were used to determine the effect of cellulose/sulfate ratio on voltage generation. The voltage results were used in a response surface model to propose and test the most suitable condition for voltage production improvement. They were further discussed to show the importance of this study for voltage generation enhancement in SMFCs.

2 Materials and methods

2.1 *Sediment samples*

The sediment was sampled from the Tecolutla estuary in the State of Veracruz, Mexico. Mud sediment was collected from the river zone located at 20°28'16" N and 97°00'25" W, close to the Tecolutla River mouth.

The granulometric analysis was performed by the pipetting method (Lewis and McConchie, 1994), and sediment was classified according to the Trefethen nomenclature triangle (Shepard, 1954). The water content of the mud was evaluated by weight loss, and the percentage of organic matter and carbonates was determined by the loss on ignition method (Lewis and McConchie, 1994).

Total carbohydrate concentration in sediment was evaluated by the phenol-sulfuric acid method (Liu *et al*., 1973). Sulfate concentration in sediment interstitial water was assessed using a turbidimetric method (Kolmert *et al*., 2000). Sediment pore water was obtained by dilution and centrifugation. The calibration curve and calculations were performed to report total carbohydrate and sulfate concentrations.

2.2 *Development of the microbial community in Winogradsky columns*

Commonly, sulfate and cellulose have been added in an amount of 0.7 to 3.5 % of the weight of sediment to build the Winogradsky columns (Rogan *et al*., 2005; Rundell *et al*., 2014; Babcsányi *et al*., 2017). The present study used estuarine sediment from Tecolutla to obtain the bacterial community in these columns. The columns were built using three tubes (22 mm diameter, 170 mm length), containing a culture medium enriched with magnesium sulfate, kraft paper, and calcium carbonate at 1 % in deionized water; sediment:culture medium ratio was 2:1. These Winogradsky columns were incubated for 50 days at room temperature and exposed to constant illumination with a 6500K color temperature LED. Afterward, the columns with the microbial community were stirred, and 8 mL were taken to inoculate the mud used for voltage generation. In these systems, the potential difference was measured between the bottom and surface layers of the sediment column (Bacchetti-DeGregoris *et al*., 2015).

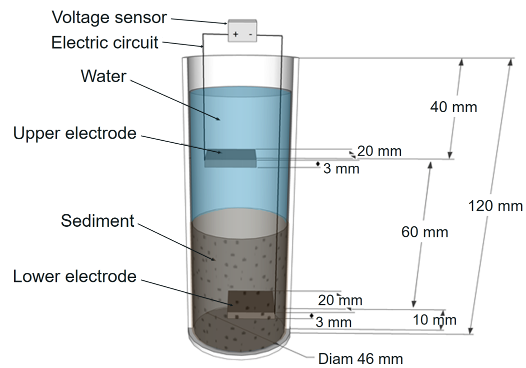


Fig. 1. Winogradsky column device with dimensions and electrodes to measure voltage generation.

2.3 *Column set-up for voltage generation*

The Winogradsky columns for voltage generation consisted of cylindrical glass recipients (46 mm diameter, 120 mm height, 125 mL volume), with an anode buried in sediment and a cathode submerged in water (Zhao *et al*., 2016; Zhu *et al*., 2016). Each electrode consisted of a graphite plate (20 mm x 20 mm x 3 mm) connected to a copper wire and a voltage sensor (Fig. 1). The column with electrodes was sterilized in an autoclave at 121°C (15 psi) for 20 minutes. Then, a glass recipient with 60 g of sterilized wet sediment and 60 mL of sterilized culture medium were prepared (see section 2.4). This glass recipient was inoculated with 8 mL of mud with the microbial community and stirred to pour its content into the column with electrodes.

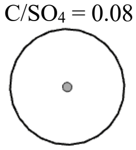
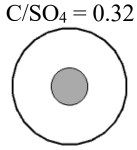
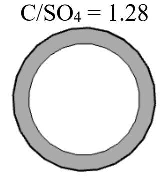
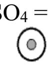
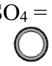
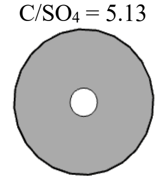
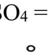
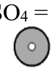
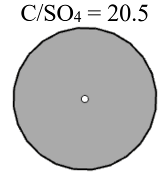
The devices were placed in the Controlled Environment Laboratory (CEL) at UAM-I, at a controlled temperature of 20.6 ± 0.5 °C and under constant illumination with a LED tube (6500K color temperature, 16 W, 1500 lumens). The voltage generated in Winogradsky columns was recorded for 45 days with a data acquisition system consisting of an Arduino UNO plate with voltage sensors connected to a data logger shield to record the data in an SD memory every minute.

2.4 *Nutrient compositions in Winogradsky column devices for voltage generation*

An initial phase was made to evaluate the system performance enriched with pulverized kraft paper towel as a source of cellulose, magnesium sulfate, and calcium carbonate, all of them at 1 % w of the wet sediment weight (60 g). The organic cellulose/sulfate ratio in this medium was 2.57. Later, the proportion of the compounds in three new devices was modified to contain less paper (0.5 % w) but the same amounts of magnesium sulfate (1 % w) and calcium carbonate (1 % w) as before, corresponding to a cellulose/sulfate ratio of 1.28. This nutrient ratio favored the generation of voltage and was selected for inoculation of subsequent factorial design treatments.

In the next stage, carbon/sulfate ratios were modified to promote the sulfate-reducing bacteria or other bacterial groups, such as acetogenic and methanogenic bacteria (McCartney and Oleszkiewicz 1993; Dar *et al*., 2008). Different cellulose/sulfate ratios were tested using a 3^2 factorial design (Gutiérrez-Pulido and Vara-Salazar 2012). This factorial design consisted of 2 factors (cellulose and sulfate) at three levels of concentration (low, medium, and high). Cellulose was used in the technical grade reagent with a known composition ($(C_6H_{10}O_5)_n$). Cellulose concentrations were: 0.125 % w (low), 0.5 % w (medium), and 2 % w (high) in relation to the wet sediment weight (60 g) placed in the column. Magnesium sulfate heptahydrate was used as a sulfate source. Calculations were made to add sulfate at the following concentrations: 0.10 % w (low), 0.39 % w (medium), and 1.56 % w (high) of the wet sediment weight (60 g), corresponding to 0.25 %, 1 %, and 4 % of magnesium sulfate heptahydrate. By combining these levels, nine treatments with different cellulose/sulfate ratios were obtained: 0.08 (A), 0.32 (B and D), 1.28 (*C*, E, and G), 5.13 (F and H), and 20.5 (I) (Table 1). Besides, calcium carbonate ($CaCO_3$) was added at 0.5 % to all treatments. The nine treatments were made in triplicate, producing a total of 27 Winogradsky columns. Some extra cells were prepared as abiotic controls, with A, *C*, E, G, and I treatments, but they did not include the microbial community inoculum.

Table 1. Culture medium compositions of 9 treatments with 5 different cellulose/sulfate ratios: 0.08 (A), 0.32 (B and D), 1.28 (*C*, E and G), 5.13 (F and H), and 20.5 (I). These treatments correspond to a 3^2 factorial design, with three levels (low, medium, and high) of two factors: cellulose (C) and sulfate (SO_4).

		Factor (c): Proportion of cellulose (C)		
	Level	Low C = 0.125 %	Medium C = 0.5 %	High C = 2.0 %
	High SO_4 = 1.56 %	A C/SO_4 = 0.08 	B C/SO_4 = 0.32 	*C* C/SO_4 = 1.28 
Factor (s): Sulfate (SO_4) as $MgSO_4$ $7H_2O$	Medium SO_4 = 0.39 %	D C/SO_4 = 0.32 	E C/SO_4 = 1.28 	F C/SO_4 = 5.13 
	Low SO_4 = 0.10 %	G C/SO_4 = 1.28 	H C/SO_4 = 5.13 	I C/SO_4 = 20.5 

The diameter of the circles represents the amount of cellulose (gray) and sulfate (white) in each treatment.

The factorial design results were analyzed using Statgraphics Centurion IX software to obtain the equation, ANOVA, and the effect of the factors on the response variable. The response surface model was used to establish the best conditions for voltage production (Gutiérrez-Pulido and Vara-Salazar, 2012). Lastly, to validate the model, Winogradsky columns were produced in triplicate with cellulose and sulfate concentrations proposed by the response surface model.

3 Results and discussion

3.1 Sediment characteristics

The granulometric composition was silt (46 %), sand (33 %), and clay (20 %), corresponding to the sandy-clay-silt sediment. It is common to find muddy sediments with different percentages of sand, silt, and clay content in coastal aquatic environments. These percentages can change depending on the site and time due to the environmental hydrodynamics (Calva-Benítez and Torres-Alvarado, 2011). The water content of the mud was 46.2 %, the organic matter represented 6.5 % (65 mg/g), and the carbonate content was 7 % (70 mg/g) of the dry weight of sediment.

Total carbohydrate concentration in sediment was 8.2 mg/g, that is, less than 13 % of the organic matter. Commonly, a large fraction of organic matter in sediments remains unidentified. Total carbohydrates make up between 8 and 48% of total organic carbon (Dauwe and Middelburg, 1998; Skellari *et al*., 2011). Winogradsky columns require a relatively low percentage of cellulose (0.7 to 2 %) as a carbon source to allow the growth of the microbial community (Rundell *et al*., 2014; Babcsányi *et al*., 2017).

Sulfate concentration in sediment was 1.3 mg/g. It may widely vary in different aquatic environments, from 10 μM to 500 μM in freshwater systems, while in marine environments, it is 28 mM (2.69 mg/g) (Holmer and Storkholm 2001). In a water mixing zone, such as the Tecolutla estuary, a concentration of 1.3 mg/g of sulfate in sediment interstitial water can be expected.

3.2 Microbial community developed in the Winogradsky columns

Microbial growth in Winogradsky columns generated color changes in the sediment. In the first five days, sediment darkening caused by the activity of sulfate-reducing bacteria, was observed. The black coloration of the sediment indicated metal sulfide precipitation (Rundell *et al*., 2014; Singh 2020). Then, green stains, typical of phototrophic sulfur bacteria, developed and spread until forming a green zone, which covered one-third of the sediment. The green coloration at the water-sediment interface may be attributed to the presence of cyanobacteria. Such color layers typically occur in Winogradsky columns (Rogan *et al*., 2005; Rundell *et al*., 2014). This microbial community was used to inoculate devices for voltage generation.

3.3 Voltage generation in Winogradsky columns

The voltage in the first devices (compounds at 1 %, cellulose/sulfate ratio 2.57) was around 160 mV and exhibited high variation with voltage increase and decrease intervals (Fig. 2).

The cellulose/sulfate ratios showed an effect on voltage generation in Winogradsky columns (Fig. 3). Voltage production was favored in treatments D and E (cellulose/sulfate ratio 0.32 and 1.28, respectively). In contrast, when an excess of sulfate was tested (treatment A; cellulose/sulfate ratio 0.08) voltage generation was relatively low while inducing an excess of cellulose (treatment I; cellulose/sulfate ratio 20.5) almost completely inhibited voltage generation.

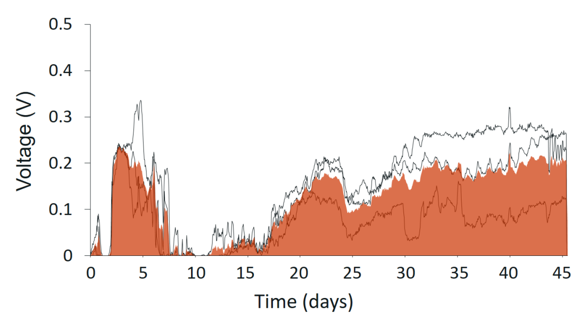


Fig. 2. Voltage generation in Winogradsky columns monitored for 45 days and enriched with paper, magnesium sulfate, and calcium carbonate, all at 1 % w of the sediment weight (cellulose/sulfate ratio 2.57). The lines correspond to 3 replicates; the shaded area is the average.

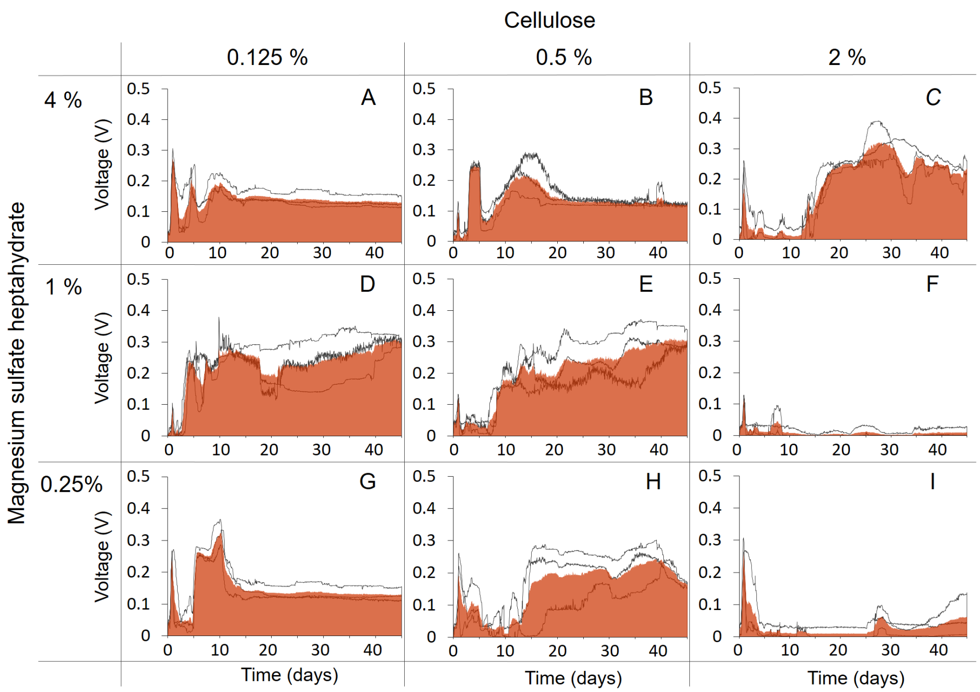


Fig. 3. Voltage generation in Winogradsky columns with different cellulose/sulfate ratios: 0.08 (A), 0.32 (B and D), 1.28 (*C*, E and G), 5.13 (F and H) and 20.5 (I). Cellulose and magnesium sulfate in percentages corresponding to low, medium, and high levels. The lines correspond to 3 replicates; the shaded area is the average.

The results showed that the potential difference was modified by the cellulose/sulfate ratio. The microbial community in a Winogradsky column can change as a consequence of nutrient composition. The change of microbial community can also modify the gradient of conditions in sediment columns. Different conditions of dissolved oxygen and redox potential have been reported along the Winogradsky columns (Pagaling *et al*., 2014; Babcsányi *et al*., 2017). If the microbial community and redox potential along the column change, the voltage generation changes as well. The potential difference between the lower and upper zones of the column influences the voltage generation (Bacchetti-DeGregoris *et al*., 2015). A more negative redox potential in the lower zone corresponds to a more reducing environment, achieved through a higher concentration of hydrogen sulfide generated by sulfate-reducing bacteria (Singh 2020). The more reducing condition in the lower zone increases the potential difference with respect to the positive redox potential in the upper zone, which generates a higher voltage. Different cellulose/sulfate ratios were tested to achieve the highest potential difference.

The ratio between organic carbon source and sulfate was modified considering the proportion of nutrients that could favor the sulfate-reducing bacteria or other bacterial groups, such as acetogenic and methanogenic bacteria. For example, the high sulfate concentration in treatment A may favor sulfate-reducing bacteria, but the carbon source's limitation restricts the development of the microbial community. In treatment I, however, the limitation of sulfate can inhibit the sulfidogenic process by competition between anaerobic bacteria (McCartney and Oleszkiewicz, 1993; Dar *et al*., 2008). Therefore, to detect a higher voltage generation, the increase in sulfate concentration must be accompanied by the addition of cellulose, as in treatments D and E (cellulose/sulfate ratio of 0.32 and 1.28, respectively) that favored voltage generation.

Sulfate concentrations naturally present in the sediment (1.3 mg/g) are negligible compared to the amounts of sulfate added to Winogradsky columns. Sulfate added at the low level (1.0 mg/g in G, H, and I treatments) was similar to that found in the environmental sediment and, therefore, its final concentration was 2.3 mg/g. In the experimental units using the high sulfate level (15.6 mg/g in A, B, and *C* treatments), the added amount was ten times greater than that found in the sediment samples.

The amount of cellulose added in treatments A, D, and G (1.3 mg/g) represents a small proportion of total carbohydrates present in the sediment, while in B, E, and H, the amount (5.0 mg/g) was similar to that found in the initital sediment samples (8.2 mg/g). Finally, in treatments *C*, F, and I, the added cellulose (20.0 mg/g) was 2.5 times higher than the carbohydrates present in the sediment. Undoubtedly, bacteria could also use the total carbohydrates naturally present in the sediment, but the effect of each treatment on voltage generation was clearly observable.

The concentration of cellulose and sulfate in nutrient enrichment had a significant influence on the results. Treatments with the same cellulose/sulfate ratio showed different voltage behaviors (Fig. 3). For example, a cellulose/sulfate ratio of 5.13 (treatments F and H) favored the voltage production in treatment H, but not in F. With the cellulose/sulfate ratio of 1.28 (treatments *C*, E, and G), higher voltage generation was recorded where the culture medium had an intermediate concentration (treatment E). In treatments B and D, whose cellulose/sulfate ratio was 0.32, a higher voltage was recorded in D than in B (Fig. 3).

Sajana *et al*. (2014) report that low and high cellulose concentrations have a negative impact on voltage generation. It was observed that adding cellulose (up to 2%) increased the voltage in sediment microbial fuel cells (SMFC), but concentrations above 4% negatively affected voltage generation. In the present work, more voltage was recorded when the cellulose concentration was lower, except for the treatment G, which had a low concentration of cellulose and sulfate; this could represent a nutrient limitation for bacteria (Dar *et al*., 2008).

In the control cells, without microbial consortium, very low voltage values were recorded, averaging 50 mV, and no voltage peak was observed. Thus, the absence of the microbial community prevents the generation of a gradient of conditions along the column, resulting in a low potential difference between the column's lower and upper areas.

3.4 The effect of factors and response surface model

The surface model was built using the data shown in figure 3. This information served as a basis for developing the mathematical regression model, which was further used to obtain a second-order polynomial equation:

$$V = 0.1761 - 0.0497\text{C} + 0.0204\text{S} - 0.0434\text{C}^2 + 0.0392\text{CS} - 0.0155\text{S}^2 \quad (1)$$

Where: V = voltage, C = cellulose concentration, and S = sulfate concentration.

Statistical analysis of the model by ANOVA is shown in Table 2. A high F-value with a low p-value specifies the significance of the regression model. The p-value evaluates the significance of each variable and the interaction of variables. Cellulose and the cellulose-sulfate interaction were statistically significant. Coefficient of determination R^2 was 0.62, which indicates a correlation between the observed values and those predicted by the model and explains 62 % voltage generation.

The analysis of the factors' effect (Fig. 4 a) indicates that when cellulose is used at low to medium concentrations, it favors voltage generation. However, with a higher amount of cellulose, voltage production decreases. Increasing the sulfate concentration has a positive effect on voltage generation. This positive effect could be related to the activity of sulfate-reducing bacteria. The electric power generation in electrochemical systems might be associated with processes related to the sulfur cycle (Lee *et al*., 2014; Bratkova *et al*., 2019). Sulfide generated by sulfate-reducing bacteria can act as an electron donor to the

Table 2. ANOVA results of the cellulose and sulfate concentrations and their interaction for voltage generation in Winogradsky columns.

Source	Sum of Squares	df	Mean Square	F-value	p-value
C: cellulose	0.0445	1	0.0445	15.15	0.001*
S: sulfate	0.0075	1	0.0074	2.54	0.127
CC	0.0113	1	0.0113	3.85	0.065
CS	0.0184	1	0.0184	6.26	0.022*
SS	0.0014	1	0.0014	0.49	0.493

* Statistically significant

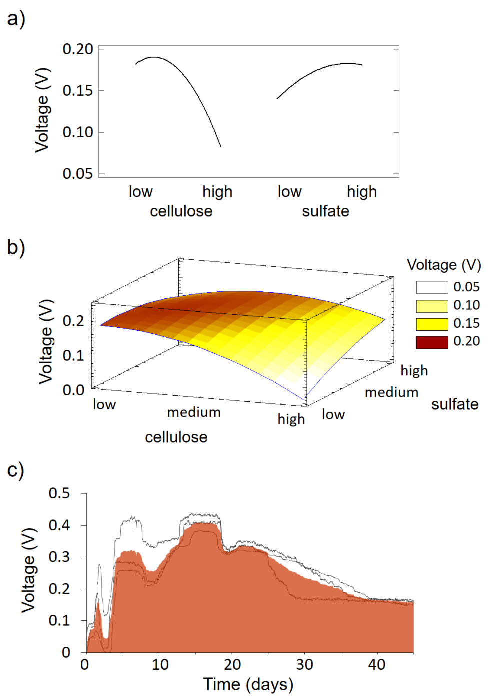


Fig. 4. Graphs of: a) the factors' effect, b) response surface model, and c) voltage generation using the optimal conditions proposed by the model.

anode (González-Paz *et al*., 2020) and change the anodic potential (An *et al*., 2013; Singh 2020).

To increase voltage generation, the response surface model suggested an optimal level (Fig. 4 b) corresponding to 0.25 % of cellulose, 0.34 % of sulfate, and a cellulose/sulfate ratio of 0.75. To validate the surface model prediction, new Winogradsky columns were prepared under conditions calculated by the response surface model, and an increase in voltage generation was observed (Fig. 4 c). A maximum voltage of 400 mV was reached, which remained for four days and decreased over time. Voltage generation at the optimal level represents an increase of 20 % compared to treatment E (Fig. 3) and double voltage production compared to the first condition evaluated in this study (Fig. 2).

Sajana *et al*. (2014) and Bhande *et al*. (2019) improved voltage generation in sediment microbial fuel cells with different cellulose concentrations, but they did not employ statistical optimization or consider the cellulose/sulfate ratio. High voltages (684 and 950 mV) were achieved in these works, but they used sediment microbial fuel cells with 150 cm height and aeration in the upper zone. Such conditions caused a more reducing environment in the lower zone and a more oxidizing one in the upper zone. The present study improved voltage generation by changing the cellulose/sulfate ratio in a Winogradsky column with a 12 cm height. A greater distance between the lower and upper electrodes increased the voltage generation. An anode buried 10 cm deep generated four times more voltage than an anode buried 2 cm deep in an SMFC (An *et al*., 2013). Therefore, changing the height and nutrient enrichment simultaneously could be a good option for enhancing voltage generation in these devices.

In other systems, like microbial fuel cells, few researchers have employed a factorial design to optimize and identify, in a systematic manner, the effect of different factors, such as the culture medium composition and operational conditions (temperature, initial pH, and salt bridge components), on voltage generation (Al-Shehri *et al*., 2013; Alshehria *et al*., 2016). The generation of voltage was increased from 684 mV to 738 mV (8% rise in voltage) with the enhanced medium (Al-Shehri *et al*., 2013). Although the R^2 value of the mathematical model was relatively low and unable to predict all voltage values, the use of the model allowed choosing the experimental conditions to improve the voltage generation.

SMFCs represent an alternative for renewable energy generation. The voltage generated in the Winogradsky column using cellulose is a good option because millions of tons of vegetable cellulose waste generated continuously could be used (Ren *et al*., 2008; Rezaei *et al*., 2009). In addition, wastewater rich in sulfates from various types of industries, such as food, pharmaceutical, paper pulp manufacturing, detergent manufacturing, and mining, could also be used for this purpose (Liang *et al*., 2013; González-Paz *et al*., 2020). Now, microbial fuel cells (MFC) and their variants, such as sediment-MFC and plant-MFC, can be employed to charge low-consumption electronic devices (Gómora-Hernández *et al*., 2020).

Liu *et al*. (2016) indicate that consistent performance in SMFCs is a prerequisite to applying these devices for power generation. The present study demonstrated the reproducibility of Winogradsky cells through voltage generation in each treatment. The condition provided by the response surface model generated a higher and more stable voltage, although a decrease in voltage was observed as of day 27. This phenomenon could be related to a depletion of nutrients. Consequently, adding more nutrients could have a positive effect on voltage generation. These results provide useful information to propose and build a continuous voltage generation system.

Conclusions

Previous researches only tested different concentrations of cellulose to evaluate voltage production in sediment microbial fuel cells (SMFCs). In this work, the amount of sulfate and the cellulose/sulfate ratio were considered for optimizing voltage generation in a special SMFC: Winogradsky columns. The results showed that the amount of voltage depends on both the concentration and the proportion of cellulose and sulfate. A low cellulose/sulfate ratio can favor sulfate-reducing bacteria, thus generating a more negative redox potential and increasing voltage generation. The cellulose/sulfate ratio between 0.32 and 1.28 promotes voltage generation for more than 35 days. The optimal conditions proposed by the response surface model produced a maximum of 400 mV and a more stable voltage generation with a cellulose/sulfate ratio of 0.75 (0.25 % of cellulose and 0.34 % of sulfate). This best condition represents the highest potential difference between the oxidizing upper zone and the reducing lower part of the column. Voltage production was achieved in small Winogradsky columns with a height of 12 cm and a distance of 6 cm between anode and cathode. The results generated in this work can be used to establish the operating conditions of an SMFC with continuous nutrient addition to achieve constant power generation.

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