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Research article

Bioelectrogenesis with microbial fuel cells (MFCs) using the microalga *Chlorella vulgaris* and bacterial communities

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ABSTRACT

Background: Microbial Fuel Cell (MFC) technology is used in various applications such as wastewater treatment with the production of electrical energy. The objective of this study was to estimate the biodepuration of oils and fats, the elimination of blue dye brl and bioelectro-characterization in MFCs with *Chlorella vulgaris* and bacterial community.
Results: The operation of MFCs at 32 d showed an increase in bioelectrogenic activity (from 23.17 to 327.67 mW/m^2) and in the potential (from 200 to 954 mV), with biodepuration of fats and oils (95%) in the microalgal cathode, and a removal of the chemical oxygen demand COD (anode, 71%, cathode, 78.6%) and the blue dye brl (73%) at the anode, here biofilms were formed by the bacterial community consisting of Actinobacteria and Deltaproteobacteria.
Conclusions: These findings suggest that MFCs with *C. vulgaris* and bacterial community have a simultaneous efficiency in the production of bioelectricity and bioremediation processes, becoming an important source of bioenergy in the future.

1. Introduction

Microbial fuel cells (MFCs) have gained much attention in recent years with microbial electricity production offering the possibility of obtaining electrical current from a wide range of soluble or dissolved organic wastes such as artificial, real and biomass wastewater Lignocellulosic [1]. MFC technology is based on the electrogenic nature of certain bacteria while treating different wastewater and producing electrical energy. Several types of sewage have been examined as substrates for feeding bacteria into MFCs being some of the future challenges and prospects regarding the energy recovery of wastewater [2].

Studies on the scale-up of MFCs containing multiple electrodes have shown the importance of optimization of electrode spacing and increasing specific surface area (surface area of the electrode per volume of reactor) to improve performance [3,4].

One of the potential applications of MFCs technology is to recover bioenergy from low-grade substrates such as wastewater [5]. The electrical effects in a biological system were observed ~100 years ago, leading to the development of the MFCs concept with intensive research conducted in the past decade [6]. We have obtained a substantial amount of fundamental information about the microbiology, electrochemistry, and materials of MFCs. To examine the technical viability of MFCs, it is critical to understand their application niche, which is strongly related to their energy performance. It is widely acknowledged that the advantage of MFCs technology is direct electricity generation; however, there is a lack of proper presentation of the energy data, and we are still not clear about how much energy MFCs can actually recover from wastewater [7].

The pollutants viz., sulfur, azo dye, nitrobenzene, chlorophenol, chromium, endocrine disrupting estrogens copper, uranium etc. present in the wastewaters itself act as mediators in transferring electrons to the anode [8,9,10,11,12].

Electrically active bacterium transports its metabolically generated electrons to insoluble substrates such as electrodes via a process known as extracellular electron transport (EET). Bacterial EET is a crucial process in geochemical cycling of metals, bioremediation and

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bioenergy devices such as MFCs. Recently, it has been found that electroactive bacteria can reverse their respiratory pathways by accepting electrons from a negatively poised electrode to produce high-value chemicals such as ethanol in a process termed as microbial electrosynthesis (MES). A poor electrical connection between bacteria and electrode hinders the EET and MES processes significantly [13]. Electron transfer in a biocatalyzed electrochemical system plays a major role in harnessing electricity and degrading multiple pollutants present in the system. Understanding the microbe-electrode interaction aids in maximizing the system performance since it plays a crucial role in electron transfer mechanism [14,15].

MFCs operating in a continuous mode have been found to be more suitable than batch or fed-batch ones for both increased COD removal and power generation [16]. However, when an MFC operates in a continuous mode, power and substrate degradation will inevitably decay if the rate of the electrolyte flow exceeds those of substrate consumption and microbial growth [17].

The electrons generated from the oxidation of organic substrates by microbes are generally transferred to a high potential electron acceptor such as dissolved oxygen in the medium. In MFCs, electrons are transported to an insoluble electrode (anode) through an electrical circuit to reach the cathode, where electron acceptors are reduced. As the current then flows over a resistance, electrical energy is directly generated from the MFCs [18].

Hence, it becomes difficult to ascertain the mechanisms and roles of the individual microorganisms contributing to power generation. Pure and mixed cultures of organisms are used to inoculate MFCs but due to high costs, pure microorganisms may not be suitable for the practical operation such as treatment of industrial effluents. Mixed cultures (i.e., soil and wastewater) containing significant amounts of electrogenic bacteria can be used as the cost-effective inoculate for MFCs. However, the nonelectrogenic bacteria (i.e., methanogenic bacteria and denitrifying bacteria) in mixed cultures consume organic substrates without generating electricity [19].

Recently, a number of bacteria such as *Shewanella putrefactions*, family of Geobacteraceae, *Rhodoferax ferrireducens*, *Bacillus subtilis*, *Geobacter sulfurreducens*, and *Escherichia coli* were reported in the literature which have ability to transfer produced electrons from oxidized fuel (substrate) to the electrode without using artificial mediator, making it possible to establish mediator-less MFCs [20,21,22,23,24,25].

Recent advances have tried to revitalize artificial photosynthesis research, but the practical feasibility of the system is still questionable, illustrating the difficulty of mimicking the photobiochemical process. In an integrated photobioelectrochemical system the CO_2 reduction with the release of O_2 was highlighted, being a solar energy-driven enzymatic system pointing to a paradigm shift in this field [26].

The oxygenic photosynthesis is carried out by microalgae and cyanobacteria, is a potential alternative to mechanical aeration. During this process, O_2 is generated through direct biophotolysis along with the simultaneous production to reduce equivalents, which also helps in the sequestration of CO_2 in the presence of sunlight. This application helps in the electrogenesis, wastewater treatment (through anodic oxidation and cathodic reduction), sequestration of CO_2 (through cathodic reduction). It has been proven that even small amounts of photosynthetic oxygen at the anode may be beneficial for generation of heterotrophs due to the advantageous energy profiles under microaerobic conditions [27,28].

In this context, the present study is based on the operation of a double chamber MFCs with microalgal biocathode. The anode chamber was inoculated with anaerobic microbial consortiums removing the blue dye brl and the cathode chamber was inoculated with cultures of the microalga *C. vulgaris* by treating the effluent waters of a chocolate factory. The electrogenic activity of MFCs with the molecular identification of bacterial communities of biofilms formed in the anolyte was evaluated.

2. Material and methods

2.1. Inoculation of microorganisms in microbial fuel cells

2.1.1. Anaerobic consortium

The anaerobic community comes from effluent sludge from "Parque Industrial río Seco, Arequipa, Perú" (71°35′59″W, 16° 21′22″S) with domestic dumps, tanneries and industrial companies, presenting a community of anaerobic microorganisms such as archaea and bacteria colocated at the anode under anaerobic conditions in the anode chamber evaluating their growth by volatile suspended solids.

2.1.2. Microalga C. vulgaris

The culture of the microalga *C. vulgaris* was colocated in the cathode chamber by inoculating (50% of the volume) with medium of synthetic culture for algae [29] containing; Peptone 292; $NaHCO_3$ 250; $MgSO_4 \cdot 7H_2O$ 18; $FeSO_4 \cdot 2H_2O$ 4; KCl 19; $(NH_4)_2SO_4$ 382; $K_2HPO_4 \cdot 3H_2O$ 346; $CaCl_2 \cdot 2H_2O$ 13 $mg \cdot L^{-1}$.

2.2. Configuration and operation of microbial fuel cells (MFCs)

The MFCs was made using the glass material, consists of two chambers with equal volume (1 L) separated by a proton exchange membrane (PEM) (Nafion 117, Sigma-Aldrich) after sequentially subjecting to a 30% H_2O_2, 0.5 M H_2O_2 and deionized water (pH = 7) for every 1 h. After pretreatment in order to increase the porosity, the PEM was fixed with washers and clamps between the two chambers. The anolyte and the catholyte were constructed with graphite plates (6.5 cm long, 5 wide and 0.8 cm thickness, surface 58.2 cm^2) being used as electrodes connected to a LED bulb of 1.5 W as resistance. In the anode two holes of uniform size of 0.1 cm in diameter were drilled to increase the surface to 58.7 cm^2. The electrodes were colocated at a distance of 5 cm on each side of the PEM.

The electrodes have a surface of 58.7 cm^2 (perforated anode) and 58.2 cm^2 (cathode). The cathode compartment was kept open to provide contact with the environment to allow the sequestration of CO_2 by photosynthetic algae. The copper wires were used to establish a contact between the electrodes. Before use, the electrodes were soaked in deionized water for a period of 24 h. The anode chamber was endowed with ports (one input and one output) sealed with silicone and paraffin to ensure an anaerobic microenvironment (Fig. 1).

The cathode chamber, inoculated with the microalga *C. vulgaris* and effluent water from a chocolate factory in the region of Arequipa, Peru presented the following data: COD = 7002 mg/L; SST = 4168 mg/L; oils and fats = 2634 mg/L; Turbidity >461 FTU; $(SO_4)^{2-}$ = 36 mg/L.

The anode chamber operated with a peristaltic pump (Stenner brand) recirculating at 0.35 L/h feeding with the wastewater (60%) and sludge from industrial effluents PIRS (COD = 33,840 mg/L, STD = 30,000 mg/L; SST = 3750 mg/L; VSS = 1612 mg/L) with a fixed bed of activated carbon (40% of the volume) in which compartment before the feeding was spread with N_2 gas free of oxygen for 2 min to maintain an anaerobic microenvironment and the pH of the wastewater was adjusted to 6 and 7 respectively in the anode and cathode chambers using concentrated orthophosphoric acid (88%) and 1 N of NaOH. At 7 d of experimentation was fed with the blue azo dye brl to evaluate the percentage of removal.

The MFCs were evaluated in the region of Arequipa during the summer season (from March 5 to April 6, 2015), under environmental conditions located at 16°24′50″ south latitude and 71°32′02″ of west longitude with an altitude of 2344 masl at ambient temperature (21 ± 3 °C) and solar radiation (1100 ± 225 W/m^2) (Table 1).

2.3. Model of Pinto et al. [30]

In the anolyte and catholyte of MFCs, the model of Pinto et al. [30] with the presence of two microbial populations in the anolyte:

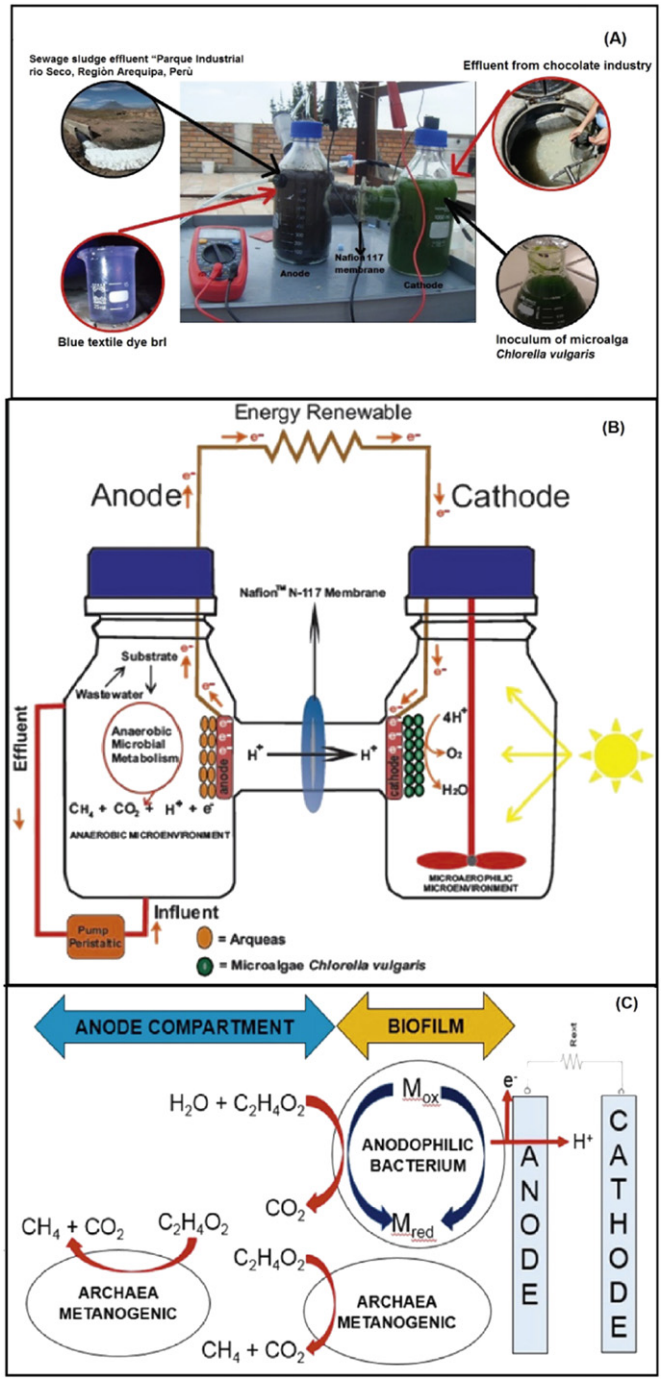

Fig. 1. Microbial fuel cells (MFCs) under environmental conditions. **A.** Photograph of MFCs. **B.** MFCs scheme. **C.** Scheme of the anolyte with biofilms.

Anodophilic bacteria (Xa) and methanogenic archaea (Xm) coexisting in the competition for the disposition of substrate (Sw = residual water) with the formation of biofilms. In the catholyte the presence of microalgae *C. vulgaris* was observed producing oxygen for the production of water.

2.4. Analysis

The electrogenic activity of the MFCs was evaluated in terms of voltage, current and electron discharge. The potential difference/open voltage circuit (OVC) and current (I) (in series, 100 Ω) these measurements were documented with digital multimeter auto range.

Table 1
Consolidated data related to the microalgae biocathode operation.

Measured parameters	
Bio-electrogenic activity (OCV, mV)/Current (mA)	954/1.99
Power density (mW/m^2)	327
Current density (mA/m^2)	340.55
Chlorophyll (μg/mg)	11.67
Chlorophyll-*a* (μg/mg)	4.07
Chlorophyll-*b* (μg/mg)	7.6
Biomass concentration (mg/mL)	5.2
pH output	
Anode	7.18
Cathode	7
COD removal efficiency (%)	
Anode	78.58
Cathode	32.62
Surface area of electrodes	
Anode	58.7 cm^2
Cathode	58.2 cm^2
Illuminance	75,000 lx
The blue brl dye removal efficiency at anode (%)	79.52

The polarization curve was plotted as a function of current density, voltage and power density measurement at different resistances (1.88–12.35 kΩ).

The bioelectrochemical characteristics of MFCs were studied with an electrochemical cell of three electrode using Ag/AgCl as reference electrode (RE), stainless Steel AISI 316 as a counter electrode (CE) and working electrode (WE). Open circuit potential measurement and cyclic voltammetry studies were performed using potentiostat (CH Instruments, USA) [31].

The pH, COD, VSS, SST, turbidity were determined according to standard methods and procedures [32] being measured in the compartment of anode and cathode during the operation.

The oils and fats were measured according to standard methods [33] in the cathode compartment. The light intensity was measured with luxometers (300 LT, Extech Corp). The biomass of the microalga was estimated at an optical density (OD, 650 nm).

$$D = 9.52 \times 10^6 \times OD_{650} + 70957$$

For the quantification of chlorophyll, 2 mL of culture of cells of *C. vulgaris* were taken from the cathode chamber, for centrifugation and alteration in a sonicator (ultrasonic bath) for 7 min (20 kHz) extracting with 2 mL of acetone. The extract was centrifuged at 3000*g* for 5 min and the optical density of the supernatant was measured at 647 and 664 nm [34]. The concentrations of chlorophyll *a* and

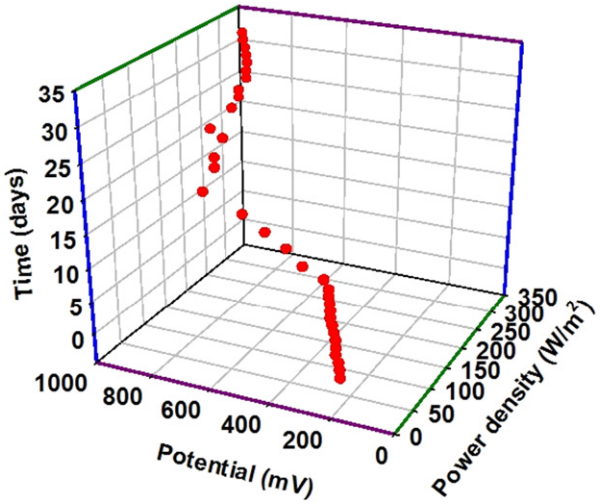

Fig. 2. Comparative profiles. Power density and voltage versus time in days.

chlorophyll *b* (μg/mL) can be calculated on the basis of values of the optical density.

$$\text{Chl } a = -1.93 \times OD_{647} + 11.93 \times OD_{664}$$

$$\text{Chl } b = 20.36 \times OD_{647} - 5.5 \times OD_{664}$$

2.5. Fluorescent staining of the structure and biofilms formed in the anolyte

For the observation of the structures of the biofilms formed in the anolith, the confocal microscopy CS SP8 inverted was used, with a compact feed unit of Argon laser at 488 nm, Leica brand with targets of 63X (Wet with immersion oil).

The biofilms samples were collected with slides, for the staining the orange fluorescence dye of acridine was used, realizing the mounting with Dako; finally, it was visualized with confocal microscopy CS SPS.

2.6. DNA extraction from anolyte biofilms, amplification of gene of 16S DNA, denaturing gradient gel electrophoresis (DGGE)

In the biofilm samples, the DNA extraction of bacteria was performed with the PowerSoil ® DNA Isolation Kit (MO BIO Laboratories, Inc.), the DNA was quantified in a Multi-Volume Analysis of Nucleic Acids Using the Epoch™ Spectrophotometer System, the purity was verified by the relationship 260/280.

For the amplification of the rRNA 16S of the genomic DNA, the universal oligonucleotides 1492R/27F and the kit of PCR GoTaq® Green Master Mix PCR PROMEGA were used with the following conditions: Initial denaturation of 94 °C for 5 min followed by 35 cycles of 94 °C for 45 s, 57 °C for 45 s and 72 °C for 1 min and 30 s, with a final extension of 5 min. The PCR product was visualized on 1% agarose gel.

The PCR product of the 16S rDNA purified was used as a mold for the analysis of bacterial communities present with the technique

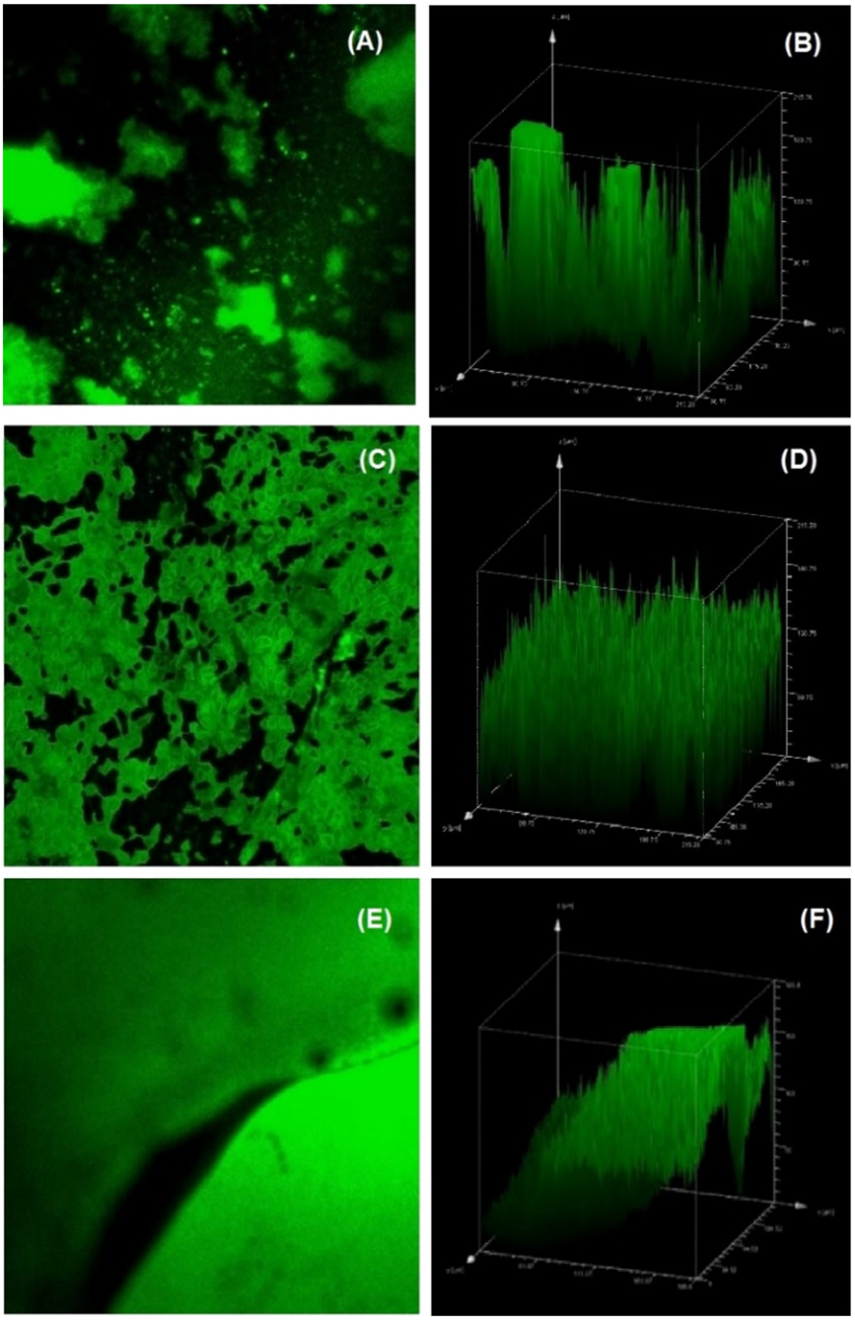

Fig. 3. Biofilms architecture in anolyte (MFCs) with images obtained in confocal microscopy. **A, C, E.** View of biofilms in confocal microscope (63X). **B, D, F.** Views of Biofilms in 3D.

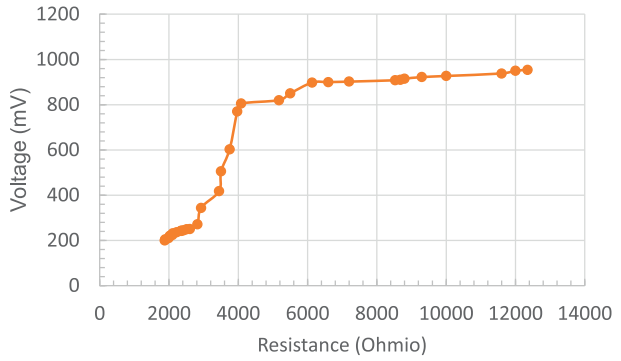

Fig. 4. Voltage (mV) versus external variable load (1880–12,350 Ω) at different days.

of denaturing gradient gel electrophoresis (DGGE). The assay was performed in base to the protocol of Demergasso et al. [35], in the first instance a PCR was performed using oligonucleotides specific for bacteria 341F-GC and 907R [36], with conditions of PCR with an initial denaturation of 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 50 °C for 45 s and 72 °C for 1 min, with a final extension of 3 min. The product of PCR was loaded in polyacrylamide gels at 6% containing a denaturing gradient of 30–60% (100% of the denaturing agent was defined as 7 M Urea and 40% Formamide). The gel was run in BioRad D Gene (BioRad) system at 60 °C, 100 V for 7 h. Later, the gels were dyed with GelRed (Biotium brand) for 1 h; being carefully separated, the gel was placed on a tray used for staining and covered with GelRed (Biotium brand) in a solution of 0.5%; during the time of reveal, the gel was kept in the dark and visualized under UV light in the transilluminator. The obtained bands were cut and reamplified with the 341F-GC/904R primers the same conditions of the PCR of DGGE. The products of PCR were purified with the kit of purification E.Z.N.A. Gel Extraction Kit, Omega bio-tek, following manual instructions, visualized on agarose gel at 1% and sent to sequencing.

The amplified and purified products obtained from the analyses of DGGE and cloning were sequenced by the method of Sanger (MACROGEN, Korea). The sequences obtained were manually edited using the bioinformatic program of ChromasPro (ChromasPro 2.1, Technelysium Pty Ltd., Tewantin QLD, Australia) and compared to the database of GenBank (http://blast.ncbi.nlm.nih.gov) using the algorithm of BLASTn [37], later the alignment of the sequences was performed using the tool of MUSCLE [38].

The phylogenetic tree was constructed using the Maximum Likelihood statistical model and the Bootstrap robustness test using the computer program of MEGA 5 [39].

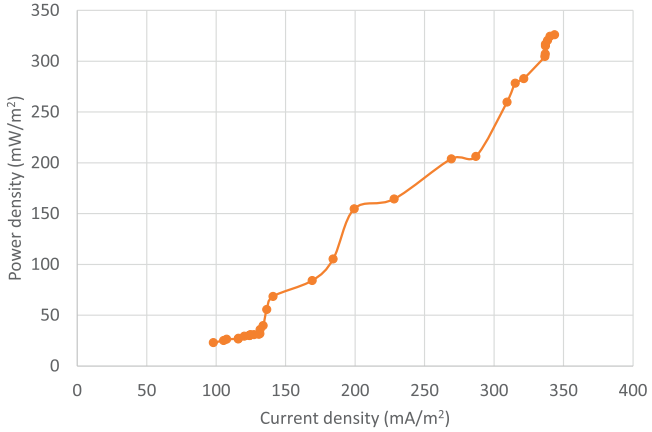

Fig. 5. Power density (mW/m^2) versus current density (mA/m^2).

Table 2
Electrochemical parameter for stainless steel in anolyte and catholyte solutions.

	Anolyte	Cathoyte
E_{mix}, mV/SHE	128	159
I_{mix}, A	1.63×10^{-4}	4.76×10^{-4}
b_a, mV/dec	206	320
b_c, mV/dec	−72	−136

3. Results

3.1. Bioelectrogenesis

The electrogenic activity depends of the growth of the microalgal biomass and the photosynthetic activity. The initial power density was 23.17 mW/m^2 with an increase of 25.26 mW/m^2 at 24 h, an increase of 27.37 mW/m^2 in 4 d, an increase from 29.44 to 105, 84 mW/m^2 from 5 to 18 d, and an increase from 105.83 mW/m^2 to 327.67 mW at 32 d. During the operation, the initial potential was 200 mV and increased slightly to 220 mV at 4 d of operation followed by an increase of 224 to 954 mV from 5 to 32 d (Fig. 2).

3.2. Microalgal growth of C. vulgaris on the cathode

The growth of the biomass of the microalga *C. vulgaris* at the cathode was controlled at regular intervals to correlate with the output power. The initial biomass concentration of the microalga *C. vulgaris* was 3.76 mg/mL with a growth of 5.2 mg/mL where the electrogenesis was higher (1.99 mA) at 32 d.

The chlorophyll concentrations were also measured, reflecting the photosynthetic activity of the photolysis of the water, releasing oxygen with supply of simultaneous energy for the use of CO_2. The initial chlorophyll concentration was 5.14 μg/mg (chlorophyll a = 3.07 μg/mg, chlorophyll b = 2.07 μg/mg). The chlorophyll content increased with time of operation and reached a maximum of 11.67 μg/mg (chlorophyll a = 4.07 μg/mg; chlorophyll b = 7.6 μg/mg).

3.3. Formation of biofilms in the anolyte

The results of the formation of the biofilms on the electrode of the anolyte are showed in Fig. 3 with a thickness of 166.7 ± 11.5 μm at 32 d.

3.4. Study of polarization

The potential (mV) measured through the external resistance (1880–12,350 kΩ), during the operation, the potential presents great

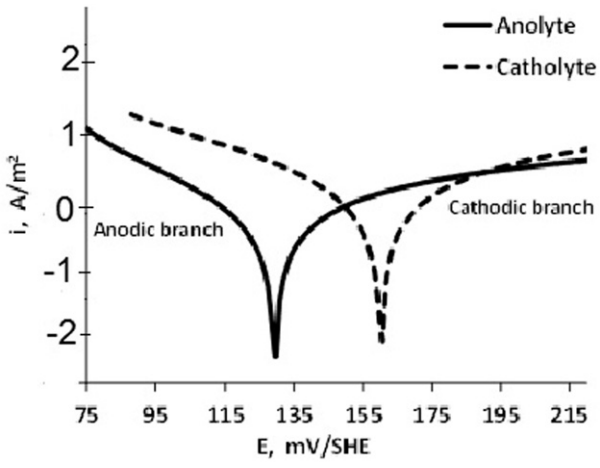

Fig. 6. Polarization curves for AISI 316 stainless steel immersed in the anolyte and catholyte solution. T = 298.15 °C, scan rate of 1 mV/s.

variations in the external resistances of 1800 to 6600 Ω, keeping constant with slight variations in the potential from 6600 to 12,350 Ω reaching the highest voltage from 980 mV to 12,350 Ω (Fig. 4). The efficiency curve of the MFCs is showed in Fig. 5. The density of maximum power of the MFCs is 325.99 mW/m^2. (Fig. 5).

3.5. Bioelectrochemical analysis

It is known that in a bioelectricity system, the electrons produced in the anode are transferred through an external circuit to be consumed on cathode to produced water [40]. In this sense, to obtain a better understanding of the activity of the main species involved during the bioelectricity generation, the behavior of the electrolytic solutions used as anolyte and catholyte were analyzed in terms of the mixed potential theory assuming a complete kinetic mechanism control for the partial reactions in both solutions. The analysis of the polarization curves shown in Table 2 and Fig. 6 was performed between a range of ±60 mV/SHE with respect to the mixed potential (E_{mix}) in order to minimize the mass transfer limitations of the anodic and cathodic partial reactions. From the results, it is noticeable that E_{mix} were 159 and 128 mV/SHE for catholyte and anolyte respectively, which are according with the experimental results shown in Fig. 7B, where low values of pH are observed in the catholyte due at the diffusion of hydrogen protons from the anolyte solution. On the other hand, the changes observed on the Tafel slope can be attributed at the adsorption of arqueas or microalgae on the electrode surface which acts as barrier to the charge transfer. The Tafel analysis shows that the anodic Tafel (b_a) slope was higher in presence of microalgae than in presence of wastewater. Additionally, it can be seen that the cathodic Tafel (b_c) slopes were more negative for the catholyte solution than that of the anolyte solution. Its behaviors are according with the mixed current density

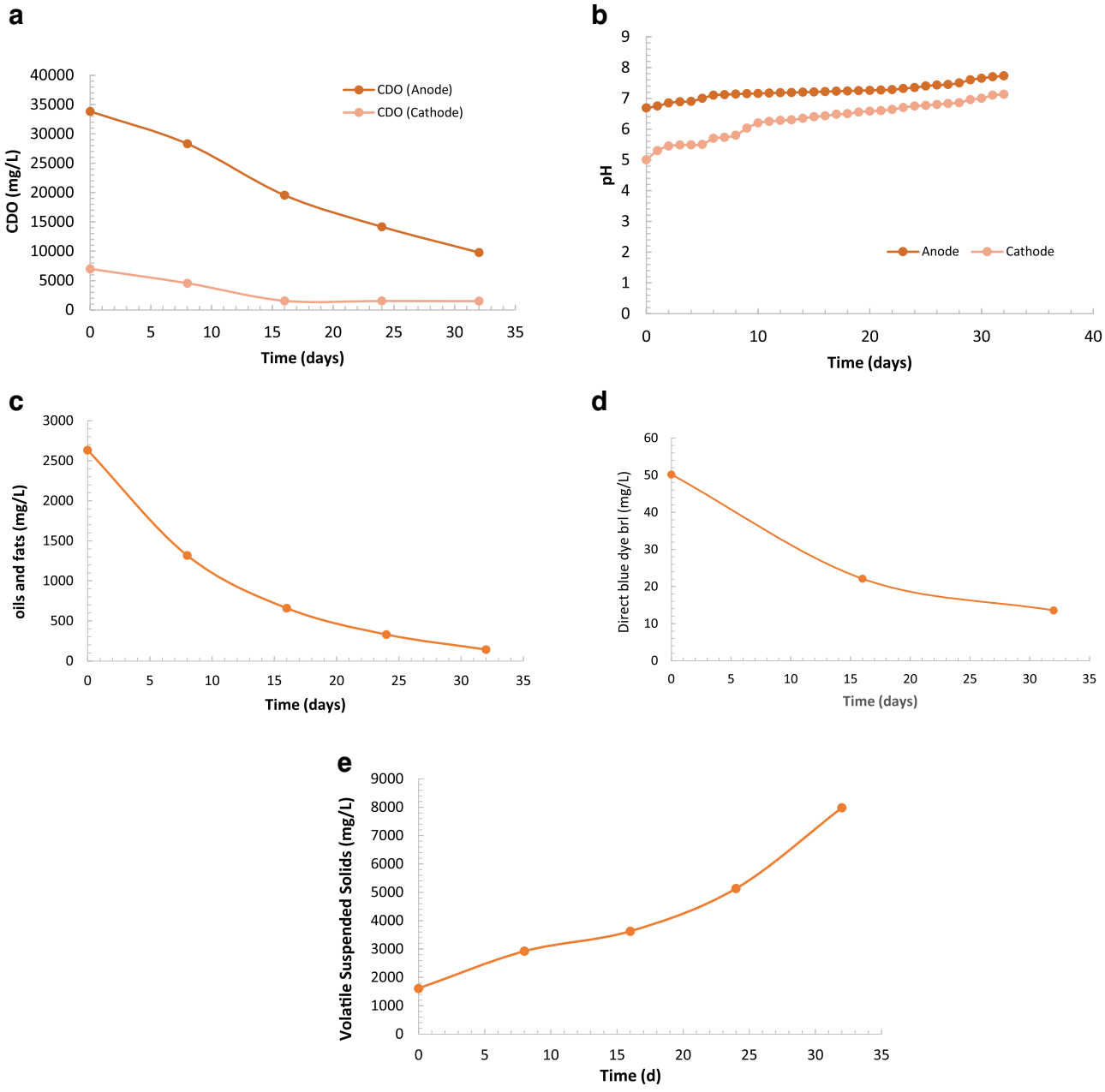

Fig. 7. Change in: (a) Removal of Chemical Oxygen Demand (COD), (b) pH, (c) Biodepuration of oils and fats (catholyte), (d) Removal of dye textile blue brl (Anolyte) and (e) Evaluation of VSS (anolite).

(i_{mix}) indicating high activity for the oxygen reduction reaction in presence of microalgae.

3.6. Effect of CDO, pH, oils and fats, dye direct blue brl and volatile suspended solids

The substrate influences not only the integral composition of the bacterial community in the anode biofilm, but also the MFCs performance including the power density (PD) and Coulombic efficiency (CE) [41]. Biocathode operation has an additional advantage of using both the chambers for treatment simultaneously under different conditions, which also helps in the removal of specific pollutants [42]. With the growth of the microalgae biomass at the cathode and the microbial metabolism at the anode. The performance of MFCs system was evaluated by Removal of Chemical Oxygen Demand (COD), pH, Biodepuration of oils and fats (catholyte), removal of dye textile blue brl (Anolyte) and Evaluation of VSS (anolyte) at regular time intervals.

The degradation of the substrate was analyzed in terms of elimination efficiency of COD (anode, 71%, cathode, 78.6%). The increase of the biomass of the microalga *C. vulgaris* in the cathode with the effective use of reducing equivalents generated in the anodic oxidation, further improved the flow of electrons in the circuit, resulting in further degradation of the substrate at the anode. The longer retention time also contributed to greater elimination of COD at the anode. However, at the cathode, the biomass of the microalga *C. vulgaris* consumed less COD due to the possibility of using CO_2 (autotrophic) and organic residues of domestic wastewater (heterotrophic) [43]. The microalgal biocatode showed less degradation of the substrate due to the mode of photoautótrofa nutrition adopted by the microalgae, sequestering to the CO_2 like main source of carbon for its metabolism that uses the organic carbon of the domestic wastewater.

The changes in the redox state (pH) and change in the concentration of acid metabolites reflect the conversion of the substrate in both chambers. The anodic pH showed a gradual increase from 6.69 to 7.73 and the pH in the catholyte showed a gradual increase from 5 to 7.13. The initial concentration of oils and fats was 2634 mg/L and at 32 d a concentration of 143 mg/L was observed resulting in a biodepuration of 95% of oils and fats in the catholyte. The initial concentration of the direct blue dye brl was 50.22 mg/L and at 32 d was 13.56 mg/L resulting a removal of 73% of the direct blue dye brl. In the system MFCs an increase of volatile suspended solids (VSS) of 1612.5 to 7980.94 mg/L was shown (Fig. 7).

3.7. Proposal of a biochemical model

The integrated model of Pinto was used as a proposal to predict the possible activities of microorganisms in the chamber of anolyte and catholyte of MFCs. The presence of multiple microbial species and wastewater plus activated sludge allows to reproduce the growth dynamics of microorganisms, the degradation of organic matter, the production of methane and current in the MFCs with the transfer of protons through the PEM and the transfer of electrons through the circuit, showing a model made by Huarachi-Olivera et al. [44] in a modified model of Molognoni et al. [45] (Fig. 8).

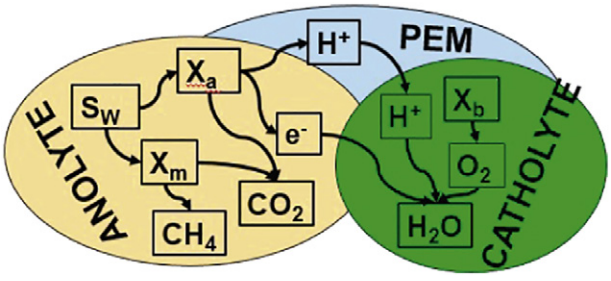

Fig. 8. Transformation of organic matter in the MFCs model. X_a = anodophilic bacterium, X_m = methanogenic bacterium, X_b = microalgae, S_W = residual water, PEM = Proton exchange membrane.

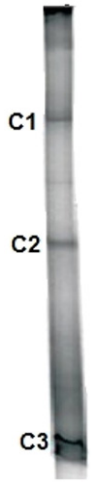

Fig. 9. The Gel of DGGE show fragments of genes 16S rDNA bacterial amplified by PCR from anolyte biofilms.

The model is expressed in terms of:

I = Model components (X_a, X_b, S_W, S_M, S_C, S_O … etc.)
J = Processes (bacterial growth, methanogenesis … etc.)
C: concentration, V: Volume, Q: Flow rate.
Balance of masses of components i:
Accumulation = Input – Output + Reaction.
$\Delta i, dCi / dt = D [(Ci) ent – (Ci) salt] + \Sigma \nu ij \rho j D = Q / V.$
$\Delta j, \Sigma \nu ij ici = 0$ processes j.
Conversion factor M_c, Mi^{-1} where C is COD, VSS.

3.8. DGGE

The results of the sequencing of the amplified obtained from the bands of DGGE (C1, C2 and C3) (Fig. 9) were using 3 clones [46], showing sequences of quality. The bacterial composition is shown in Table 3, with a maximum identification of 99 and 98% with taxa related to Deltaproteobacteria and Micromonosporales in anolyte biofilms of MFCs.

The phylogenetic tree was constructed with the sequences obtained from the clones with the program MEGA5, obtained from the database. The analysis based on the sequences of 16S rDNA revealed two classes of bacteria: Actinobacteria and Deltaproteobacteria with the following clones denominated Clon 1, Clon 2 and Clon 3 (Fig. 10).

4. Discussion

The MFCs acclimated to external resistors (1880–12,350 kΩ), and could shorten the delay phase for the development of an exoelectrogenic biofilms [47,48].

The mean current density in continuous flow mode (97.94 mA/m^2) and power density (23.1 mW/m^2) at a start increasing to maximum values of the current density of 343.47 mA/m^2 and power density

Table 3
Bands of representative clones in DGGE.

Clone name	Band	Closest relative	Phylogenetic group	Similarity (%)
Clone 1	C1	*Geobacter bemidjiensis* (CP001124)	Deltaproteobacteria	99%
Clone 2	C2	*Jishengella sp.* (LC158546)	Actinobacteria	99%
Clone 3	C3	*Verrucosispora sp.* (HQ123438)	Actinobacteria	98%

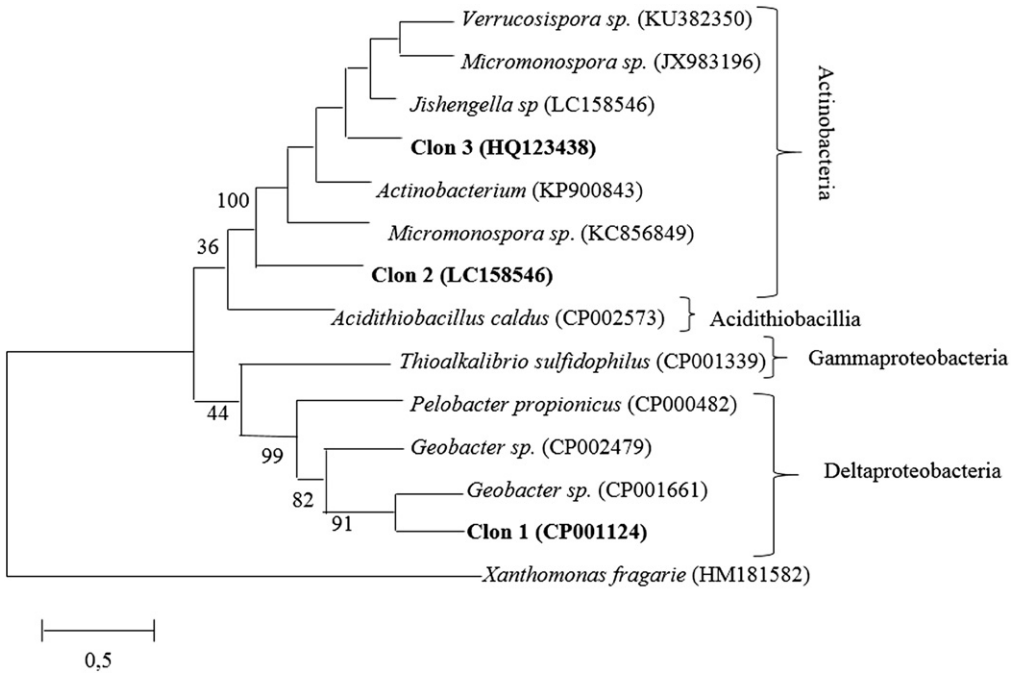

Fig. 10. Phylogenetic tree of the sequences of 16S rDNA obtained from the anolyte biofilms, determined by the distance analysis of Jukes-Cantor. Percentages of "bootstrap" greater than 50% are showed. The scale represents the number of substitutions per nucleotide. Sequence of *Xanthomonas fragarie* was chosen as outgroup.

325.9 mW/m^2. The efficiency in the removal of COD was reduced (anode, 71%, cathode, 78.6%), generating current due to lower concentrations of substrate [49].

In MFCs, in chambers of anolith and catholyte, the following COD removals (anode, 71%, cathode, 78.6%) were obtained in comparison to results of Ismail and Jaeel [50] in sustainable energy generation, obtaining removals of COD 84% and 90% obtained from MFCs inoculated with activated sludge and *Bacillus subtilis*; in another study by Sanchez-Herrera et al. [51], a removal of 93% and 86.5% of COD with planktonic cells were obtained.

The bleaching of the direct blue dye brl in MFCs was measured in terms of removal giving a percentage of 73% in the anolyte, compared to results of Zavala-Rivera et al. [52] obtaining a removal of 61% of the direct blue dye brl, and in a study by Conceição et al. [53] a removal of 99.6% of the indigo blue dye was obtained. The dyes function as electron acceptors and the presence of glucose as a co-substrate facilitates their reduction to intermediate amines with simultaneous decolorization under anaerobic conditions [54].

The biofilms formed in the anolyte according to studies with confocal microscopy of fluorescence showed a thickness of 166.7 ± 11.5 μm at 32 d. Therefore, in the MFCs, the electric current is generated from a continuous flow of electrons that is extracted from the anodic substrate by bacterial metabolism in the biofilms. Thus, the rate of electron transfer is based on the electrochemical behavior of biofilms of bacterial metabolism. The bioelectroactive species (for example, cytochromes of outer membrane, Pili conductivity and extracellular excretions) play a role in the transfer of electrons between the biofilms and the anolyte electrode [55,56,57]; some abiotic factors may also influence the performance of bioelectroactive species during the electron transfer process [58,59], for example, oxidative and reductive peaks in the cyclic voltammetry of the anode biofilm varying as a function of pH [58]. Further, the biofilms of *Geobacter* with several cytochromes of outer membrane produce different currents under different electrode potentials [60]. Therefore, the behavior of the electron transfer of the Anode Biofilms is considered as a useful tool to understand the fact that the actual generation differs greatly between MFCs, short circuit condition (MSC) and microbial electrolysis cell (MEC).

In this research, in molecular tests through DGGE in the biofilms formed in the anolith three species of bacteria were identified *Geobacter bemidjiensis* (CP001124), *Jishengella sp.* (LC158546) and *Verrucosispora sp.* (HQ123438) obtaining an efficiency in the production of bioelectricity with an increase of 200 to 954 mV corroborated with the investigations of Kim et al. [61] and Chaudhuri and Lovley [62] discovering the role of some microbes to transfer the electrons directly to the anode being operably stable, producing a coulomb efficiency according to Chaudhuri and Lovley [62] and Scholz and Schroder [63], with the following microorganisms like *Shewanella putrefaciens* [61], *Geobacteraceae sulfurreducens* [64], *Geobacter metallireducens* [65] and *Rhodoferax ferrireducens* [62] all being bioelectrochemically active, being able to form biofilms on the surface of the anode and transfer electrons directly by conductance through the membrane.

5. Conclusions

The MFCs allow the generation of bioelectricity by the flow of electrons (from bacterial metabolism) from the anolyte to the catholyte with the diffusion of protons through the membrane Nafion 117 with a maximum bioelectrochemical efficiency of 954 mV, degrading the organic material in terms of removal efficiency of chemical oxygen demand (COD) (Anode, 71%, cathode, 78.6%) with a removal of 73% of the blue dye brl in the anolyte and a biodepuration of 95% of fats and oils in the catholyte in 32 d of evaluation, forming biofilms with a thickness of 166.7 ± 11.5 μm in the electrode of the anolyte, identifying the bacterial communities by denaturing gradient gel electrophoresis (DGGE) linked to *Geobacter bemidjiensis* (CP001124), *Jishengella* sp. (LC158546) and *Verrucosispora sp.* (HQ123438).

Conflicts of interest

The authors declare no conflicts of interest.

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References

[1] Pant D, Van Bogaert G, Diels L, et al. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 2010;101(6):1533–43. https://doi.org/10.1016/j.biortech.2009.10.017.
[2] Pandey P, Shinde VN, Deopurkar RL, et al. Recent advances in the use of different substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery. Appl Energy 2016;168:706–23. https://doi.org/10.1016/j.apenergy.2016.01.056.
[3] Liu H, Cheng S, Huang L, et al. Scale-up of membrane-free single-chamber microbial fuel cells. J Power Sources 2008;179(1):274–9. https://doi.org/10.1016/j.jpowsour.2007.12.120.
[4] Li Z, Yao L, Kong L, et al. Electricity generation using a baffled microbial fuel cell convenient for stacking. Bioresour Technol 2008;99(6):1650–5. https://doi.org/10.1016/j.biortech.2007.04.003.
[5] Logan B, Rabaey K. Conversion of wastes into bioelectricity and chemicals using microbial electrochemical technologies. Science 2012;337(6095):686–90. https://doi.org/10.1126/science.1217412.
[6] Arends JBA, Verstraete W. 100 years of microbial electricity production: three concepts for the future. J Microbial Biotechnol 2012;5(3):333–46. https://doi.org/10.1111/j.1751-7915.2011.00302.x.
[7] He Z. Microbial fuel cells: now let us talk about energy. Environ Sci Technol 2013; 47(1):332–3. https://doi.org/10.1021/es304937e.
[8] Dutta PK, Rabaey K, Yuan Z, et al. Spontaneous electrochemical removal of aqueous sulfide. Water Res 2008;42(20):4965–75. https://doi.org/10.1016/j.watres.2008.09.007.
[9] Mu Y, Rabaey K, Rozendal RA, et al. Decolorization of azo dyes in bioelectrochemical systems. Environ Sci Technol 2009;43(13):5137–43. https://doi.org/10.1021/es900057f.
[10] Wen Q, Yang T, Wang S, et al. Dechlorination of 4-chlorophenol to phenol in bioelectrochemical systems. J Hazard Mater 2013;244-245:743–9. https://doi.org/10.1016/j.jhazmat.2012.11.002.
[11] Tandukar M, Huber SJ, Onodera T, et al. Biological chromium(VI) reduction in the cathode of a microbial fuel cell. Environ Sci Technol 2009;43(21):8159–65. https://doi.org/10.1021/es9014184.
[12] Kumar AK, Reddy MV, Chandrasekhar K, et al. Endocrine disruptive estrogens role in electron transfer: bio-electrochemical remediation with microbial mediated electrogenesis. Bioresour Technol 2012;104:547–56. https://doi.org/10.1016/j.biortech.2011.10.037.
[13] Kalathil S, Pant D. Nanotechnology to rescue bacterial bidirectional extracellular electron transfer in bioelectrochemical systems. RSC Adv 2016;6(36):30582–97. https://doi.org/10.1039/C6RA04734C.
[14] Boesen T, Nielsen LP. Molecular dissection of bacterial nanowires. MBio 2013;4(3): 270–313. https://doi.org/10.1128/mBio.00270-13.
[15] Mohan SV, Velvizhi G, Krishna KV, et al. Microbial catalyzed electrochemical systems: a bio-factory with multi-facet applications. Bioresour Technol 2014;165:355–64. https://doi.org/10.1016/j.biortech.2014.03.048.
[16] Sevda S, Dominguez-Benetton X, Graichen FH, et al. Shift to continuous operation of an air-cathode microbial fuel cell long-running in fed-batch mode boosts power generation. Int J Green Energy 2016;13(1):71–9. https://doi.org/10.1080/15435075.2014.909363.
[17] Scott K, Yu EH, Ghangrekar MM, et al. Biological and microbial fuel cell. In: Sayigh A, editor. Comprehensive renewable energy. Elsevier; 2012. p. 277–300. https://doi.org/10.1016/B978-0-08-087872-0.00412-1.
[18] Rabaey K, Verstraete W. Microbial fuel cells: novel biotechnology for energy generation. Trends Biotechnol 2005;23(6):291–8. https://doi.org/10.1016/j.tibtech.2005.04.008.
[19] Logan BE, Regan JM. Microbial fuel cells-challenges and applications. Environ Sci Tech 2006;40(17):5172–80. https://doi.org/10.1021/es0627592.
[20] Kim BH, Kim HJ, Hyun MS, et al. Direct electrode reaction of Fe(III)-reducing bacterium, *Shewanella putrefaciens*. J Microbiol Biotechnol 1999;9:127–31.
[21] Kaufmann F, Lovley DR. Isolation and characterization of a soluble NADPH-dependent Fe(III) reductase from *Geobacter sulfurreducens*. J Bacteriol 2001; 183(15):4468–76. https://doi.org/10.1128/JB.183.15.4468-4476.2001.
[22] Liu ZD, Li HR. Effects of bio-and abio-factors on electricity production in a mediatorless microbial fuel cell. Biochem Eng J 2007;36(3):209–14. https://doi.org/10.1016/j.bej.2007.02.021.
[23] Nimje VR, Chen CY, Chen CC, et al. Stable and high energy generation by a strain of *Bacillus subtilis* in a microbial fuel cell. J Power Sources 2009;190(2):258–63. https://doi.org/10.1016/j.jpowsour.2009.01.019.
[24] Du F, Xie B, Dong W, et al. Continuous flowing membrane less microbial fuel cells with separated electrode chambers. Bioresour Technol 2011;102(19):8914–20. https://doi.org/10.1016/j.biortech.2011.07.056.
[25] Angelov A, Bratkova S, Loukanov A. Microbial fuel cell based on electroactive sulfate-reducing biofilm. Energ Conver Manage 2013;67:283–6. https://doi.org/10.1016/j.enconman.2012.11.024.
[26] Majumdar P, Pant D, Patra S. Integrated Photobioelectrochemical systems: a paradigm shift in artificial photosynthesis. Trends Biotechnol 2017;35(4):285–7. https://doi.org/10.1016/j.tibtech.2017.01.004.
[27] Rosenbaum M, He Z, Angenent LT. Light energy to bioelectricity: photosynthetic microbial fuel cells. Curr Opin Biotechnol 2010;21(3):259–64. https://doi.org/10.1016/j.copbio.2010.03.010.
[28] Mohan SV, Srikanth S, Raghuvulu SV, et al. Evaluation of the potential of various aquatic eco-systems in harnessing bioelectricity through benthic fuel cell: effect of electrode assembly and water characteristics. Bioresour Technol 2009;100(7):2240–6. https://doi.org/10.1016/j.biortech.2008.10.020.
[29] Valiente V. Compendio de medios de cultivos, soluciones y extractos para microalgas y cianobacterias. 1st ed. Cuba: CNIC; 1995.
[30] Pinto RP, Srinivasan B, Manuel MF, et al. A two-population bio-electrochemical model of a microbial fuel cell. Bioresour Technol 2010;101(14):5256–65. https://doi.org/10.1016/j.biortech.2010.01.122.
[31] Ghosh A, Basu S, Verma A. Graphene and functionalized graphene supported platinum catalyst for PEMFC. Fuel Cells 2013;13(3):355–63. https://doi.org/10.1002/fuce.201300039.
[32] American Public Health Association (APHA) American Water Works Association (AWWA). Standard methods for the examination of water and wastewater. Washington, DC: Water Environment Federation; 1998.
[33] American Public Health Association APHA. Métodos normalizados para el análisis de aguas potables y residuales. Madrid, Spain: Daz de Santos; 1992.
[34] Jeffrey SW, Humphrey GF. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz J 1975;165:191–4. https://doi.org/10.1016/S0015-3796(17)30778-3.
[35] Demergasso C, Galleguillos P, Escudero L, et al. Molecular characterization of microbial populations in a low-grade copper ore bioleaching test heap. Hydrometallurgy 2005;80:241–53. https://doi.org/10.1016/j.hydromet.2005.07.013.
[36] Muyzer G, Teske A, Wirsen CO, et al. Phylogenetic relationships of Thiomicrospira species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Arch Microbiol 1995;164:165–71. https://doi.org/10.1007/BF02529967.
[37] Altschul SF, Gish W, Miller W, et al. Basic local alignment search tool. J Mol Biol 1990; 215(3):403–10. https://doi.org/10.1016/S0022-2836(05)80360-2.
[38] Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004;32(5):1792–7. https://doi.org/10.1093/nar/gkh340.
[39] Tamura K, Peterson D, Peterson N, et al. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011;28(10):2731–9. https://doi.org/10.1093/molbev/msr121.
[40] Srikanth S, Mohan SV. Change in electrogenic activity of the microbial fuel cell (MFC) with the function of biocathode microenvironment as terminal electron accepting condition: influence on overpotentials and bio-electro kinetics. Bioresour Technol 2012;119:241–51. https://doi.org/10.1016/j.biortech.2012.05.097.
[41] Chae KJ, Choi MJ, Lee JW, et al. Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. Bioresour Technol 2009; 100(14):3518–25. https://doi.org/10.1016/j.biortech.2009.02.065.
[42] Mohan SV, Srikanth S. Enhanced wastewater treatment efficiency through microbially catalyzed oxidation and reduction: synergistic effect of biocathode microenvironment. Bioresour Technol 2011;102(22):10210–20. https://doi.org/10.1016/j.biortech.2011.08.034.
[43] Devi MP, Subhash GV, Mohan SV. Heterotrophic cultivation of mixed microalgae for lipid accumulation and wastewater treatment during sequential growth and starvation phases: effect of nutrient supplementation. Renew Energy 2012;43: 276–83. https://doi.org/10.1016/j.renene.2011.11.021.
[44] Huarachi-Olivera R, Dueñas-Gonza A, Yapo U, et al. Bioelectro-caracterización en cátodo microalgal aerobio y ánodo microbial anóxico en celdas de combustibles microbianas (CCMs) para biorremediación. VI Congreso Argentino de la Sociedad de Toxicología y Química Ambiental de Argentina (SETAC, Capitulo Argentino); 2016. [Córdoba, Argentina, 11 al 14 de Octubre de 2016].
[45] Mologni D, Puig S, Capodaglio AG, et al. A biochemical model for microbial fuel cells treating wastewater. The 3rd European meeting of the international society for microbial electrochemistry and technology; 2016. [Rome, Italy 26th–28th September 2016].
[46] Liu WT, Huang CL, Hu JY, et al. Denaturing gradient gel electrophoresis polymorphism for rapid 16S rDNA clone screening and microbial diversity study. J Biosci Bioeng 2002;93(1):101–3. https://doi.org/10.1016/S1389-1723(02)80063-9.
[47] Hong Y, Call DF, Werner CM, et al. Adaptation to high current using low external resistances eliminates power overshoot in microbial fuel cells. Biosens Bioelectron 2011;28(1):71–6. https://doi.org/10.1016/j.bios.2011.06.045.

[48] Zhang L, Zhu X, Li J, et al. Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances. J Power Sources 2011; 196(15):6029–35. https://doi.org/10.1016/j.jpowsour.2011.04.013.
[49] Huang L, Cheng S, Rezaei F, et al. Reducing organic loads in wastewater effluents from paper recycling plants using microbial fuel cells. Environ Technol 2009; 30(5):499–504. https://doi.org/10.1080/09593330902788244.
[50] Ismail ZZ, Jaeel AJ. Sustainable power generation in continuous flow microbial fuel cell treating actual wastewater: influence of biocatalyst type on electricity production. Scientific World Journal 2013. https://doi.org/10.1155/2013/713515.
[51] Sanchez-Herrera D, Pacheco-Catalan D, Valdez-Ojeda R, et al. Characterization of anode and anolyte community growth and the impact of impedance in a microbial fuel cell. BMC Biotechnol 2014;14(1):102. https://doi.org/10.1186/s12896-014-0102-z.
[52] Zavala-Rivera C, Dueñas-Gonza A, Huarachi-Olivera R, et al. Anaerobic removal of the brl direct blue dye in upflow anaerobic sludge blanket (UASB) with activated carbon. Rev Colomb Biotecnol 2015;17(2):55–64. https://doi.org/10.15446/rev.colomb.biote.v17n2.54276.
[53] Conceição V, Freire FB, de Carvalho KQ. Treatment of textile effluent containing indigo blue dye by a UASB reactor coupled with pottery clay adsorption. Acta Scientiarum Technol 2012;35(1):53–8. https://doi.org/10.4025/actascitechnol.v35i1.13091.
[54] Van der Zee FP, Villaverde S. Combined anaerobic–aerobic treatment of azo dyes-a short review of bioreactor studies. Water Res 2005;39(8):1425–40. https://doi.org/10.1016/j.watres.2005.03.007.
[55] Richter H, Nevin KP, Jia HF, et al. Cyclic voltammetry of biofilms of wild type and mutant *Geobacter sulfurreducens* on fuel cell anodes indicates possible roles of OmcB, OmcZ, type IV pili, and protons in extracellular electron transfer. Energ Environ Sci 2009;2:506–16. https://doi.org/10.1039/B816647A.
[56] Gorby YA, Yanina S, McLean JS, et al. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc Natl Acad Sci 2006;103(30):11358–63. https://doi.org/10.1073/pnas.0604517103.
[57] Newman DK, Kolter R. A role for excreted quinones in extracellular electron transfer. Nature 2000;45:94–7. https://doi.org/10.1038/35011098.
[58] Qiao Y, Li CM, Bao SJ, et al. Direct electrochemistry and electrocatalytic mechanism of evolved *Escherichia coli* cells in microbial fuel cells. Chem Commun 2008;11: 1290–2https://doi.org/10.1039/b719955d.
[59] Busalmen JP, Esteve-Nuñez A, Feliu JM. Whole cell electrochemistry of electricity-producing microorganisms evidence an adaptation for optimal exocellular electron transport. Environ Sci Technol 2008;42(7):2445–50. https://doi.org/10.1021/es702569y.
[60] Fricke K, Harnisch F, Schroder U. On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. Energ Environ Sci 2008;1:144–7. https://doi.org/10.1039/B802363H.
[61] Kim HJ, Park HS, Hyun MS, et al. A mediatorless microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microb Technol 2002;30: 145–52. https://doi.org/10.1016/S0141-0229(01)00478-1.
[62] Chaudhuri SK, Lovley DR. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. Nat Biotechnol 2003;21(10):1229–32. https://doi.org/10.1038/nbt867.
[63] Scholz F, Schröder U. Bacterial batteries. Nat Biotechnol 2003;21(10):1151–2. https://doi.org/10.1038/nbt1003-1151.
[64] Bond DR, Lovley DR. Electricity production by *Geobacter sulfurreducens* attached to electrodes. Appl Environ Microbiol 2003;69:1548–55. https://doi.org/10.1128/AEM.69.3.1548-1555.2003.
[65] Min B, Cheng S, Logan BE. Electricity generation using membrane and salt bridge microbial fuel cells. Water Res 2005;39:1675–86. https://doi.org/10.1016/j.watres.2005.02.002.