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Electrical energy generation in a double-compartment microbial fuel cell using *Shewanella* spp. strains isolated from *Odontesthes regia*

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Abstract

This study is focused on electrical energy generation in a double-compartment microbial fuel cell. Carbon felt impregnated with multi-walled carbon nanotubes was used as an anode, which contained gold nanoparticles and *Shewanella* spp. grown under aerobic conditions was used as a biocatalyst. The electrodes, used before and after biofilm growth, were characterized by scanning electron microscopy and cyclic voltammetry. The results revealed the formation of *Shewanella* spp. colonies on the electrode surface and electrochemical activity under aerobic and anaerobic conditions. During biofilm growth in Luria Bertani medium, a stabilized average power density of 281 mW m^{-2} was recorded. Subsequently, the cell reached a maximum current density of 0.11 mA cm^{-2} after 72 h of operation and a coulombic efficiency of 65% under anaerobic conditions.

Keywords: Microbial fuel cell, *Shewanella* spp., Aerobic anodic chamber, Multi-walled carbon nanotubes, Gold nanoparticles

Introduction

In recent years, many researches have been focused on the study of microbial fuel cells (MFC) for conversion of chemical energy to electrical energy [1, 2]. MFCs generate energy through electron transfer from the microbial cell to the fuel cell anode. The ability of microorganisms to generate an electrochemical potential is naturally occurring in several microbial species such as the Gram-negative *Shewanellaceae* and *Geobacteracea*, which are called exoelectrogenic bacteria as they tend to produce electrochemically active biofilms on the electrodes (in particular the anodes) [2–4]. Researchers have reported electrochemical activity in the biofilm formation under anaerobic conditions [5, 6]. The systems that use these

bacteria require special conditions, such as the absence of oxygen. Therefore, their application is limited to use sludge inoculums and to apply MFC on seabed. For this reason, the study on energy generation for active bacteria in aerobic and anaerobic environments such as *Shewanella* spp., can widen the scope and application of these systems.

Shewanella spp., are Gram negative bacilli which have bacillary mobility with a single polar scourge, an important phenotypic feature is the production of hydrogen sulfide [7]. *Shewanella* spp. are distributed throughout the world, mainly in marine environments. On the one hand, they are capable of reducing substances such as nitrate, nitrite, thiosulfate and trimethylamine-N-oxide [8]. The biofilm-forming ability has been described in detail for *Shewanella oneidensis* and *Shewanella putrefaciens* [9].

In order to obtain a high-power density of MFCs, electrodes with good electrical conductivity, large surface area and biocompatibility [1] are required. For this reason,

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carbonaceous electrodes have been widely used as well as the modification of their surfaces with metals: Ni/Co-based oxide/activated carbon [10], Pd/carbon cloth [11], and Fe/CNT [12]. Despite their high performance, these electrodes have certain restrictions related to their low biocompatibility, laborious synthesis and high cost, which limit their use. In this regard, the use of commercial carbon felts (CF) turns out to be promising due to their large surface area and low cost [2]. Studies using this type of CF reported high power density values of 1 and 0.52 $W\,m^{-2}$ [13–15]. However, these values are far less than the power densities values of some metal electrodes such as Cu and Pt with 40 and 78 $W\,m^{-2}$, respectively. Thus, their surface modification has been proposed as a problem-solving strategy to improve the electric conductivity of electrodes. Gold nanoparticles (AuNPs) are considered a good alternative to facilitate electron transfer and increase the biocompatibility of the electrode due to their high conductivity [16]. In addition, the use of carbon nanotubes as good charge carriers with low resistivity of $\sim 10^{-5}\,\Omega\,cm$ and good chemical stability [17] can increase surface area and electron transfer as have been reported in studies applied to MFC devices [18–20].

Therefore, this study evaluates the possibilities to generate electrical energy in a MFC that uses surface-modified CF with multi-walled carbon nanotubes (MWCNT) and AuNPs as anode and an isolate strain of *Shewanella* spp. as inoculum.

Materials and methods
Materials
MWCNTs (> 95% purity) used in the experiment were purchased from Chengdu Organic Chemicals Co.; glucose (≥ 85%) was purchased from Microgen Culture Media-CDH; nutrient agar from Panreac AppliChem; blood agar base (dehydrated) from Thermo Scientific™ Oxoid™ Columbia, all are analytical grade reagents for microbiological control. Oxidase test, catalase test, SIM (sulfide, indole, motility) agar, urea agar base, Simmons citrate agar, defibrinated sheep blood, and Gram staining set were used for microbial studies, polydiallyldimethylammonium chloride (20 wt%), nafion 5% (w/w) and L-tyrosine were purchased from Sigma Aldrich; potassium hexacyanide ferrate (II) (99%), potassium hexacyanoferrate (II) trihydrate purum (≥ 99%), potassium hexacyanide ferrate (III) (99%), potassium hexacyanoferrate (III) (99%), potassium chloride (> 98%), potassium chloride (≥ 98%) and sulfuric acid (96%) were purchased from Merck. All chemical reagents were used as received without further purification.

Isolation, identification and maintenance of Shewanella spp. strain
Thirty species of "pejerrey" *Odontesthes regia* that live in Ancon Bay (*latitude*; 11°46′28.524″ S and *longitude* 77°9′ 43.992″ W), Peru, were collected in February. These organisms were sliced and placed in 450 mL glass jar, with a third of the volume filled with the sliced pejerrey and then covered with seawater. All jars were incubated at 30 °C for their decomposition and bacterial growth for 24 h.

For bacterial isolation, the samples were incubated for 24 h in seawater, then 1 mL of each of the jars was extracted, seeded in triplicate, cultured in test tubes with SIM medium, and incubated at 30 °C for 24 h to obtain pure cultures. Those cultures with the bacterial colonies exhibiting the presence of hydrogen sulfide (black matter) were differentiated by streak plate technique, then transferred to individual plates with agar supplemented with fish soup (15 $g\,L^{-1}$ of agar and 50 vol% of fish soup) at 30 °C for 24 h.

Phenotypic identification, mobility, Gram staining and morphology were determined as described by Austin [21]. Biochemical tests were used for the identification of oxidase, catalase, indole production, hydrogen sulfide production, urea hydrolysis, gelatin hydrolysis, nitrate reduction, nitrite reduction, and hemolysis [22]. For the maintenance of the *Shewanella* strain, it was preserved in plates with nutrient agar and in agar slant tubes with nutrient agar.

Raman microscopy strain isolated from Shewanella spp.
Shewanella spp. was incubated in 250 mL flasks, containing 25 g of Luria Bertani (LB) broth in 100 mL of ultrapure water. It was applied in a rotating bath (37 °C, 100 rpm) for 24 h. After incubation the grown bacterial cells were concentrated. For this, the bacterial solution was washed by centrifugation at 1000 rpm in phosphate buffer at pH 7.2 and stored under sterile conditions. The collected bacterial cells were suspended on a silk screen-printed gold electrode, dried for 24 h and then analyzed with a Raman spectroscopy. The rough gold surface with bacteria was mounted directly under the microscope lens and the bacterial colonies were excited using a 638 nm laser line. Surface Enhanced Raman spectroscopy analysis was performed through a Horiba Scientific XploRA spectrometer with a Charge Coupled Device detector. A 1200 lines mm^{-1} diffraction grating was used and measured in a range of 500–1200 cm^{-1}, with a 50X objective and 50% laser power. The spectra were collected consecutively at 3 min per scanner. The positions of the spectral peaks were calibrated using silicon wafer of crystal as a reference.

Bacterial population and growth conditions
The *Shewanella* spp. strain was seeded on nutrient agar by streak-plating method and incubated at 30 °C for 24 h. They were transferred to a flask containing 100 mL of culture medium and, after 12 h, 3 mL of this solution were transferred to a flask containing 750 mL of LB broth. Finally, the bacteria were grown under aerobic conditions and constant agitation of 150 rpm for 36 h.

Strain of 1×10^8 CFU mL^{-1} of *Shewanella* spp. was used as anodic biocatalyst in MFCs.

Preparation of CF electrode modified with MWCNT-AuNPs (MWCNT-AuNPs/CF)

Prior to carbon fiber surface modification, the synthesis of the AuNPs was performed using 100 mL of a 10^{-4} M precursor solution of HAuCl$_4$ at pH 9.5 adjusted with NaOH. The reduction was carried out with 3 mL of 0.01 M NaBH$_4$ at room temperature, obtaining ruby-red colloidal gold. Ten milliliters of 1 mM tyrosine were used as stabilizing agents for nanoparticles [23]. Finally, the solution was heated to remove excess of unreacted borohydride ions, and the colloid was allowed to age for 24 h. The nanoparticles were centrifuged three times at 12000 rpm with a solution of NaOH at pH 9.5.

The modification of MWCNT with recently synthesized AuNPs was performed using the layer-by-layer self-assembly technique [24] and three assemblies were applied to obtain three layers of gold on the surface of the MWCN T. For this purpose, 20 mg of MWCNT were mixed with 20 mL of 1% polydiallyldimethylammonium chloride solution and the mixture was taken to an ultrasonic bath for 10 min. Then, the solution with nanotubes was washed with ultrapure water and 20 mL of AuNPs solution were added to the flask; the mixture was taken to an ultrasonic bath and the non-assembled nanoparticles were removed by centrifugation; the volume extracted was replenished with a NaOH solution at pH 9.5 for its last wash. The procedure

was repeated 2 more times to obtain three layers of AuNPs anchored on MWCNT surface. The modified carbon nanotubes were dried in an oven at 80 °C for 3 h.

Subsequently, an ink was prepared with 2 mg of MWCNT-AuNPs, 15 μL of nafion and 500 μL of distilled water. This mixture was agitated in an ultrasonic bath for 30 min to obtain a homogeneous dispersion. The procedure of ultrasonic bathing was repeated as often as necessary in order to maintain dispersion. Finally, the surface of a CF electrode of dimension $1 \times 4 \times 1$ cm was impregnated with the ink through the drop casting technique and dried at 80 °C for 30 min.

Characterization of MWCNT-AuNPs ink and MWCNT-AuNPs/CF

A cell with three-electrode setup was used for the electrochemical characterization. A sample of MWCNT-AuNPs ink was placed on a glassy carbon (GC) disk electrode with CF of $1 \times 6 \times 1$ cm used as counter electrode and Ag/AgCl/KCl (sat) as reference electrode, and cyclic voltammetry (CV) was performed in a solution of 0.5 M H$_2$SO$_4$. The measurements were made on an IviumStat potentiostat/galvanostat (Ivium Technologies. Eindhoven, Netherlands). Electrochemical characterization of the charge transfer capacitance of the CF modified with MWCNT-AuNPs was tested in an equimolar solution of 10 mM of Fe (CN)$_6^{4-}$/Fe (CN)$_6^{3-}$ that used 0.1 M KCl as supporting electrolyte at 10 mV s^{-1}. CV was performed at scan rate of 1 mV s^{-1} and at potential range from − 0.7 to 0.4 V (measured against an

Fig. 1 MFC under aerobic conditions
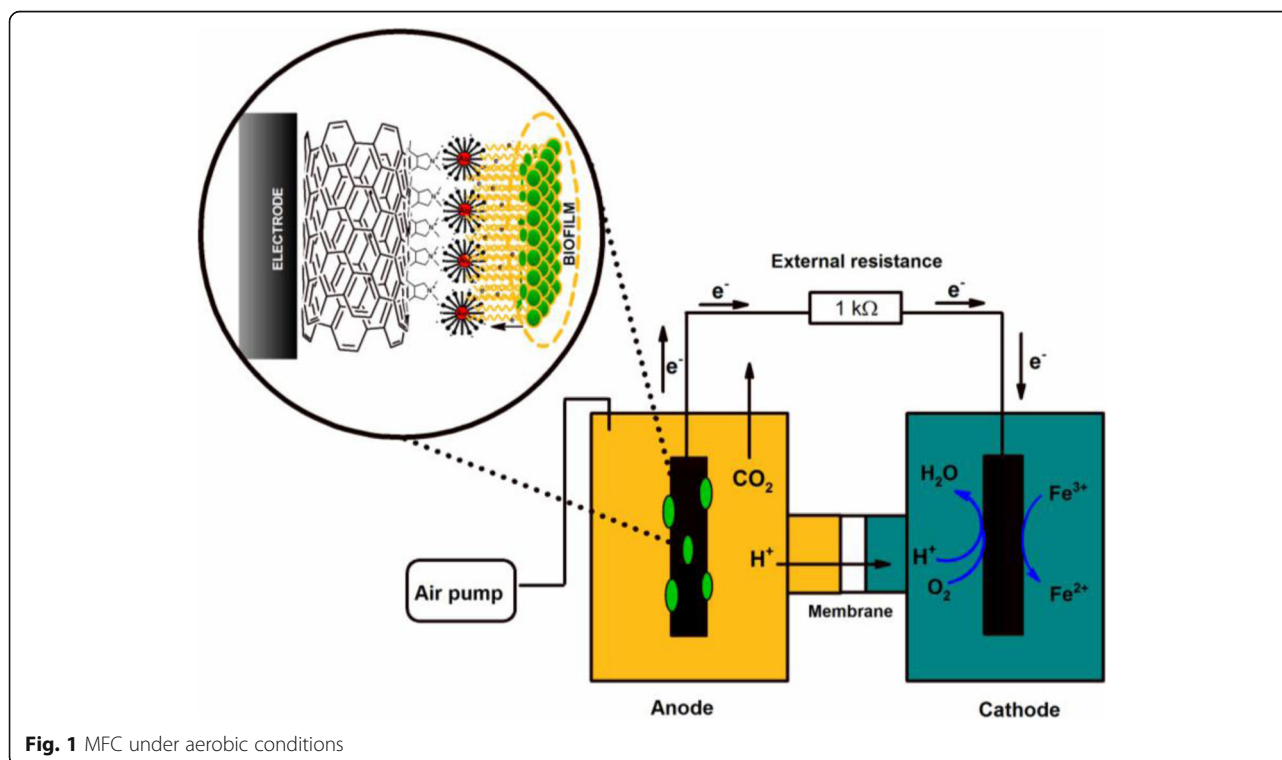
Ag/AgCl/KCl(sat) reference) in order to evaluate electrochemical activity of the used MFC.

MFCs and electrical measurements

MFCs had two polyhedral-shaped polyethylene containers (11.5 cm length × 11.7 cm width × 7.5 cm height). The total volume of each chamber was 900 mL, but the volume used was 675 mL. A proton exchange membrane (CMI-7000) separated both chambers. The membrane thickness was 0.45 ± 0.03 mm and the available surface area was 2.54 cm^2. Before being used, the membrane was immersed in 5% NaCl for 12 h in order to activate the membrane.

MWCNT-AuNPs/CF anode (1 × 4 × 1 cm) and CF without modified cathode (4 × 7 × 1 cm) were used. The anode/cathode area ratio of 1/7 was established to avoid possible resistance interferences derived from the limit current density at the cathode. Ag/AgCl/KCl(sat.) reference electrode was placed in the anodic chamber. Both chambers were joined through an external resistor of 1000 Ω. Two cells were prepared with different culture media. On the one hand, synthetic wastewater (SWW) (4 g glucose, 310 mg NH_4Cl, 130 mg KCl, 4.97 g NaH_2PO_4 and 2.75 g $Na_2HPO_4 \cdot H_2O$ in 1 L, pH 7.2) and LB (adjusted to pH 9 with NaOH) were used in anodic cell under aerobic conditions. On the other hand, in the cathodic chamber, a solution of $Fe(CN)_6^{3-}$ dissolved in 0.1 M KCl was used (Fig. 1). The electricity produced by electrical cells was controlled for 12 d while measuring voltage difference between the anode and the cathode through the external resistance, using a HANTEK® 365A data logger connected to a computer. The system was operated at room temperature.

SEM of bioanode

Scanning electron microscopy (SEM) was used for observing modified surface morphology of anodes. The electrodes were dried with hot air and stored in a desiccator. The images were obtained using a Philips 505 SEM at 10,000X magnification and applying an incident beam energy of 10 keV with the working distance of 6.5 mm. SEM characterization was repeated in order to observe biofilm formation on the carbon surface.

MFC operating under anaerobic conditions

After 12 d of biofilm growth in LB medium, the electrode was removed and stored for 15 d at 4 °C. Subsequently, the electrode was installed in the MFC with fresh 0.1 M acetate electrolyte under anaerobic conditions and the reactivation time was evaluated by the analysis of redox process versus immersion time.

Results and discussion

Isolation and identification of Shewanella spp.

After 24 h, the marine samples deposited in the glass jars showed decomposition and at 48 h the process of putrefaction began. *Shewanella* spp. was isolated and biochemically identified from one of the marine samples. Table 1 shows the results of phenotypic and biochemical tests. After staining, bacteria appear as motile Gram-negative bacilli. The characteristics of the *Shewanella* strains on the SIM medium showed black, punctate colonies, 1–3 mm of diameter, giving off the smell of hydrogen sulfide. The colonies had mucous consistency. The published results of a *S. putrefaciens* strain ATCC 8071 were compared to the biochemical test results obtained from the isolated strains.

Phenotypic test results showed that *Shewanella* spp. was characterized by motile Gram-negative flagella with positive oxidase and catalase and H_2S production. These

Table 1 Phenotypic characteristics of *Shewanella* spp. strain isolated compared to the reference strain ATCC 8071

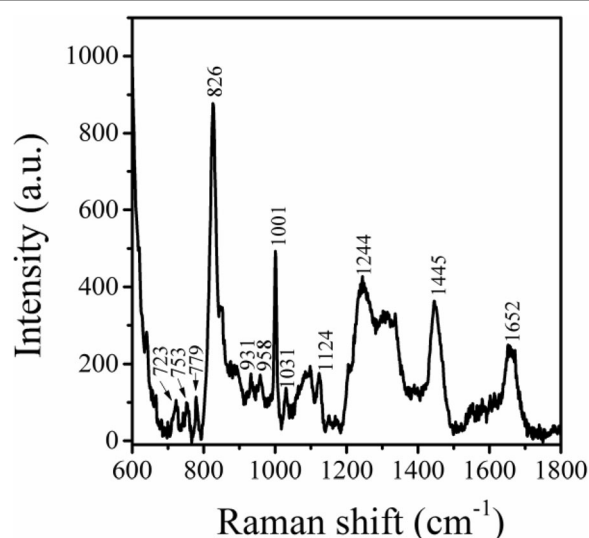| Biochemical tests	*S. putrefaciens* ATCC 8071	*Shewanella* spp.
Mobility	+	+
Oxidase	+	+
Catalase	+	+
Indole production	–	–
H_2S production	+	+
Urea hydrolysis	–	–
Gelatin hydrolysis test	+	+
Nitrate test	+	+
Nitrite test	+	+
Hemolysis	–	–
Gram staining	–	–

+ Positive, – Negative

Fig. 2 Raman spectrum of *Shewanella* spp. on screen-printed gold electrodes collected by a diode laser (638 nm)

Table 2 Structures of the main Raman bands (cm^{-1}) of *Shewanella* spp. isolated sample

Raman shift (cm^{-1})	Tentativa assignment of peaks	Refs.
723	Adenine (glycosidic ring)	[29, 30]
753	Tryptophan (symmetric stretch of benzene)	[31]
779	glycosidic ring	[29]
826	Tyrosine protein (Fermi resonance doublet)	[31]
931, 958	C − COO$^-$ and C − C stretching	[30, 31]
1001	Tryptophan (symmetrical benzene and pyrrole ring stretch)	[30, 31]
1124	NH$_3^+$deformation	[30]
1244	Amide III	[28, 29]
1445	C − H$_2$ deformation	[28, 30]
1652	Amide I	[30]

findings are consistent with those mentioned by Sten-strom and Molin [25] and Pekala et al. [26].

In addition, *Shewanella* spp. was analysed in the fin-gerprint region (600–1800 cm^{-1}) of the spectra [27, 28]. The spectra obtained for *Shewanella* spp. (Fig. 2) show peaks with values close to those reported by other inves-tigations that used *Shewanella* species (Table 2) [28–31].

Synthesis and characterization of CF electrode modified with MWCNT-AuNPs

The synthesized AuNPs were characterized by UV-Vis absorption spectroscopy. AuNPs show the maximum ab-sorption peak around 520 nm (Fig. 3a) [23]. In addition, d*ynamic light scattering* analyzer (NanoBrook 90Plus, Brookhaven Instruments., New York, USA) was used to measure particle size (real refractive index n$_s$ 0.21 and imaginary refractive index k$_s$ 3272), obtaining an effect-ive diameter of 30 nm (Fig. 3b).

The characterization of GC electrode modified with MWCNT-AuNPs was performed by using CV. Figure 3c shows the comparative voltammograms of MWCNT/GC and MWCNT-AuNPs/GC, observing the gold oxidation
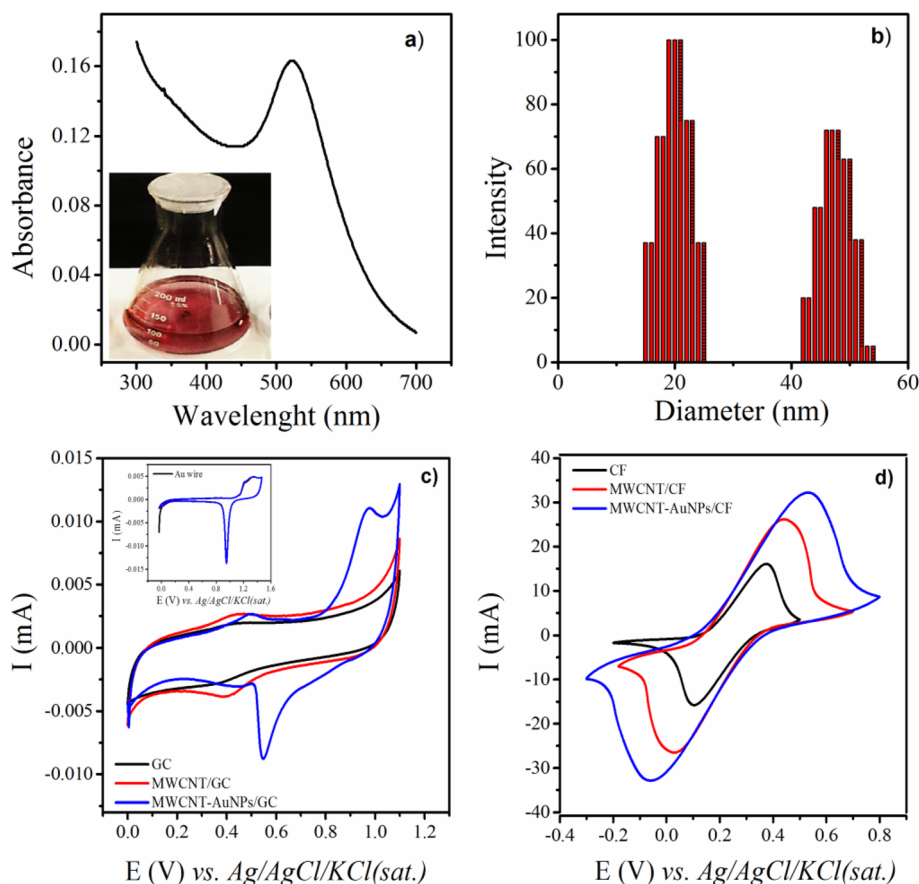

Fig. 3 a UV-Vis absorption spectrum of AuNPs. **b** Data obtained of AuNPs in the particle size analyzer. **c** CV in 0.5 M H$_2$SO$_4$ of GC, MWCNT/GC and MWCNT-AuNPs/GC, inset: gold wire. **d** in equimolar aqueous solution at 10 mM Fe(CN)$_6^{4-}$/Fe(CN)$_6^{3-}$ with 0.1 M KCl supporting electrolyte
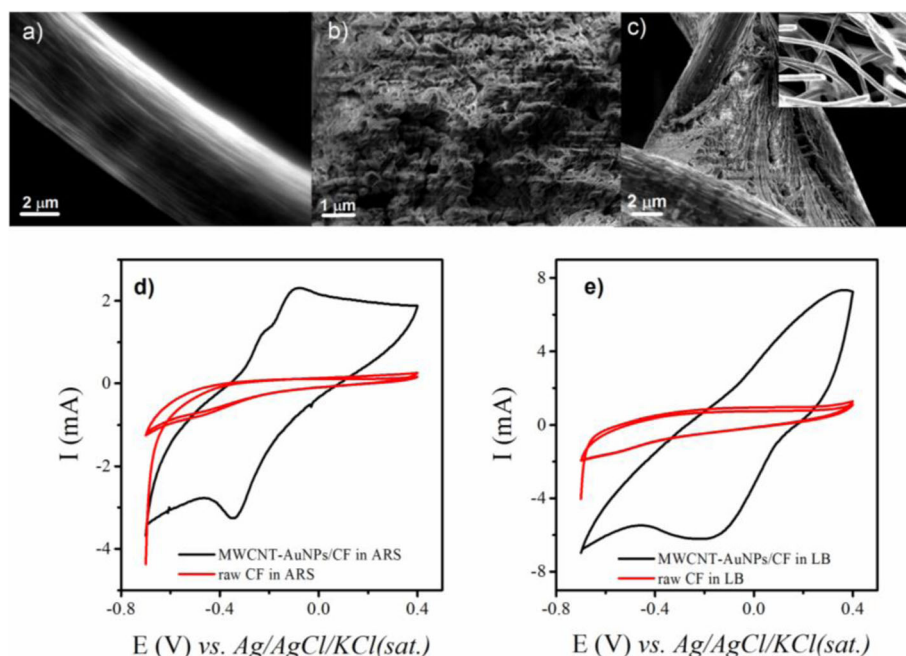
Fig. 4 a SEM images of CF. **b** biofilm on MWNCT-AuNPs/CF in SWW. **c** biofilm on MWNCT-AuNPs/CF in LB. **d** CVs in SWW of CF and MWNCT-AuNPs/CF with biofilm. **e** with LB

and reduction peaks at 0.95 and 0.55 V vs. Ag/AgCl/KCl(sat.), respectively, coinciding with the redox potentials obtained from a gold electrode used as reference (see inset Fig. 3c). Moreover, the running CVs of the CF electrodes modified with MWCNT and MWCNT-AuNPs in $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}/KCl$ solution show a significant increase in peak current with respect to the unmodified electrode, from 15.4 to 27.7 and 31.5 mA (Fig. 3d). This increase in current may be related to the increase in the electroactive area and the conductivity generated by MWCNT and AuNPs in MWCNT-AuNPs (0.012 mg m^{-2} of Au). However, the displacement of the potential is an indicator of a greater difficulty of the electrolyte diffusion in nanotubes. Assuming a proportional relationship between the heights of anodic current and the area (Butler-Volmer equation), it was obtained an initial electroactive area of 0.12 m^2 for unmodified CF and 0.43 m^2 for MWCNT-AuNPs/CF.

The SEM micrograph analysis shows the fibers of the unmodified CF (Fig. 4a). Figure 4b shows the fibers completely covered with the biofilm formed after 5 d of culture in SWW medium. Likewise, it was noted the formation of a biofilm that extends over the fibers in the felt immersed in LB (Fig. 4c). This characteristic should be considered in the evaluation of the electrode efficiency. Therefore, these results show the biocompatibility of the materials used and the morphological modification, which occurs during the biofilm formation and growth on the surfaces of CF modified with MWNCT-AuNPs.

In addition, the voltammograms of Fig. 4d and e, corresponding to the CF electrode immersed in SWW and LB media, respectively, show two wide oxidation peaks around - 0.2 and – 0.1 V and a reduction band around – 0.3 V vs. Ag/AgCl/KCl(sat). Redox process shows the facultative nature of the *Shewanella* spp. bacteria, due to their electrochemical activity under aerobic conditions. The potentials obtained in the voltammograms are close to the potentials reported in other investigations that used *Shewanella* under anaerobic conditions [3, 5, 32]. Thus, the results would reveal that electrochemical activity also occurs in aerobically growing bacteria and this activity probably depends on the type of substrate since the results show a better visualization of the redox reaction in SWW (Fig. 4d).

Evaluation of bioelectrochemical energy production in SWW and LB

The difference of potential versus the time was recorded during 12 days of biofilm growth over the anode of MWNCT-AuNPs/CF (Fig. 5a and b). Potential of an unmodified CF was measured simultaneously in another microbial cell. It was noted a potential increased with the passing of the hours and in the power production profile the power density generation changes with the type of substrate. The MFC was evaluated during the first 12 d, and a stabilized power density value 71 and 281 mW m^{-2} were obtained in the SWW and the LB
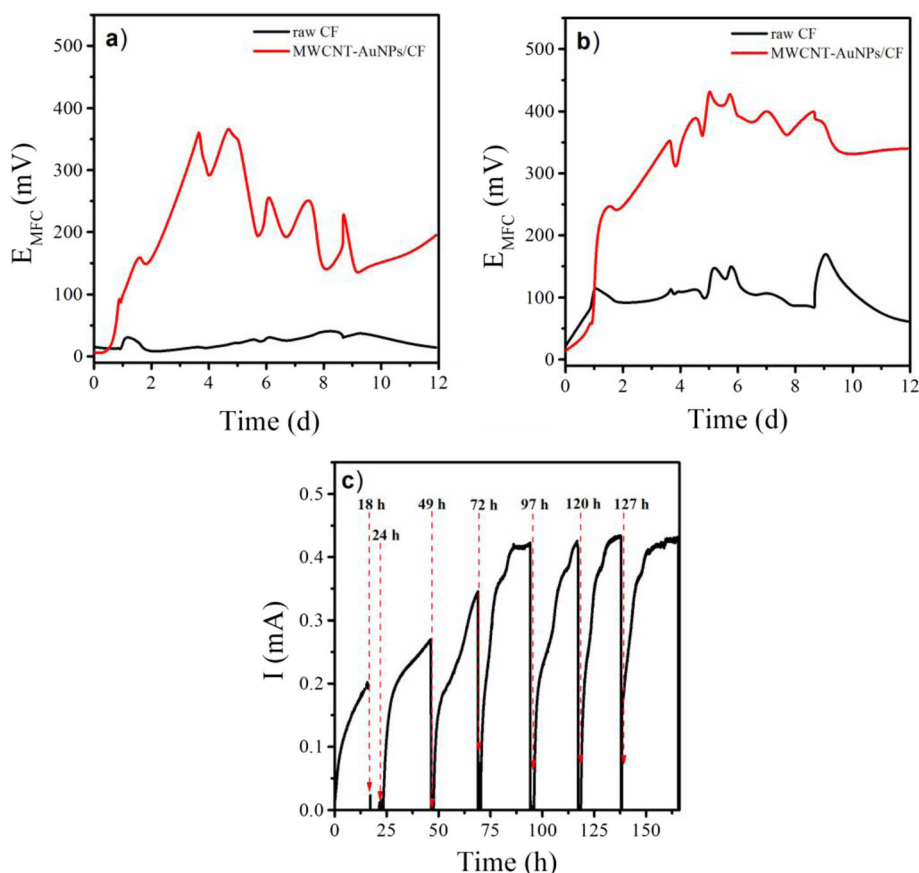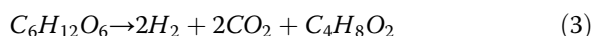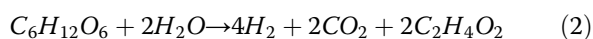
Fig. 5 a Cell performance over time in aerobic conditions of CF and MWCNT-AuNPs/CF in SWW. **b** in LB. **c** Chronoamperometry of biofilm on MWCNT-AuNPs/CF, applied potential at 0.2 V vs. Ag/AgCl/KCl(sat.) in anaerobic conditions. The arrows indicate the addition of 0.1 M acetate

substrates, respectively. These values were determined using the following equation (Eq. (1)) [33]:

$$P = \frac{E_{MFC}^2}{A_{An}R_{ext}} \tag{1}$$

where, P is the stabilized power density, E_{MFC} is the MFC stabilized potential, A_{an} is the anodic area and $R_{ext} = 1\,k\Omega$ is the external resistance employed.

Moreover, the power generated in the LB medium is almost twice that obtained in the SWW medium. The results may be related to the components of culture media, since a percentage of glucose (the main component of the SWW) carries out a parallel reaction that generates fermentation products according to the following (Eqs. 2 and 3) [34, 35]:

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2C_2H_4O_2 \tag{2}$$

$$C_6H_{12}O_6 \rightarrow 2H_2 + 2CO_2 + C_4H_8O_2 \tag{3}$$

While in the LB medium, due to its main content of tryptone (enzymatic digest of casein), microorganisms use the available nitrogen source, avoiding fermentation.

Therefore, the LB medium turned out to be favourable for the generation of electrical energy.

Figure 5c shows the current produced in the anode. Each dotted line represents the time when the substrate in the MFC was replaced with fresh acetate substrate. Initially, no current was recorded, but with time, there is a progressive increase in current at an approximate rate of $0.023\,\mathrm{mA\,m^{-2}\,h^{-1}}$ reaching a maximum current density in the steady state of $0.11\,\mathrm{mA\,cm^{-2}}$ after 72 h. The coulombic efficiency was determined by chronoamperometry. In this case, a 700 mL cell was used to which 0.8 g of acetate was added along with recording time and current data produced by the MFC during a cycle of 16.4 h. Integration of these data from the equation, $Q = I\,t$, resulting in $Q_a = 1193\,\mathrm{C}$. In the meantime, the chemical oxygen demand (COD) was analyzed at the beginning and end of the experiment. The coulombic efficiency is determined from the comparison of the measured charge against the charge produced by the combustion of the consumed acetate during the time of the experiment.

The COD values analyzed were: COD at 0 h = 1076 $\mathrm{mg\,L^{-1}}$; COD at 16.4 h = 646 $\mathrm{mg\,L^{-1}}$. Thus, the total charge obtained from the combustion of the acetate was,

Qc = 1841 C. Coulombic efficiency is thus calculated as Qa/Qc or 1193/1841 = 65%.

Furthermore, the evaluation by CV of the electrodes immersed in the new substrate allowed to observe the redox process after 18 h of measurement (Fig. 6a and b). The potentials recorded at 0.08 and – 0.28 V represent the redox process that occurs in microbial reaction. Redox potentials were not observed during the first hours due to the new substrate that inhibited bacterial activity. Figure 6c shows peak current variations during the bacteria adaptation. The increase in the peak current is related to the growth in the number of redox species in the electrodes that represent an increment in the electrochemical activity of bacteria. This result favors production of electric current.

According to the results, it is conclusive that the incorporation of MWCNT and AuNPs contributes significantly to the increase in energy of the MFC, it is possible to associate this increase with several factors, among them, the increase in energy density, as well as the decrease of ohmic resistance favoring its high electrical conductivity. The presence of AuNPs can improve the biocompatibility of exoelectrogenic bacteria to form a stable biofilm on the electrode surface, increasing its coulombic efficiency. Table 3 shows the comparative results with those obtained in the present work.

The metabolic process of bacteria, which produces energy and gases along with growth of biofilm of uncontrolled thickness, codeposition of particles from salts and other interferers can affect the stability of the electrode, so future studies aimed at evaluating the effects mentioned in the cell lifetime are necessary.

Conclusions

It is possible to design a double–compartment MFC, separated with a cationic membrane, using *Shewanella* strains isolated from *O. regia regia* as inocula. The surface modification of CF electrodes with AuNPs and
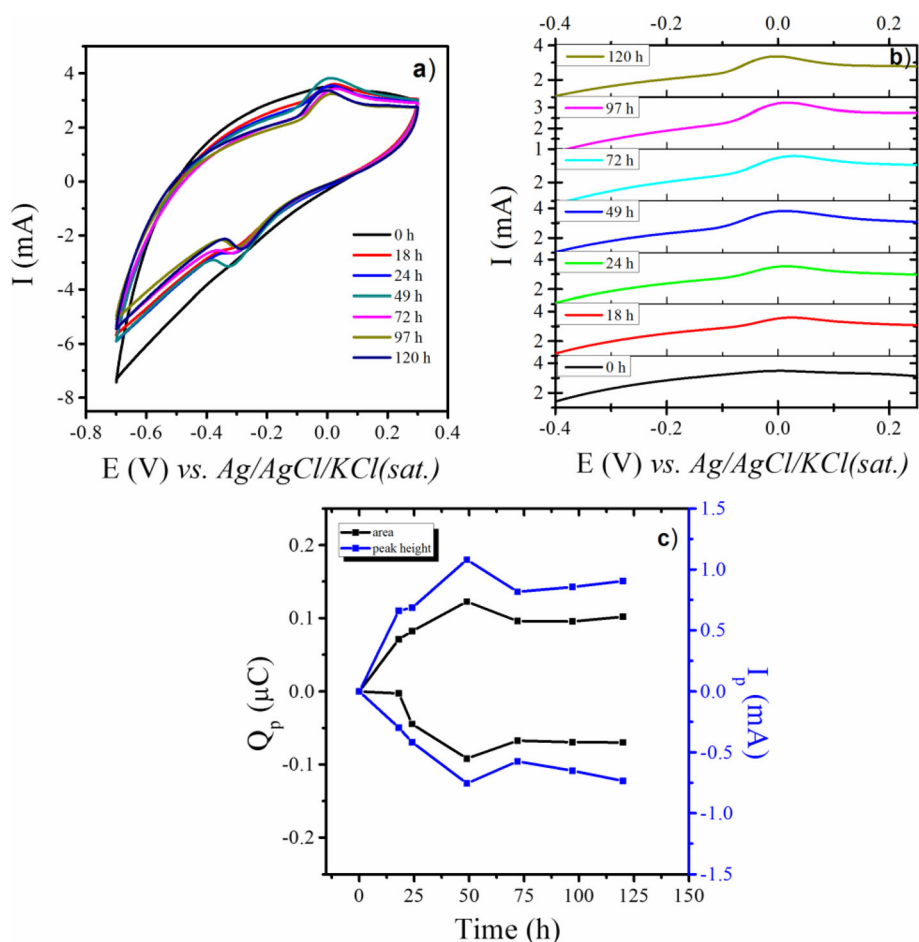

Fig. 6 a CV of CF with microbial biofilm immersed in a electrochemical cell containing industrial wastewater, at scan rate of 1 mV s^{-1} vs. Ag/AgCl/KCl(sat.). **b** Amplification of the anode peak of the modified CF with biofilm augmentation over time. **c** Height (I$_p$) and area (Q$_p$) for the oxidation and reduction peaks of redox system, depending on their potential measured at 1 mV s^{-1} vs. Ag/AgCl/KCl(sat.). For visualization purposes, reduction peaks were considered as negative values

Table 3 Comparative values of energy density obtained in studies of MFCs

Anode	Inoculum/substrate	Power density (mW m^{-2})	Refs.
CF modified with PANI	Mixed culture/acetate	216	[36]
CF modified with graphene oxide (GO)	*S. putrefaciens*/LB + Lactate	240	[37]
CF modified with GO–zeolite	Sludge/acetate	281	[38]
Iron–plated CF	*S. oneidensis* MR-1/LB + lactic acid	113	[39]
CF modified with MWCNT–AuNPs	*Shewanella* spp./LB	281	This study
CF modified with MWCNT–AuNPs	*Shewanella* spp./SWW	135	This study

MWCNT proved to be useful for increasing electrical conductivity and electrode biocompatibility.

Additionally, studies by CV allowed the observation of electrochemical activity of *Shewanella* spp. biofilm formation under aerobic and anaerobic conditions. The placement of the electrode with biofilm grown in a new system with real wastewater presented the same electricity generation capacity. This finding opens the possibilities of the use of electrodes with stable biofilms in substrates with oxygen content. Besides, these results extend the possibilities of research on applications for other optional type of bacteria in MFC.

Authors' contributions
Sandy Luz Calderon performed the experimental work in the laboratory, obtained data, and produced the report. Pilar García, isolated, cultivated and characterized the strains of bacteria, doping for use in microbial fuel cells. Angelica Baena Moncada, contributed with the knowledge of supported nanoparticles on carbon materials and its adaptation on MFC, and the revision of the manuscript. Ana Lucía Paredes, participated in the discussion of results and wrote the manuscript. Adolfo La Rosa Toro Gómez, coordinated the work team, contributed with experience and knowledge on microbiological fuel cells and contributed to the discussion of results. All authors read and approved the final manuscript.

Availability of data and materials
All data generated or analysed during this study are included in this published article and its supplementary information files.

Competing interests
The authors declare that they have no competing interests" in this section.

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