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ENVIRONMENTAL PERFORMANCE OF MICROBIAL FUEL CELL BASED LIVE SLUDGE FOR VOLTAGE PRODUCTION AND CONGO RED DYE REMOVAL

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Abstract: In this work, Single chamber Microbial fuel cells (SCMFCs) are a versatile technology is depends on the interaction mechanisms of bacteria, to produce bioelectricity simultaneously and treat Congo red (CR) dye from aqueous solution at different pH (6.5-8). Electricity generation from the biodegradable organic substrate (sucrose) accompanied by decolorization of azo dye was investigated in the batch test results showed that more than 99% decolorization demonstrated at UV-Visible Spectrophotometer (500 nm) was achieved within 20 days and maximum output voltage (889 mv) had been obtained in an open circuit at a pH value of 7.5. Microbial community analysis showed that species in live sludge and the impact of bacteria grown on removal and voltage.

Keywords: *live sludge; SCMFs; Congo red; voltage*

1. Introduction

Increasing the number of citizens or activity and energy demand led to a negative effect on climate changes and environmental degradation. Therefore, a lot of researchers are intensely interested in using alternative sources that are eco-friendly, cost-effective to obtain renewable energy [1, 2] such as biomass, hydropower, geothermal, wind, and solar radiation. Microbial fuel cells (MFCs) or biological fuel cells are sustainable technology that offers green energy and clean water from wastewaters [3-5]. MFCs are a bio-electrochemical system consisting of an anode (anaerobic) and a cathode (aerobic) separated by a membrane, microorganism (catalyst), and electrical circect. In general, at an anode chamber, microbes are used to oxidate substrate; produce proton, electron, and carbon dioxide. Firstly, the electron generated from the microbe's metabolic activity goes to the cathode via an external wire. While the protons transfer to the cathode through a membrane and combine with electron acceptor (oxygen) and electrons to form water [6].

Azo dye application in the textile industry represents 70% of among dyes used. It is characterized by double bonds (-N=N-) as the chromophore in the molecular structure. [7].This dye does not impact the water bodies and aesthetic appearance; however, It also poses a significant harmful threat to the ecology. In the current research, study effect of pH on degradation one of the common azo dyes class is Congo red from aqueous solution designing a single-chamber MFCs inoculated with live sludge as fuel by. Decolorization and voltage

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generation, pH, and conductivity were measured every day.

2. Materials and Procedures

2.1. Congo Red Dye (CR)

CR is an azo dye class, CR is soluble in water, ethanol, and very slightly soluble in acetone. It can be a pH indicator dye that gives the red-colored at a pH of more than five and blue at a more acidic pH. It has a chemical formula, and the molecular weight (MW) is $C_{32}H_{22}N_6Na_2O_6S_2$ and 696.663, respectively. Its formula in 3dimensions is as follows.

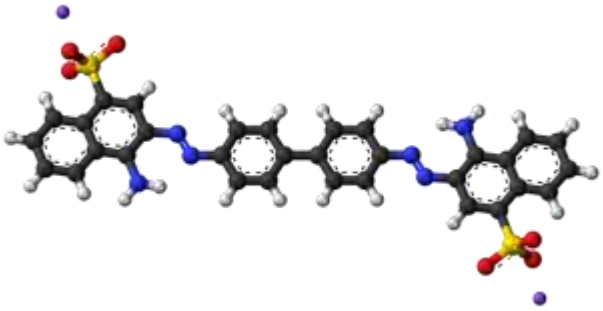

Figure 1. 3D chemical structure of CR.

2.2. Single Chamber Microbial Fuel Cells (SCMFCs)

SCMFCs constructed from the container has a high 24cm and volume of 6L (operation volume 5L), container operates under anaerobic conditions. Agar salt bridge pipe incorporating with container on high 1cantimeter from the bottom, on the other side of the container located sample port at high 4cm. Graphite plate electrodes (surface area 38.5 cm^2) were used, one electrode as anode located in the container, and the other electrodes are put at the elbow pipe that acts as a cathode. Copper wire is used to connect these electrodes, and prior to their use in the SCMFCs experiment, the electrodes were cleaned with pure water to enable microbe adhesion and electron transfer. Fig.2 Show is a schematic diagram of SCMFCs.

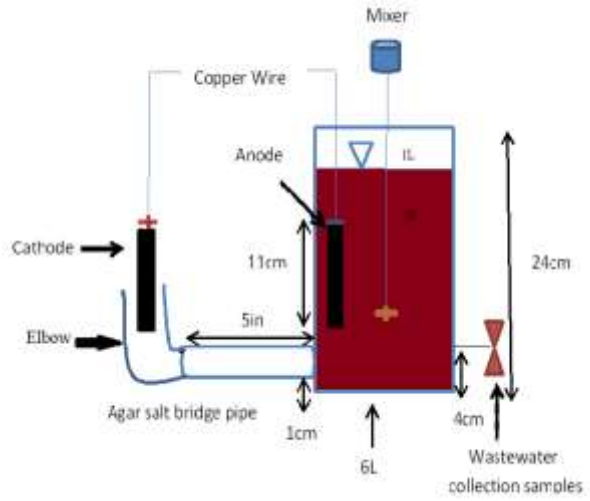

Figure 2. Schematic diagram of SCMFC

2.3. Preparation of Agar Salt Bridge Pipe

Bacteriological agar was used to make a salt bridge, agar was used as a solid media. The agar used has a molecular weight of 336.337 g/moL and a chemical formula of $C_{14}H_{24}O_9$. The bridge was made by dissolving 15% in a 1M NaCl solution. The boiling solution (agar + salt) was then poured into a PVC pipe (L 5 inches and D 1.5 inches). The salt bridge is solidified in the refrigerator before being connected to the SCMFCs. The agar salt bridge is shown in Fig. 3.

Figure 3. Agar salt bridge pipe.

2.4. Preparation of Dye Aqueous Solution

The media was prepared by dissolving 200 mg/ L of dye and mixed with 2.343gram of $C_{12}H_{22}O_{11}$ (468 COD\L) as fuel. 200 mL of filter sludge was added to the mix, after filtering by Whatman filter

paper (Grade 542) to remove impurities. Also, nutrients were added to the anode chamber (7.75 gram of NH_4CL, and 3.25 gram of KCL) to increase the activity of bacteria, Fig.4 shows the stock solution of CR and sludge filter.

Figure 4. (a) Congo red solution and (b) Sludge filter.

2.5. Effect of pH

Four SCMFCs shown in Fig.5 were operated at room temperature feed simultaneously with the same substrate but with varying pH values (6.5, 7, 7.5, and 8) to study the effect on microorganism's growth. Sodium Hydroxide (NaOH) and Hydrochloric acid (HCl) were used to control the pH to select the best pH to give maximum voltage output and removal efficiency.

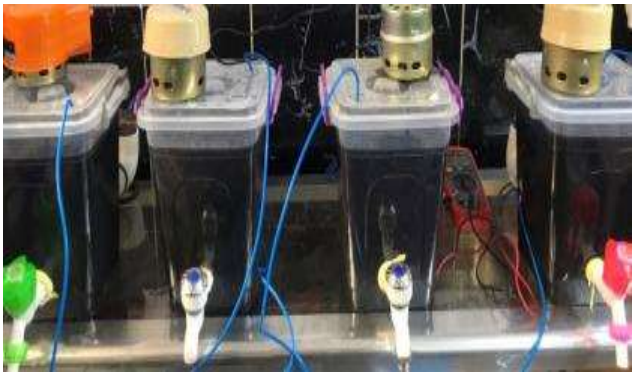

Figure 5. Four SCMFC with different pH.

2.6. Sludge Sample

The live sludge was collected from the Al-Rustamiyah treatment plant (the old project, Baghdad, Iraq). To recognize species of microbes present, initially, the sludge was serially diluted with pure water. 0.1 ml of liquots are put over various nutrients agar and cultivated in an incubator (ICP 500, Germany) at a temperature of 303 K for 3 to 7 days. Using a strake with 32 chambers, rely on biochemical tests and an intelligent system (Api 32 system) to diagnose bacteria species in a short period. These tests are carried out at the Ministry of Environment laboratories, college of Science/Baghdad University, and Environmental engineering department/ Al- Mustansiriya University.

Figure 6. Sludge was taken [8].

2.7. Analytical Methods

Multi-meter (UNI-T type), an electrical conductivity (EC) (WTW, Cond 3110, Germany), and a digital pH meter were used to monitor the voltage, Ph, and EC. The dyes' absorbance was measured with a UV–visible scanning spectrophotometer (Thermo-Genesys 10 UV, USA) throughout a wavelength range of 400–800 nm. The maximum wavelength calculated for CR dye by the calibration curve was found 500. Before the dyes samples were analyzed, the samples were filtered to remove any particulate matter using Whatman filter paper (

Grade 542). Fig. 8 shows how to prepare samples for analysis and spectrophotometer device.

The following equation was used to determine removal efficiency:

$$R\% = \frac{X-Y}{X} \times 100 \quad (1)$$

Figure 7. Sample filter and Spectrophotometer

3. Result

3.1. Effect of pH

Fig.8 explains variation in pH. Initially, at the anode chamber, the pH value decreases due to high H+ ion generation and then gradually increases due to the high current generation because more H+ ions are utilized in the cathode chamber by the catholyte. After a period, remains stable, due to the constant transport of H+ ions and the high current generation. Finally, the anode chamber becomes more acidic due to substrate degradation and slow H+ ion production [9]. The resulting pH value is within the normal allowable range of concentration in the river (6.5-9.5) [10].

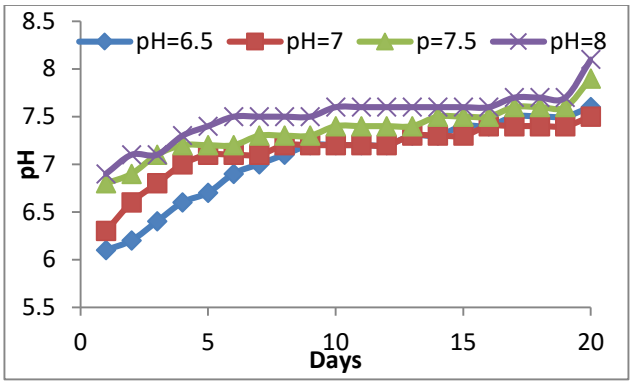

Figure 8. Variation in pH

3.2. Voltage Production

After four SCMFs were operated in batch mode, CR (200mg\l), the output voltage had been obtained in an open circuit for 20 days is shown in Fig.9 and Fig.10.The maximum voltage obtained at pH value 7.5 was 889 mv, while for pH values 8, 7, and 6.5, the maximum voltage production was 690, 679, and 604, respectively. The decrease output voltage for all MFCs gradually through the operation because the consumption of the organic matter and drop bacterial activity also has an effective role in MFC voltage generation.

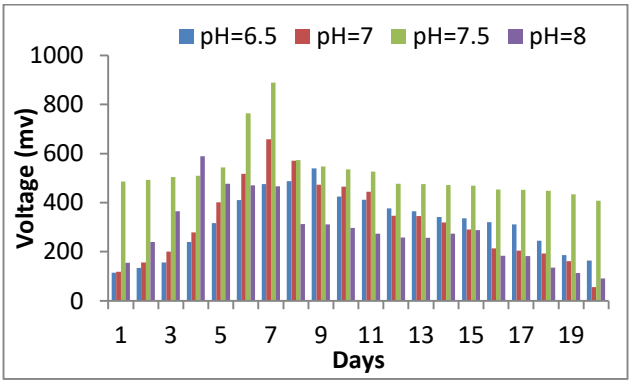

Figure 9. Output voltage (mv) during the light day for 20 days

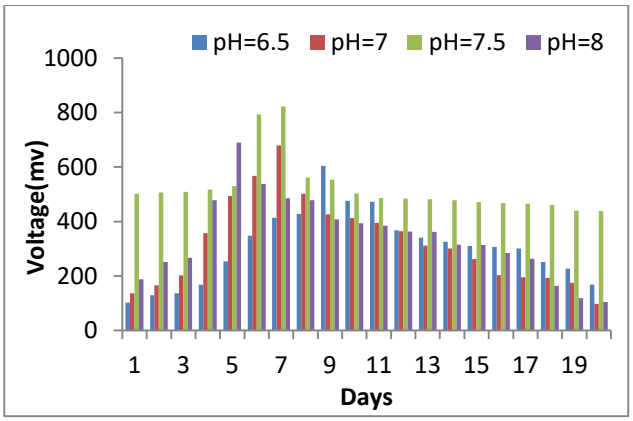

Figure 10. Output voltage (mv) during the light day for 20 days

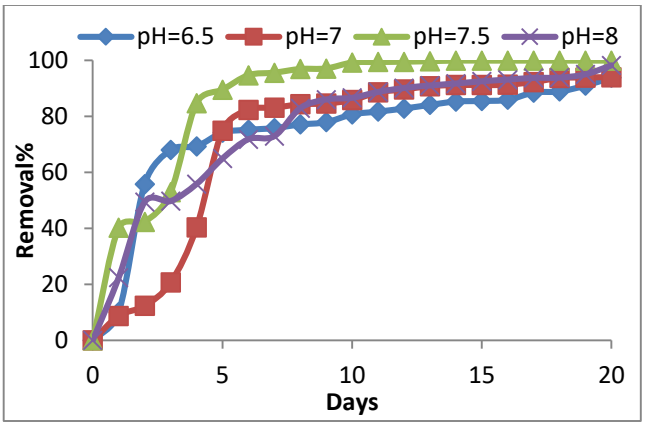

Figure11. The removal efficiency of CR

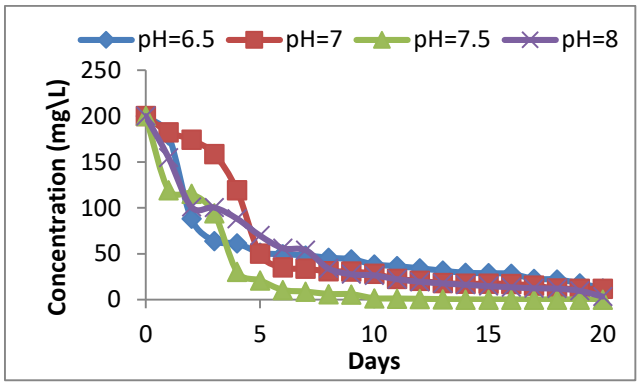

Figure 12. The decline of concentration of CR

3.3. Decolorization

The removal efficiency of dye at pH (6.5, 7, 7.5, and 8) is (93.8, 94, 99.9, and 98) where the concentration reached (12.1, 11.8, 0.09, and 3.5) as shown in Fig.11 and Fig.12. The initial concentration for all cells was 200 mg/L, then it gradually decreased with time because of a drop in the metabolism process of microbes that is responsible for the removal of dye. Bacteria activity gradually decreases due to a drop in substrate. Sun et al., 2013 study the mechanism of SCMFCs to degradation for CR dye by depending on a microfiltration membrane, Congo red (300mg\L) mixed with glucose used as fuel and anaerobic sludge. The electrode used was graphite felt (anode) and carbon paper (cathode). The researchers were obtained removal efficiency was 70% [11]. Dai et al., 2020 also study decolorization of Congo red in SCMFC and achieved removal efficiency of more than 88% by depending on Graphite fiber brush and Graphite fiber with platinum as the anode and cathode respectively, anaerobic sludge, 25ºC, initial pH 7.00, and initial concentration 200 mg\L [12].

3.4. Effect of Electrical Conductivity(EC)

EC was measured for samples collected every day. As a result, observed a high connecting between EC and voltage generation, the initial EC was measured to be between 6.34 and 9.96 mS/cm then it increased gradually to reach 11.5 to 16.4 mS/cm on the 20th day for the pH 8. This increase in EC results from the settling of salts on the cathode and anode and solution as well, Fig. 13 shows increasing EC during the operation time. Salt is necessary because the high saline condition in dye baths may promote the combination of colors and cellulose fiber. Salt is extensively used in the dying industry because the high saline condition can enhance the combination of dyes and cellulose fiber in dye baths. Salt concentrations in dye-stuff industry effluent were typically between 15-20 percent

[12, 13]. Proton transport is accelerated by high-value EC, which lowers the system's internal resistance. Tan et al., 2012 [13] showed 20 g/L of NaCl was added to SCMFCs enhanced overall performance by decreasing 33% of internal resistance and boosting voltage production by 30%.

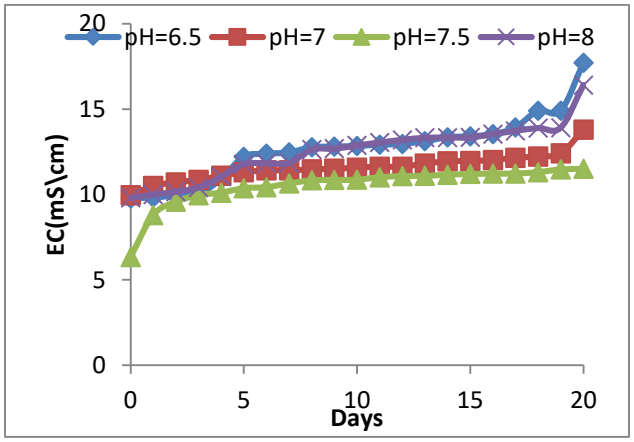

Figure13. Variation in EC during the process

3.4. Live Sludge

The microorganisms or microbes discovered in the sludge were heterogeneous (Table 1 and Fig. 14) mostly consisting of facultative anaerobic bacteria, amoeba, and protozoa, among others. The bacterial growth began to increase gradually (Lag phase), then increased rapidly until it reaches a maximum (log phase), then becomes steady due to substrate decreasing[14, 15]. Fig. 15.show bacteria growth pattern.

Table 1. Species of microorganisms identified in live sludge.

Species of microorganisms	CFU/ml
Dispersed bacteria	25×10^{9}
Filamentous microorganism	23×10^{9}
Flagellated protozoa	20×10^{9}
Stalked ciliate	19×10^{9}
Stalked ciliate (Vorticella)	1.3×10^{9}
Stalked ciliate cluster	2.4×10^{6}
Stalked ciliate (Opercularia)	5×10^{5}
Free-swimming ciliate (Spirostomum)	4.3×10^{8}
Suctoria	3.9×10^{8}
Carnivorous free-swimming ciliate	3.5×10^{8}
Probably chilodonella	2.4×10^{6}
Reproducing free-swimming ciliate	2.2×10^{6}
Stentor	5.3×10^{5}
Crawling ciliate	3.1×10^{5}
Amoeba	3×10^{5}
Testate"Shelled"amoeba	4.2×10^{4}
Boelloid rotifer (Rotifera)	2.2×10^{4}
Water bear (Tardigada)	1.8×10^{3}
Round worm (Nematoda)	1.5×10^{3}
Bristle worm (Aeolosoma)	1.4×10^{3}
Water bear "skin" with eggs	1.0×10^{3}

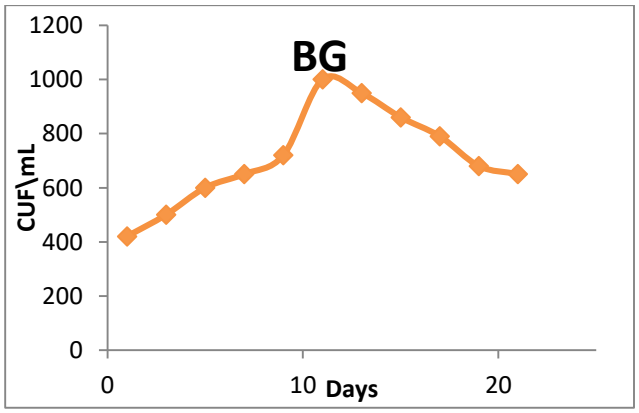

Figure 15. Bacteria growth

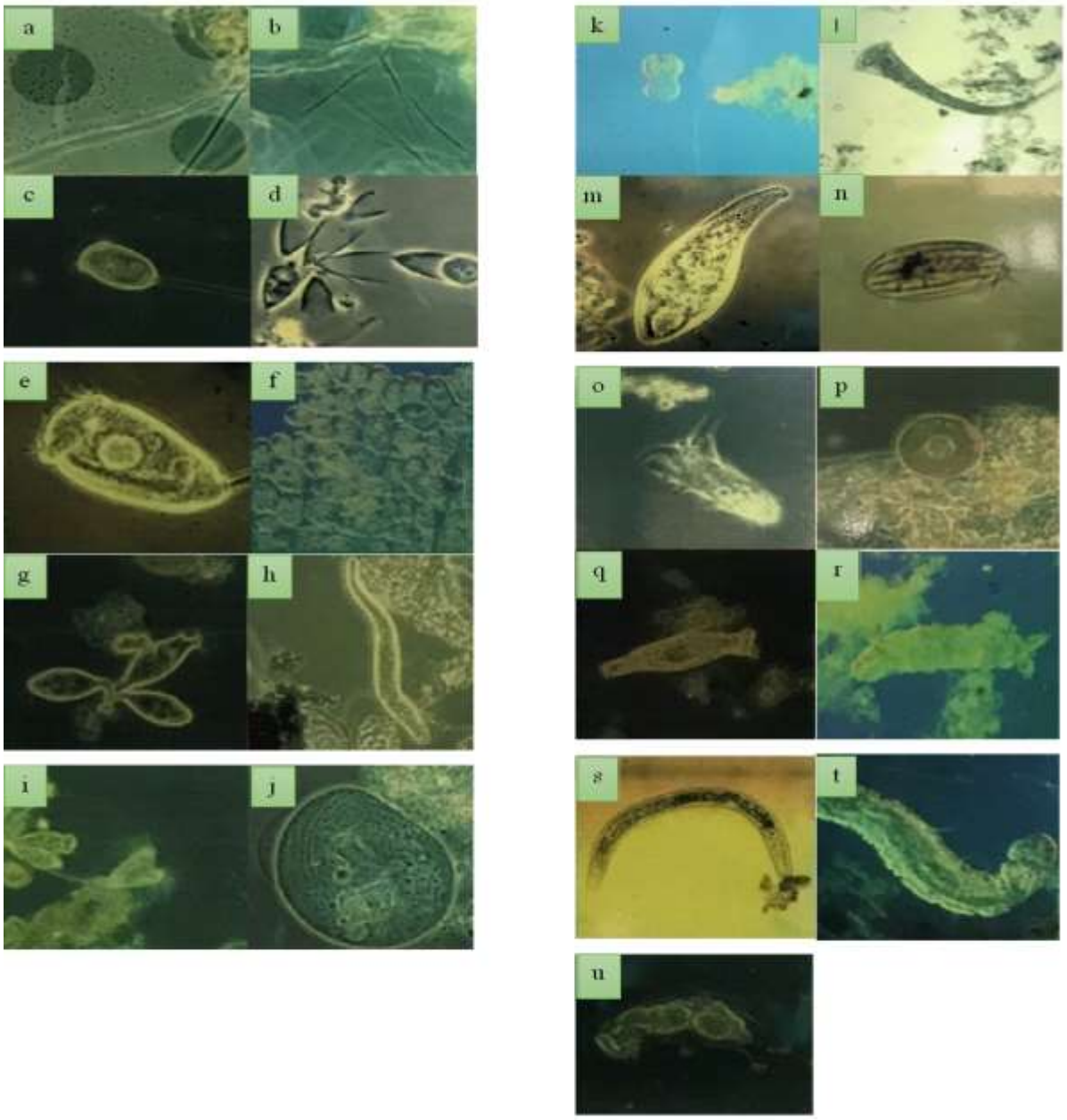

Figure14.(a) Dispersed bacteria, (b) Filamentous microorganism, (c) Flagellated protozoa, (d) Stalked ciliate, (e) Stalked ciliate (Vorticell), (f) Stalked ciliate cluster, (g) Stalked ciliate (Opercularia), (h) Free-swimming ciliate (Spirostomum), (i) Suctoria, (j) free-swimming ciliate with oral basket(Probably chilodonella), (k) Reproducing free-swimming ciliate, (l) free-swimming ciliate(Stentor), (m) Crawling ciliate, (n) Carnivorous free-swimming ciliate, (o) Amoeba, (p) Testate"Shelled" amoeba, (q) Boelloid rotifer (Rotifera), (r) Water bear (Tardigada), (s) Roundworm (Nematoda), (t) Bristle worm (Aeolosoma), and (u) Water bear "skin" with eggs.

4. Conclusion

This study is an indication of the high efficiency it has achieved of microbes for degradation of Congo red dye and bioelectricity generation during 20 days under anaerobic experimental conditions. The constructed single chamber microbial fuel cells showed good performance in batch mode (889 mv maximum output voltage and 99% removal efficiency). To enhance increasing voltage production different parameters, including electrode configuration, electrode surface, temperature, and other types of salt or other members, will be studied in further research.

Acknowledgments

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Conflict of interest

The authors hereby declare that there is no conflict of interest in the publishing of this paper.

Abbreviations

MFCs	Microbial fuel cells
SCMFC	Single chamber microbial fuel cell
CR	Congo red
mv	Mili volt
MW	Molecular weight
EC	Electrical conductivity
R%	Removal efficiency
x	Inicial absorbance
y	Observed absorbance

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