



## Electricity generation in a microbial fuel cell using iron oxide nanoparticles

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Rapid industrialization and population growth have generated a worldwide interest in renewable energy resources to meet. In this context, microbial fuel cells serve the dual purpose of electricity generation and wastewater treatment in a sustainable way. Here, we conducted a set of experiments in two-chambered microbial fuel cell (MFC) to study its efficiency in chemical oxygen demand (COD) removal and electricity generation. The MFC was run at constant pH of 5.5 and mesophilic temperature of 30-32°C using mixed consortia of sediment as inoculum and candy industry wastewater as substrate. Of the five different initial substrate concentrations of 2000, 4000, 6000, 8000 and 10000 mg COD/L studied, the highest COD removal efficiency of 96.0% and electricity generation of 810 mV was recorded at the initial substrate concentration of 4000 mg COD/L. The experiments conducted also revealed that iron oxide nanoparticles concentration of 0.10 g/L with an average size of 25.64 nm, increased the electricity generation potential to 870 mV by 6.9%. Among the different species of bioelectricity generating bacteria colonized, *Corynebacterium variabile* SMS-14 was documented as the most dominant species.

**Keywords:** Bioelectricity, Candy industry wastewater, COD removal efficiency, *Corynebacterium variabile*, Exoelectrogens, Renewable energy resources, Sustainable development, Wastewater treatment

Fossil fuels, the main source of energy, constitutes 80% of consumption worldwide. Uncontrolled exploitation of fossil fuel is the main cause for accumulation of CO<sub>2</sub> in the atmosphere and subsequent warming up of the earth<sup>1-5</sup>. The dual issues of energy crisis and environmental deterioration have prompted many countries to develop sustainable energy sources<sup>6-9</sup>. Further, the global electricity demand is assessed to increase up to 70% by 2035<sup>6</sup>. In this backdrop, harnessing renewable bioenergy was considered as one of effective ways to alleviate the impending problems. Many research works have been carried out using prototype bio-electrochemical system (BES). The advanced version of the microbial fuel cell (MFC) used in the present investigation has got the dual function of wastewater treatment and energy recovery<sup>10,11</sup>. The MFCs have attracted the

attention of all stakeholders throughout the world on many counts. Moreover, bioelectricity generated from MFC has been recognized as a clean fuel, and may serve to reduce the load on the fossil fuel demand<sup>12,13</sup>. The special features of this technology are direct conversion of wastewater into electricity, efficient operation at ambient and even at low temperatures and non-requirement of gas treatment.

Many research studies have examined bioelectricity generation in MFC using various types of substrates like food processing wastewater<sup>14</sup>, hospital wastewater<sup>15</sup>, meat processing wastewater<sup>16</sup>, domestic wastewater<sup>17,18</sup>, dairy wastewater<sup>19</sup>, palm oil effluent with acetate<sup>20</sup>, paper wastewater<sup>19</sup>, pharmaceutical wastewater<sup>21</sup>, agriculture wastewater<sup>22</sup>, distillery wastewater<sup>23,24</sup>, wine wastewater<sup>25</sup>, artificial wastewater<sup>26</sup> and biopesticide wastewater<sup>13</sup>. A large number of research works<sup>3,4,27-30</sup> have been carried out in different microbial fuel cell configurations with one

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or two chambers and also by using a membrane or without a membrane. In literature, reports on using wastewater for such experiments are limited. On the other hand, application of nanoparticles with nanoscale of 1-100 nm is widely used to accelerate various biochemical reactions<sup>31,32</sup>. However, the use of nanoparticles in enhancing the electricity generation in MFC has not been experimented.

Hence, we have made an attempt to produce electricity using two-chambered microbial fuel cell using wastewater from candy industry. In addition, we tried to assess the effect of nanoparticles in enhancing electricity generation in the MFC.

## Materials and Methods

### Anodic inoculum

The sediment sample was taken at a depth of 100 cm from the mangroves of Pichavaram located in the Cuddalore district of Tamil Nadu State and heated for an hour at 110°C and used as inoculum. The physicochemical parameters of the used mangrove soil sediment were clay, sand and silt (50, 22 and 36 %); temperature 28°C; pH 6.83; and salinity 28.50 ppt.

### Substrate

The substrate used was wastewater collected from a candy industry in Tamil Nadu State. The wastewater used in this investigation had the following characteristics: alkalinity – 50 mg/L; biological oxygen demand (BOD) 4600 mg/L; chemical oxygen demand (COD) 10470 mg/L; pH 5.2; volatile fatty acid (VFA) 2000 mg/L; volatile suspended solids (VSS) 1215 mg/L; and total solids (TS) 5200 mg/L.

### Synthesis of iron oxide nanoparticles

The iron oxide nanoparticles used in this study were synthesized by borohydride method following Sun *et al.*<sup>33</sup>.

### Experimental setup

The MFC with two chambers was constructed using two plexi jars of 1300 mL capacity with a working volume of 1000 mL. The anode and cathode were made of graphite plates and pre-treated. The electrodes were connected to the external circuit through a copper wire<sup>34</sup>.

### Batch experiments

Batch experiments were conducted in a two-chambered MFC. Between the two chambers, the designated anode chamber was filled with candy industry wastewater which was inoculated with 50 g

of pre-treated mangrove sediment under aseptic conditions. The aqueous potassium permanganate solution (electron acceptors) was taken in the cathode chamber as cathodic solution. In the present experiment, we evaluated the effect of initial substrate concentration and iron oxide nanoparticles concentration on bioelectricity generation. The substrate's initial pH was maintained at 5.5 using 1N NaOH or 1N HCl. The experiments were continued in triplicate at mesophilic temperature of 30-32°C.

### Sampling and analysis

The sample was collected and analysed at once for total solids (TS), biomass concentration as volatile suspended solids (VSS), pH and chemical oxygen demand (COD) concentration following standard methods<sup>35</sup>. The potential measurements were recorded between anode and cathode of the MFC using a multimeter after stabilization of the readings. The characterisation of nanoparticles was made by Fourier transform infrared (FTIR) spectrophotometer (Burker ALPHA, India) and also by employing X-ray diffraction (XRD) using X-ray diffractometer (PANalytical, X'pert PRO, India). The microbial species involved in bioelectricity generation were identified using scanning electron microscope (SEM) (JEOL-JSM-5610LV, Japan). The identified microbial species were isolated and characterized using polymerase chain reaction and density gradient gel electrophoresis (PCR-DGGE) and 16S rRNA analysis.

## Results and Discussion

### Effect of initial substrate concentration

In this experiment, the treatment performance and electricity generation of the MFC were assessed by removal of COD and TS from the substrate. When the batch experiment was initiated with five different initial substrate concentrations of 2000, 4000, 6000, 8000 and 10000 mg COD/L, the highest COD removal efficiency registered was 76.5, 96.0, 85.0, 66.0 and 64.0%, respectively (Fig. 1A). It is, from the above obtained results, when the initial substrate concentration was increased from 2000 to 4000 mg/L, the COD removal efficiency was also increased. When the initial substrate concentration was increased further from 6000 to 10000 mg/L, a decrease in COD removal efficiency was recorded (Fig. 1B). It was found out that among the five different initial substrate concentrations used the initial substrate

concentration of 4000 mg/L recorded the highest COD removal efficiency. Phenomenon of this nature could be attributed to the anodophiles found in the substrate might have made use of such substrate concentration highly suitable for colonization and subsequent metabolizing of carbon. The finding of the present study corroborates with the results of Venkatamohan *et al.*<sup>27</sup> on chemical wastewater treatment and electricity generation in MFC. The decline in COD removal efficiency with the initial substrate concentration of above 4000 mg/L might be

due to substrate inhibition as reported by Sridevi *et al.*<sup>36</sup> and Yogeswari *et al.*<sup>37</sup>. Similarly, for five different initial substrate concentrations of 2000, 4000, 6000, 8000 and 10000 mg COD/L, the TS removal efficiency (%) recorded were 72.31, 76.92, 75.0, 71.34 and 68.93%, respectively, at the end of 92 h. The relatively lower concentration of TS and VSS recorded in the present experiment than the previous research work of Mullai *et al.*<sup>7</sup> indicated the formation of low sludge production in MFC than conventional anaerobic treatment.

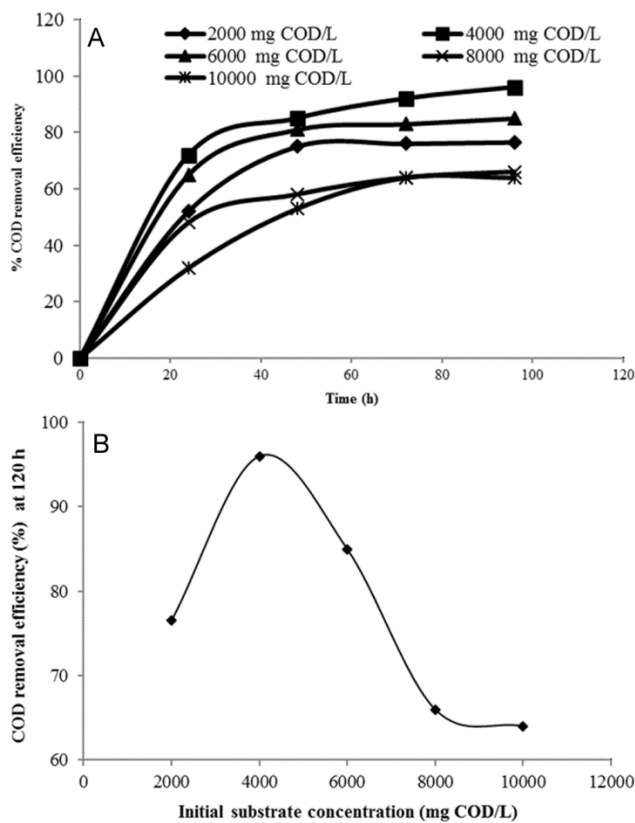


Fig. 1 — COD removal efficiency. (A) Performance of MFC anode chamber; and (B) Effect of initial substrate concentration

Among the five different initial substrate concentrations *viz.*, 2000, 4000, 6000, 8000 and 10000 mg COD/L, the greater potential generated under the applied external resistance of  $R=100\Omega$  was 777, 810, 798, 265 and 125 mV, respectively at the end 48<sup>th</sup>, 42<sup>nd</sup>, 42<sup>nd</sup>, 17<sup>th</sup> and 17<sup>th</sup> hour using potassium permanganate ( $\text{KMnO}_4$ ) as electron acceptor (Fig. 2A). When the experiment was conducted with five different initial substrate concentrations, the substrate concentration of 4000 mg COD/L registered a maximum potential of 810 mV. The highest potential generation recorded might be due to the availability of favourable nature of carbon sources for the microbes to transfer electrons at higher rate between microbial film and the solid electrode. Moreover, such a higher potential registered might also be due to the strong oxidation ability of cathodic solution<sup>38</sup>. During the experimental period, the potential produced by the MFC varied between 3 and 810 mV (Fig. 2A) and the current generated fluctuated between 0.03 and 8.10 mA. A wide variation in current density and power density was obtained and it ranged from 10.2 to 43.6 mA/m<sup>2</sup> (Fig. 2B) and 19.45 to 353.65 mW/m<sup>2</sup>, respectively (Fig. 2C). Such fluctuations in the power density might be attributed to the irregular rates of electron transfer to the anode and it was one of the major

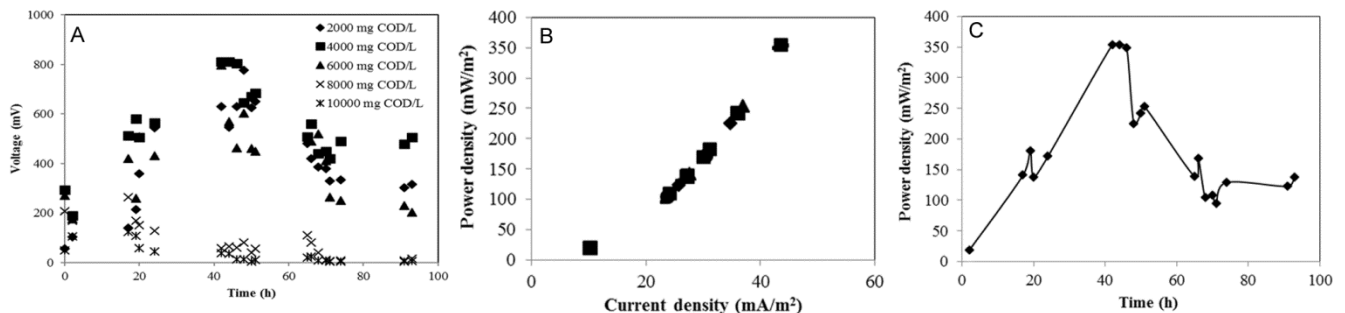


Fig. 2 — (A) Voltage variation at 100 $\Omega$  resistance during MFC operation; (B) Voltage and current density profile during MFC operation; and (C) Variation in power density without nanoparticles at 4000 mg COD/L

limiting factors. These fluctuations might also be due to the occurrence of different microbial groups in the anaerobic system<sup>27</sup>. In the same substrate with 4000 mg/L, a decrease in potential generation along with increase in time might be due to the decrease in oxidation ability of cathodic solution<sup>38</sup>. The pH of the substrate is an essential parameter and determines the nature of anaerobic process. The pH which was initially maintained at 5.5 (Table 1) for all the initial substrate concentrations was found to gradually increase till 48<sup>th</sup> hour and thereafter it declined gradually. The reason for such an increase in pH might also be adduced to proton transport rate from anode to cathode chamber<sup>39</sup>. A decline in pH values after 48<sup>th</sup> hour might be due to volatile fatty acids production.

#### Characterization of nanoparticles

The average size of iron oxide nanoparticles used in this experiment was found to be 25.64 nm and the SEM image is shown in Fig. 3A. The formation of iron oxide nanoparticles formation was confirmed by the Fourier transform infrared (FTIR) spectrum

(Fig. 3B) and the characteristic absorption bands recorded were 3423.43 cm<sup>-1</sup>, 1635.61 cm<sup>-1</sup>, 1396.50 cm<sup>-1</sup>, 1357.11 cm<sup>-1</sup> and 701.37 cm<sup>-1</sup>. Similar absorption bands have been reported by many researchers *viz.*, Tartaj *et al.*<sup>42</sup>, Khayatian *et al.*<sup>43</sup>, Mahdavi *et al.*<sup>44</sup> and Khalil<sup>45</sup>. Moreover, a peak at 701.37 cm<sup>-1</sup> confirmed that the synthesized nanoparticles were iron oxide (Fe<sub>3</sub>O<sub>4</sub>) nanoparticles.

#### Effect of nanoparticles

Iron plays a pivotal role in the synthesis of hydrogenase which in turn becomes crucial for the release of protons and electrons. In this experiment, since the initial concentration of the substrate at 4000 mg/L was found to be ideal it was taken for the experiment (Table 1). For different iron oxide nanoparticles concentration, such as 0.010, 0.050, 0.100, 0.150 and 0.200 g/L, the corresponding COD removal efficiencies were 85.0, 93.0, 98.0, 75.0 and 68.0 % (Fig. 4 A & B). For the same initial substrate concentration of 4000 mg COD/L, with five nanoparticle concentrations, the maximum potential recorded was 93.0, 850, 870, 567 and

Table 1 — Comparison of key findings in MFC operation

Parameters	Present study		Venkatamohan <i>et al.</i> 2008 <sup>27</sup>	Wang <i>et al.</i> 2013 <sup>40</sup>	Hassan <i>et al.</i> 2018 <sup>41</sup>
	Candy industry wastewater without nanoparticles	Candy industry wastewater with iron oxide nanoparticles	Chemical wastewater	Synthetic municipal wastewater	Young leachate
Initial pH	5.5	5.5	5.5 – 6.2	7.5	7.0
Initial substrate concentration (mg COD/L)/ OLR (kg COD/m <sup>3</sup> -d)	4000	4000	1.404	287.0	9000
COD removal efficiency (%)	96.0	98.0	61.11	87	91.0
Voltage (mV)	810	870	731	790	421.5

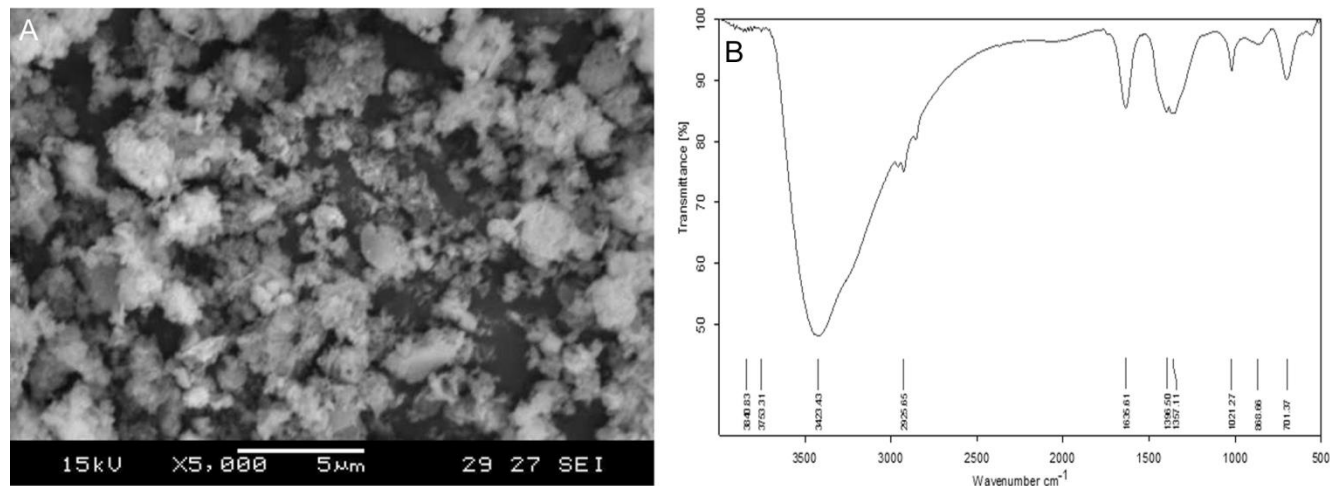


Fig. 3 — (A) SEM image of iron oxide nanoparticles; and (B) FTIR spectrum of iron oxide nanoparticles

140 mV, respectively at 36, 56, 71, 75 and 29 h (Fig. 5 A & B).

In the control, the value of COD removal efficiency and potential recorded which was 96% and 810mV, respectively marginally increased to 98% and 870 mV after the addition of nanoparticles (Table 1). The potential difference increased with

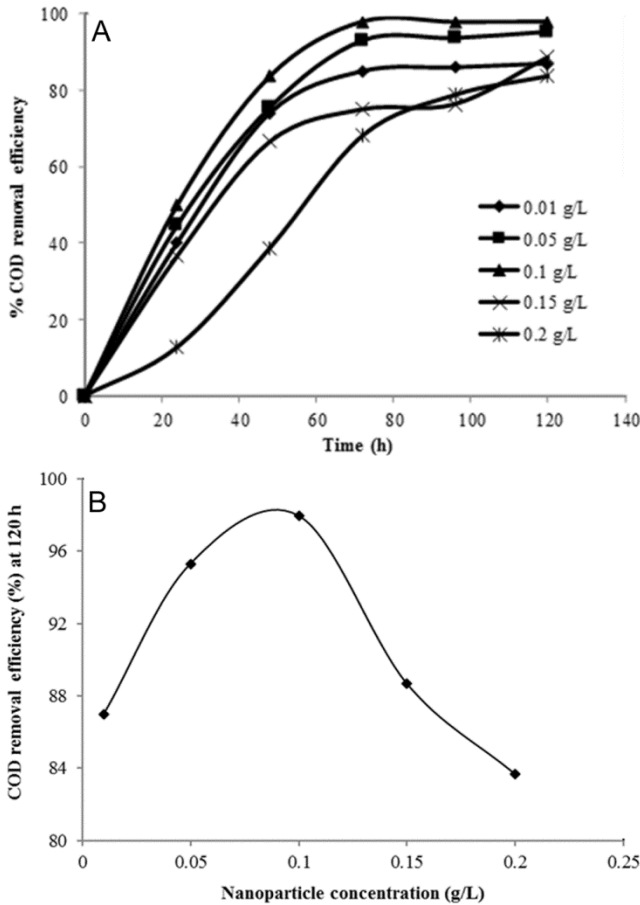


Fig. 4 — Performance of MFC anode chamber with respect to COD removal efficiency at (A) different nanoparticles concentration; and (B) 120<sup>th</sup> hour

increase in nanoparticles concentration and reached maximum at 0.100 g/L of iron oxide nanoparticles and then decreased. The increase in potential difference might be attributed to super paramagnetic properties of iron oxide nanoparticles and also increase in surface area. The decrease in COD removal efficiency and potential difference when increasing the concentration above 0.10 g/L might be due to toxic effect of metal nanoparticles at higher concentration which in turn decreased the growth of microorganisms. The range in power and current densities obtained was 151.41 to 407.98 mW/m<sup>2</sup> and 28.5 to 46.8 mA/m<sup>2</sup>, respectively (Fig. 5C). The addition of iron oxide nanoparticle concentration in the anodic chamber could influence the hydrogen production and in turn protons and electrons by influencing the activity of hydrogenase enzyme. Similar to that of the present investigation, Santoro *et al.*<sup>46</sup> have also used iron-based catalyst.

#### Bioelectricity generating microbial community

Bioelectricity producing microbial community identified through PCR-DGGE was affiliated to *Corynebacterium variabile* SMS-14 (KJ668601), *Escherichia coli* SAM-14 (KJ668602) *Klebsiella milletis* MYSKD (KJ668603) and *Bacillus thuringiensis serovar kurstaki* SMS (KJ668604). Among the other organisms, *Corynebacterium variabile* SMS-14 was recorded as a dominant organism. SEM images illustrated the nature of the mixed consortia which generated bioelectricity without (Fig. 6A) and with the addition of nanoparticles (Fig. 6B). In the SEM, many cells in clumps with varied shape and size were found. Many of the exoelectrogens were spherical and rod shaped with either clumped or in free form. SEM images also revealed that some cracks on the surface indicated the utilization of nutrients and hydrogen.

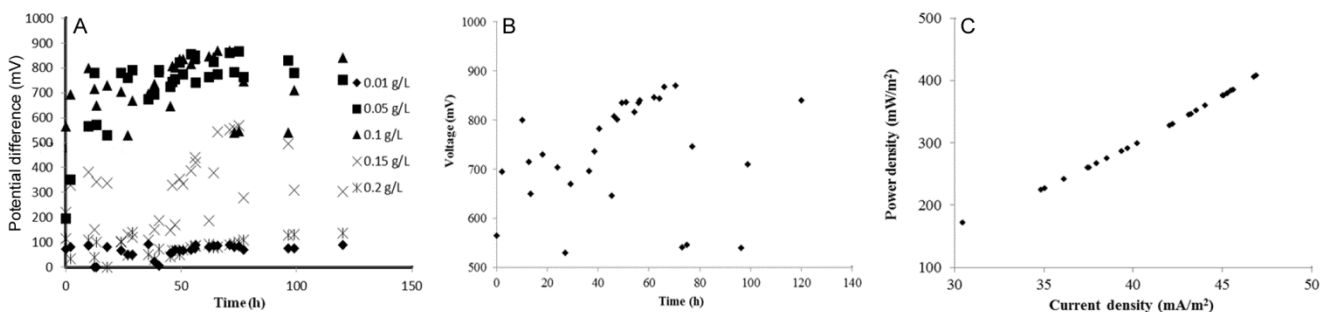


Fig. 5 — (A) Voltage variations at 100 $\Omega$  resistance during MFC operation with different nanoparticles concentrations; (B) Voltage variations with nanoparticles concentration of 0.100 g/L; and (C) Variations in current and power density with nanoparticles concentration of 0.100 g/L at 4000 mg COD/L

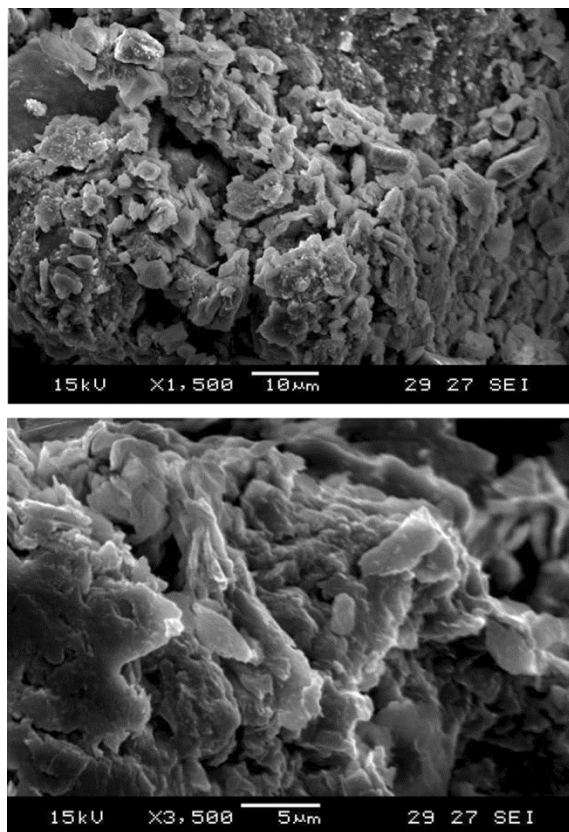


Fig. 6 — SEM image of bioelectricity producing (A) microbes from anodic biofilm; and (B) mixed consortia from anode surface after the addition of nanoparticles

### Conclusion

The present investigation substantiates the importance of application of iron oxide nanoparticles as catalyst in improving the electricity generation. Among the five different concentrations of iron oxide nanoparticles used, the iron oxide nanoparticles concentration of 100 mg/L was found to increase the electricity generation potential from 810 mV to 870 mV. The findings, though lab-scale in nature, throw much insight in designing MFC in commercial level and with the view of scaling up the electricity generation to find remedy for the impending energy crisis and in taking measures in protecting environment.

### Conflict of Interest

Authors declare no conflict of interests.

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