



Bio-electrodegradation of 2,4,6-Trichlorophenol by mixed microbial culture in dual chambered microbial fuel cells

Nishat Khan,¹ Mohammad Danish Khan,¹ Mohd Yusuf Ansari,² Anees Ahmad,¹ and Mohammad Zain Khan^{1,*}

Environmental Research Laboratory, Department of Chemistry, Aligarh Muslim University, Aligarh 202 002, UP, India¹ and Petrochemical Laboratory, Department of Petroleum Studies, Aligarh Muslim University, Aligarh 202 002, UP, India²

Received 15 May 2018; accepted 27 August 2018

Available online 25 October 2018

2,4,6-Trichlorophenol (TCP) was bioelectrochemically treated in anodic and cathodic compartments of two identical dual chambered microbial fuel cells MFC-A and MFC-B under anaerobic and aerobic conditions, respectively, and energy was recovered in the form of electricity. It was observed that MFC-B with bio-cathodic treatment of TCP outperformed the MFC-A with bio-anodic treatment. The maximum power density for MFC-A with bio-anode was found to be 446.76 mW/m² while for MFC-B with bio-cathode it was 1059.58 mW/m². The MFC-B consistently showed higher coulombic efficiency, power density and chemical oxygen demand removal efficiency indicating the better performance of the MFC-B as compared to the MFC-A. Scanning electron micrograph also confirmed better accumulation of microbes on the anode of MFC-B and hence its better performance in terms of energy recovery. Some major genera present in the microbial community were quantified using quantitative real-time polymerase chain reaction technique. It also confirmed the dominance of electroactive species in the bio-anodic sludge of MFC-B over the bio-anodic sludge of MFC-A. Cyclic voltammogram also asserted better electrochemical activity of the bio-cathode in the treatment of chlorinated phenol toxicants in MFC-B system. The study shows that MFC can be a viable option in treatment of recalcitrant chemical compounds like TCP with the generation of energy in the form of electrical power.

© 2018, The Society for Biotechnology, Japan. All rights reserved.

[**Keywords:** Bioelectrochemical treatment; Chlorophenol; Cyclic voltammetry; Microbial fuel cell; Power density]

Chlorophenolic compounds are among the most toxic pollutants distributed widely in the aquatic environment. They are common recalcitrant environmental pollutants and have gained increasing interest in the recent years in view of their environmental concerns (1,2). Because of their high toxicity, carcinogenicity, mutagenicity and bioaccumulation potential, chlorophenols have been classified as priority pollutants by the United States Environmental Protection Agency (USEPA) (3,4). Chlorophenols are weakly acidic compounds which have irritant effect on skin and mucous membrane (5) and the tendency to transfer through the cell membrane and accrue inside the bodies of aquatic organisms because of their lipophilic nature (6). They have been widely used in the preparation of pesticides, paints, pharmaceuticals, wood preservation and paper and pulp industry (7,8) which results in their unremitting presence in the natural water bodies.

2,4,6-Trichlorophenol (TCP) is a degradation product of pesticides such as pentachlorophenol, lindane and hexachlorobenzene and is one of the most significant polychlorophenolic pollutants (9). In polychlorophenols, the position of the chlorine atom relative to the hydroxyl bond tends to contribute towards their toxicity, carcinogenic property, structural stabilization and environmental persistence which make their removal from wastewater very crucial

(10). TCP has been reported to cause respiratory problems like cough, chronic bronchitis, chest wheezing, and altered pulmonary functions (1). Hence, TCP removal from wastewater is of utmost importance.

Several strategies have been followed over the years to remove these pollutants from the environment. Various physical and chemical methods like activated carbon adsorption, chemical oxidation, and incineration; biological and electrochemical methods have been employed to treat TCP containing wastewater. Most of these methods suffer from higher treatment cost, generation of secondary pollutants or incomplete mineralization (11,12). Biological methods are considered better over physicochemical methods except the microbes can be easily inhibited by the toxicity of TCP (13). Several research groups have reported the treatment of TCP under both aerobic and anaerobic conditions (14–16). Biological methods also generally have the disadvantage of excess sludge production (17). Recently a new technique has been developed and successfully used for the bioremediation of wastewater. Microbial fuel cell (MFC) is a novel bioelectrochemical technology that converts the chemical energy stored in the biodegradable chemical compounds into electrical energy by the action of microbes acting as catalyst (18,19). MFC is a low-cost, low maintenance technology with an added advantage of low sludge production (20). It works on the principle of redox reaction occurring simultaneously on anode and cathode. The electrons produced by the oxidation of substrate on anode flow through the external circuit to generate electric

* Corresponding author. Tel.: +91 8800704835; fax: +91 8266057545.
E-mail address: dr_mzain.fa@amu.ac.in (M.Z. Khan).

current (21). Various toxic chemicals like dyes (22), phenol (23) among others have been reportedly treated in MFC.

Several works have been reported with toxic chemicals being treated in bio-anodic and bio-cathodic compartments of dual chambered microbial fuel cell (DCMFC) but have been seldom compared. In the present study, we compare the bio-treatment of TCP in bio-anodic and bio-cathodic compartments of DCMFC along with the production of electrical power. Attempts have been made to link the power output to chemical oxygen demand (COD) of TCP wastewater. The performance of both MFC setups was evaluated by estimating power density (PD), coulombic efficiency (CE) and COD removal efficiency. The bioelectrochemical behaviour was studied using cyclic voltammetry. The abundance of microbial communities was studied using quantitative real-time polymerase chain reaction (qPCR).

The findings of this work can help in designing the efficient setup for large scale treatment of TCP containing wastewater with concurrently achieving electric output.

MATERIALS AND METHODS

Experimental setup Two identical airtight H-shaped MFC setups with an effective working volume of 70 ml for each chamber were commissioned with an anodic and a cathodic chamber separated by a proton exchange membrane. The carbon cloths with the surface area of 20 cm² were used in both the setups as electrodes. In order to improve the performance of MFC, the electrodes were pre-treated prior to use by first soaking in methanol followed by rinsing with distilled water and then dried in muffle furnace for 30 min at 450 °C (24). In order to enhance the porosity of the proton exchange membrane, Nafion-117 was pre-treated in accordance with Liu and Logan (25). The electrodes were connected to an external load of 470 Ω (22,26) through copper wire. To avoid the exposure of experimental setup to changing environmental conditions, the setup was kept in a separate temperature controlled room and periodically stirred to mix the sludge evenly.

Inoculation and operation The inoculum was collected from Okhla Sewage Treatment Plant, New Delhi with mixed microbial community. To acclimate the system before starting the experiment, the MFC-A was inoculated with anaerobic sludge with TCP and mineral salt media with glucose to support the growth of microorganisms. The cathodic chamber of the MFC-A was filled with buffer and opened to the ambient air without any external aeration. Conversely, MFC-B was inoculated with anaerobically acclimated sludge in the anodic chamber with glucose to support the microbes and act as electron donor while aerobically acclimated inoculum added in the cathodic chamber along with TCP. The anaerobic sludge was thick with dark brownish appearance while aerobic sludge was light brown in colour. The mineral salt media used to support microbial community was prepared in accordance with Khan et al. (27). Phosphate buffer solution (NaH₂PO₄, Na₂HPO₄; pH 7.2) was added at the start of the experiment to maintain the initial pH close to neutral. After the sludge was successfully acclimatised for a period of 30 days, the solution was replaced with the first experimental TCP concentration of 50 ppm and the circuit was closed. The concentration of TCP was stepwise increased from 50 to 500 ppm during the study. A constant glucose concentration of 1 g/L was maintained and replenished every time the voltage dropped below 100 mV throughout the study. The systems were operated for a period of 15 days for each concentration and the samples were collected periodically for different analyses. All the experiments were performed in duplicate in the temperature range of 30 ± 2 °C.

Analytical methods The voltage across the external load was recorded using a digital multimeter (Kehao KH200, Xiamen Kehao Automation Co., Ltd., Fujian, China). PD was determined from voltage and reported in mW/m². The efficiency of electron recovery as current was determined in terms of CE. The concentration of TCP was determined from absorbance recorded at 310 nm of the samples collected over time and the removal efficiency was calculated using formula:

$$\% \text{ RE} = \left(\frac{C_i - C_f}{C_i} \right) \times 100 \quad (1)$$

where C_i and C_f are the initial and final concentrations of TCP.

pH and conductivity were recorded periodically using pH and conductivity meter (KI-262-C, Khera Scientific Instruments Pvt. Ltd., New Delhi, India) while cell growth in both the setups was monitored by recording optical density (OD) at 600 nm. The COD of the collected samples was evaluated in accordance with the standard method (28) from absorbance using UV-Vis spectrophotometer (Perkin Elmer Lambda 45, PerkinElmer, CA, USA). To study the degradation of TCP during the course of time, the collected samples were centrifuged and scanned in the range of

200–700 nm using UV-Vis Spectrophotometer (Perkin Elmer Lambda 45). The concentration of chloride ions released due to the dehalogenation of TCP was determined over time in accordance with the Indian standards (29).

To study the redox reaction occurring at the surface of electrodes in both the setups, cyclic voltammetry was performed using electrochemical analyser (Autolab PGSTAT302N, Metrohm Autolab, Utrecht, Netherlands) by measuring current output at a defined potential range in accordance with Sultana et al. (30) with anode, cathode, and Ag/AgCl as working, counter, and reference electrode, respectively, and scan rate of 10 mV/s. Scanning electron microscopy (SEM) coupled with electron dispersive X-ray (EDX) (JSM-6510LV, Jeol, Tokyo, Japan) was used to examine the microbial attachment and the formation of the biofilm on the surface of carbon cloth electrodes along with determination of elemental composition. The fragments were cut from the electrodes and rinsed with phosphate buffer solution (pH 7.2) multiple times to stabilise the microbial community on electrode surface and then dried at 120 °C for 3 h for SEM analysis (24).

qPCR was performed to estimate the relative abundance of major genera. The technique provides a highly sensitive and rapid detection of microbial DNA thereby indicating the existence of target microorganisms. DNA SPIN KIT from MP Bio-medicals (Solon, OH, USA) was used to extract the DNA from the sludge collected from MFC-A and MFC-B anodes as per Khan et al. (31).

Before the qPCR analysis was performed, the effect of possible PCR inhibitors present in the DNA extract was minimized by diluting the DNA extract with molecular grade water. The inhibition free qPCR amplification was obtained with 1:10 diluted DNA sample which was used for all qPCR analysis using BioRad CFX C1000 (BioRad, Hercules, CA, USA). A reaction mix of 10 μL each was prepared from DNA (3 μL), each primer (0.5 μL), nuclease free water (1 μL) and qPCR reagent (5 μL) (SsoFast EvaGreen Supermix, BioRad). The target groups for qPCR analysis were 16S rRNA, sulphate reducing bacteria, methanogens and *Geobacter* species. The qPCR programmes followed were in accordance with Khan et al. (27). The primers used for qPCR were in accordance with our previous work (30,32).

RESULTS AND DISCUSSION

Operating parameters for the bioelectrochemical system The operating conditions were recorded throughout the study. The pH in the anodic chamber for both the reactors was set at neutral at the start of the experiment for every concentration. Fig. S1 shows the variation of pH, OD and conductivity over time in the anodic chamber for 100 ppm concentration of both the setups. The pH ranged from 4 to 7 throughout the experiment for both the setups. The overall conductivity for all the concentrations ranged from 0.4 to 0.8 mS/cm for MFC-A and 0.3–0.75 mS/cm for MFC-B and followed an increasing trend with time. The falling pH and increasing conductivity indicate the increasing ion concentration over time (mainly H⁺). The figure shows higher conductivity in case of MFC-A than MFC-B which could be because in MFC-A both glucose and TCP are present and being metabolised into protons and chloride ions whereas in MFC-B only glucose is being metabolised thereby increasing the ionic concentration in the former. Lower pH in MFC-A as compared to MFC-B can again be explained by the fact that in MFC-B the protons are moving to the cathodic chamber to maintain the electrical neutrality as electrons flow through the circuit. However in case of MFC-A, since the chloride ions are being released continuously in the anodic chamber the flow of protons is retarded due to electrical imbalance created leading to the accumulation of the protons in the anodic chamber thus lowering the pH. This lower pH further affects the performance of MFC-A as the microbial population comes under stress. The OD for MFC-A ranged from 1.3 to 1.9 while for MFC-B, it was observed to be varying from 1.7 to 2.6. The lower OD in case of MFC-A indicates that the toxic nature of TCP had derogatory effect on the growth of microbial community. The mixed liquor volatile suspended solid of the pre-acclimated sludge was 3.5 and 4.5 g/L for anaerobic and aerobic sludge, respectively. However, the mixed liquor volatile suspended solid for MFC-A and MFC-B were found to be varying from 1 to 3.5 and 1–4.5 g/L, respectively, throughout the experiment.

Bioelectricity generation The treatment of TCP under anaerobic and aerobic conditions of MFC-A and MFC-B,

respectively, was compared in terms of voltage output. Fig. 1A, B represents the output voltage variation (mV) of MFC-A and B with reference to time (h) for different concentrations of TCP. Similar voltage trend has also been reported by Huang et al. (8). The system was fed with glucose every time voltage dropped below 100 mV. A control experiment was also performed with only glucose as substrate (no TCP was added). It was observed that energy generation was low when both glucose and TCP were added in anaerobic conditions (MFC-A). The maximum voltage of 648 mV was reached for 100 ppm concentration of TCP. However, for aerobic treatment of TCP (MFC-B) when only glucose was anaerobically metabolised, the maximum output voltage was 35% higher than when TCP was also present in the anaerobic chamber. The maximum voltage for system with aerobically treated TCP was 998 mV at the concentration of 100 ppm. The lower voltage output for system with anaerobically treated TCP could be due to the toxicity of TCP towards microbes due to which the performance was limited. Substrate control experiment with only glucose and inocula (no TCP) gave the maximum open circuit voltage of 1071 mV. Control experiment with only substrate (glucose and TCP without anaerobic sludge) did not show any biocatalytic activity towards degradation. The decreased voltage

outputs of system under study with reference to the control also suggested the inhibitory effect of TCP on microbial community. This also explains the decreased performance of both experimental setups with the increasing concentration of TCP. The difference in the performance of the two setups could also be due to the fact that under both anaerobic and aerobic conditions TCP is being treated differently. On one hand in anaerobic conditions, the presence of TCP in the anodic chamber leads to the utilization of some electrons produced to be consumed for reductive dechlorination of TCP which affect the electron flow through the circuit and thus current generation. It also affects degradation by accumulation of lower biodegraded products in the same compartment thus affecting further treatment. On the contrary, in case of aerobic treatment in MFC-B, no limitation to electron flow is presented. Also, the presence of oxygen assists the further oxidation of degraded products which decreases accumulation of the degraded moieties as also reported for chlorophenols by other researchers (18). Similar results were also observed by Aifei et al. (10) when they treated TCP in an air-cathode MFC and Khan et al. (22) when they treated azo-dyes in single chambered MFC and Rikame et al. (33) without the addition of mediators. High voltage output in MFC-B could be attributed to better treatment of TCP by aerobic bacteria.

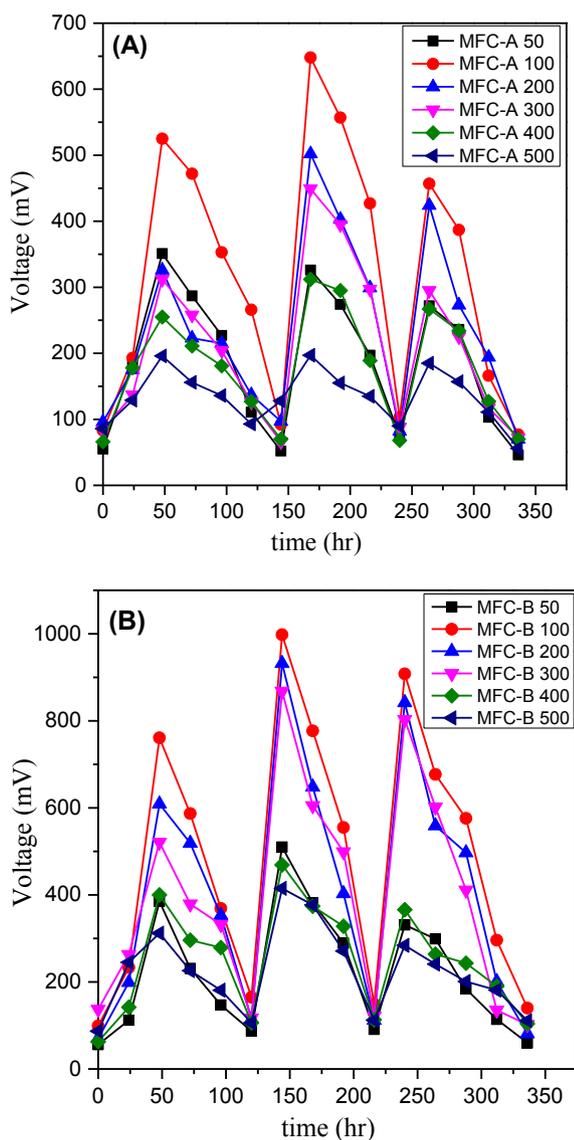


FIG. 1. Voltage variation during course of time for (A) MFC-A and (B) MFC-B.

Power density, chemical oxygen demand removal and coulombic efficiency

The performance of the MFC setups was further evaluated in terms of PD, COD removal efficiency and CE. The highest PD for both the setups was observed at 100 ppm concentration. For MFC-A bio-anodic treatment, the highest PD was recorded to be 446.76 mW/m² while for MFC-B it was found to be 1059.58 mW/m². The peak in the PD curve represents the fresh addition of co-substrate (glucose). The PD peaked every time the fresh substrate was added and gradually decreased as the substrate was consumed by the microbial community. Lower PD in case of MFC-A as compared to the MFC-B could be due to the fact that the presence of TCP in the same chamber as substrate lowered the performance of microbes due to the toxic nature of TCP as compared to the system with separate chambers for both TCP and glucose. Moreover, TCP may act as an electron acceptor thereby lowering the voltage when present in the anodic compartment. Similar high power densities have also been reported by Cheng and Logan (34) and Feng et al. (35) when they treated domestic wastewater in MFC with carbon cloth and carbon fibre used as electrodes and Khan et al. (22) when they treated azo-dye in MFC.

The efficiency of the MFC setups in treating waste was estimated in terms of COD removal efficiency. It was observed that the highest COD removal for both the setups was obtained for the TCP concentration of 100 ppm. When TCP was degraded in the anodic chamber of MFC-A, the highest COD removal achieved was 72.48%. On the other hand, when TCP was treated aerobically in a separate chamber the highest COD removal efficiency was 89.64%. The highest PD achieved corresponding to COD indicates that energy output was achieved at the expense of COD removal. The performance of MFC-B in terms of PD and COD removal efficiency was consistently better compared to the MFC-A. This could be attributed to various factors like better sludge porosity, more active and sustainable bacterial community growing in this system. Fig. 2A and B links the PD to COD removal efficiency during the study.

The efficiency of both the setup in terms of energy recovery was calculated in terms of CE. The CE for both the reactors was found to be highest at 100 ppm concentration indicating that at this concentration both the setups showed best performance. However, the performance decreased as the concentration of TCP further increased which could be due to its toxic nature inhibiting microbes and affecting the overall performance. The highest CE for MFC-A

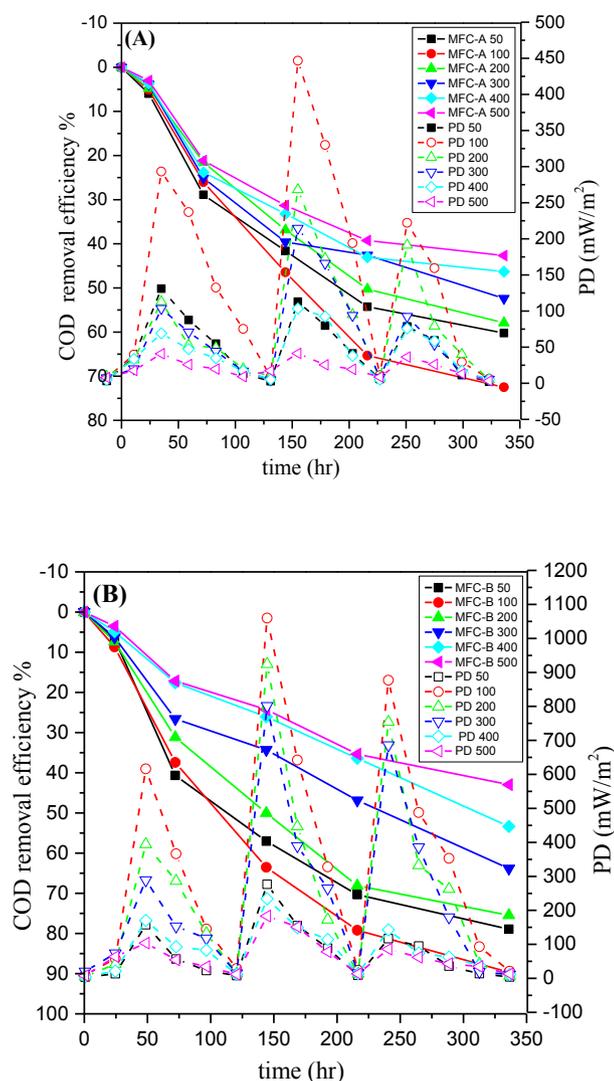


FIG. 2. PD and COD removal efficiency variation over time for (A) MFC-A and (B) MFC-B.

was 5.45% while for MFC-B it was calculated to be 7.23%. Fig. 3 presents the CE of MFC-A and MFC-B at various concentrations of TCP. The consistently better performance of MFC-B as compared to MFC-A suggests that the presence of toxic compound in the anodic chamber affects the electron production thereby affecting the CE and MFC performance.

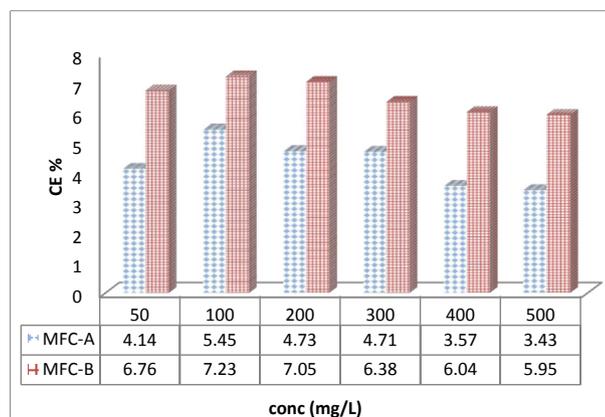


FIG. 3. Coulombic Efficiency variations at different concentrations for MFC-A and MFC-B.

Formation of chloride ions The first step in the treatment of chlorinated phenols is dehalogenation that results in an increased concentration of chloride ion in the wastewater. The variation in the concentration of chloride ion with time for 100 ppm TCP concentration has been presented in Fig. S2. As shown in the figure, chloride ion concentration increased over the course of time during the experiment suggesting dehalogenation to be the first step in the process of treatment of TCP. The chloride ion concentration increased rapidly in the first week of experiment and became nearly constant after 8 days of treatment. The plot also shows that in the case of MFC-B, the chloride ion concentration was more dramatically increasing than MFC-A indicating better dehalogenation and thereby better treatment of TCP in MFC-B.

Degradation study of 2,4,6 TCP using UV/Vis spectroscopy The degradation of TCP in both the setups was studied by scanning the effluent sample in the wavelength range of 200–700 nm using UV/Vis spectrophotometer. Fig. S3A and B shows the UV/Vis spectra for MFC-A and MFC-B for the TCP concentration of 100 ppm. The peak absorptions as shown in the figure were observed at 245 nm and 310 nm. The highest intensity was observed on the first day of the experiment as can be seen in the graph and decrease in absorbance with time indicates the degradation of TCP. Fig. S3B clearly shows that in case of MFC-B the maximum degradation was achieved within a week of start of the experiment and no peak was observed after 10 days of experiment indicating better treatment of TCP in the setup. Also, it can be noted from Fig. S3A that in case of MFC-A, a peak is observed at 270 nm in the sample collected after 72 h which could be the peak of phenol indicating that TCP is being degraded to phenol over time. However, on further treatment the loss of peak indicates the further treatment of phenol over time. The absence of phenolic peak in MFC-B could be due to the rapid conversion of produced phenol into even simpler compounds.

The TCP removal efficiency was evaluated for the samples collected from MFC-A and MFC-B. It was observed that the TCP removal efficiency was better for MFC-B indicating that in MFC-B, TCP was better treated as compared to MFC-A. The percent TCP removal for MFC-A and MFC-B is presented in Fig. S4A and B, respectively. The highest TCP removal efficiency was observed to be 79.75% for MFC-B at the TCP concentration of 100 ppm. For MFC-A, again the highest TCP removal efficiencies were obtained for 100 ppm concentration and were found to be 60.39%. However, as the concentration increased the TCP removal efficiency decreased which can be explained by the fact that the increasing concentration has inhibitory effect on the growth of microbial community leading to the diminishing effect on the performance of the system. The better performance at 100 ppm as compared to the 50 ppm concentration could be due to the better acclimatization of microbes over time.

Characterization of sludge by scanning electron microscopy The surface morphology and elemental composition of the electrodes used in the anodic compartment of both the setups have been studied by SEM coupled with EDX. Fig. 4A–C shows SEM images of plain carbon cloth and the carbon cloth anodes obtained from MFC-A and MFC-B, respectively. The SEM images obtained for both the reactors clearly show the accumulation of microbial community on the electrode surface. The dominance of microorganisms (bacterial community) in MFC-B as compared to the MFC-A confirms the better performance of the former than the latter. The difference in the microbial population in both the setups could be due to the difference in the environmental conditions provided. The lower microbial population in MFC-A could be due to the toxic effect of TCP which in turn affected the performance of the system while in

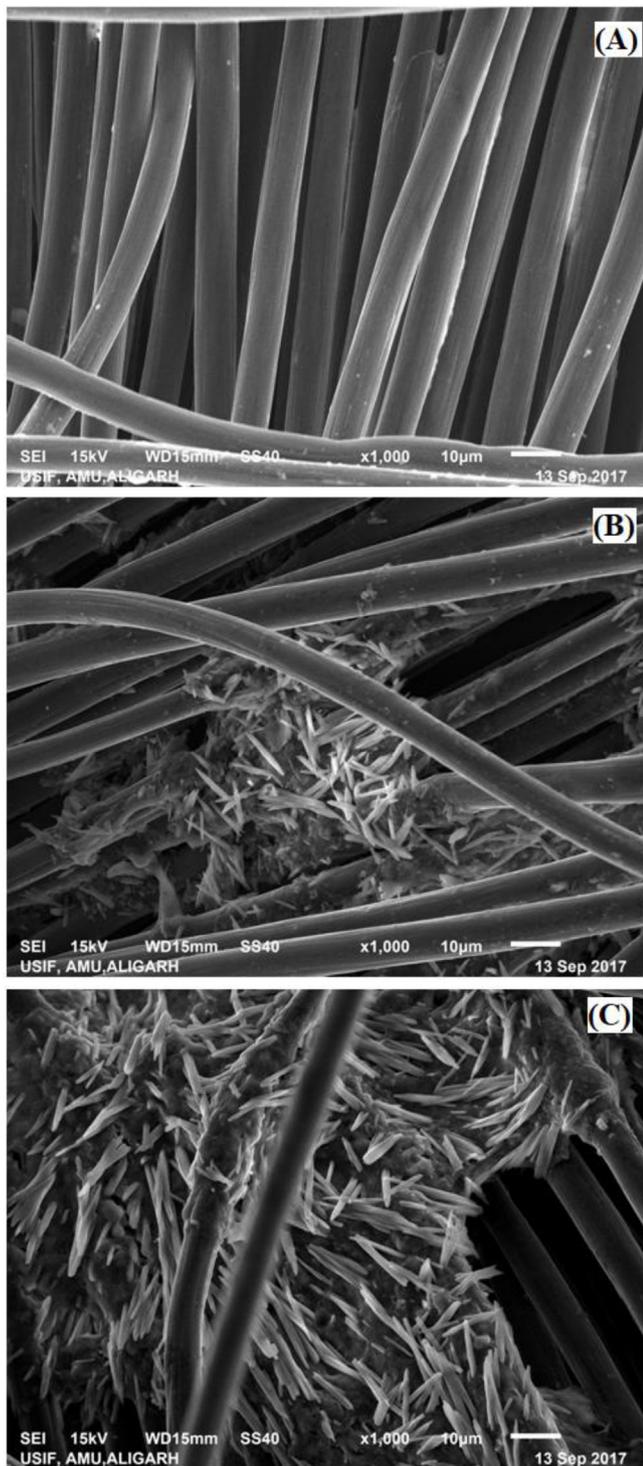


FIG. 4. SEM images (scale length 10 µm) for (A) plain carbon cloth, (B) anode of MFC-A and (C) anode of MFC-B, and EDX plot for anodes of MFC-A (D) and MFC-B (E).

MFC-B only availability of glucose in the anodic chamber assisted the growth of microbial community and thus improved the voltage output of the system. The microbes observed in the SEM images of the anodic biofilm developed on the surface of carbon cloth used in both MFCs were mostly spindle-shaped *fusobacterium*. Similar microbial community in MFC has also been reported by other research groups (36–38). The presence of exoelectrogens (*Geobacters*) was confirmed by qPCR.

The elemental composition of the sludge in the two reactors was studied using EDX. Fig. 4D and E shows the EDX plot of MFC-A and

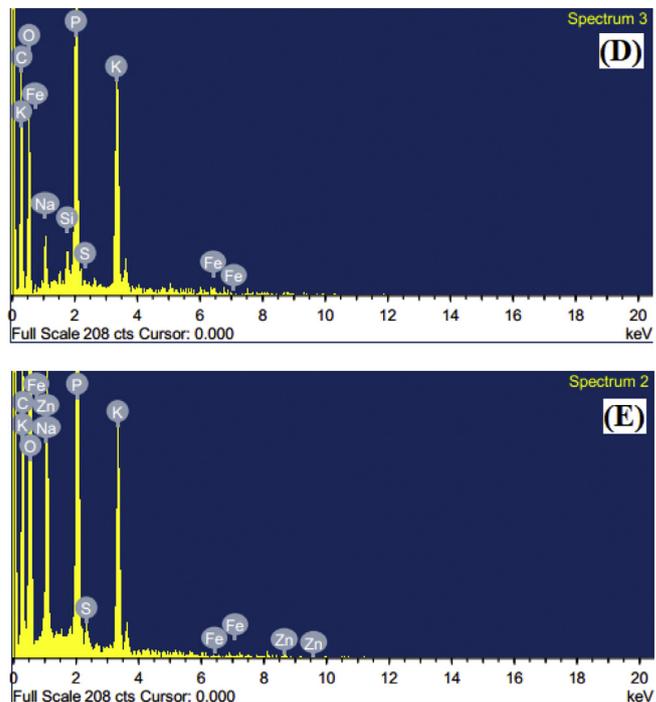


FIG. 4. (continued).

MFC-B, respectively. The elemental composition of sludge in MFC-A was C, O, Na, Si, P, S, K, Fe, Zn, while in case of MFC-B it was C, O, Na, P, S, K, Fe, Zn. The similarity in the sludge composition could be due to same anodic material and similar micronutrients added in both the setups.

Molecular biology of microbial communities The microbial communities present in the anodic chambers of both the setups were analysed and quantified using qPCR technique, a method of estimating real-time abundance of different microbial communities. The microbial communities targeted for the estimation of their absolute abundance in the systems were namely *Methanosarcina*, *Methanoseata*, *Methanococcus*, *Methanobacterium* and *Methanomicrobium* along with sulphate reducing bacteria and exoelectrogenic *Geobacter* as presented in Fig. 5. It was observed that the methanogenic population was relatively similar in both the reactors. However, sulphate reducing bacteria (potential

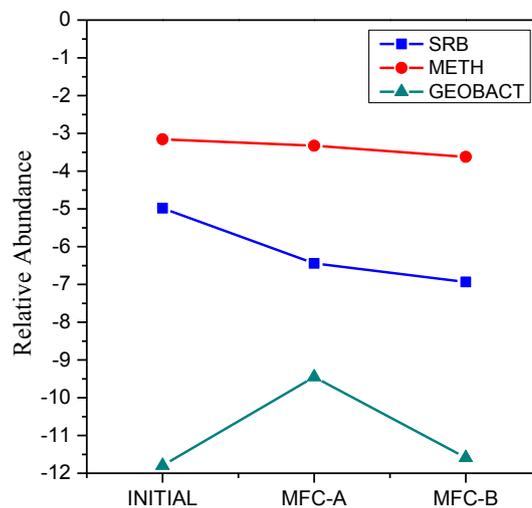


FIG. 5. Microbial community quantification for MFC-A and MFC-B using qPCR.

methanogens competitors) (39) were observed to be in higher abundance in MFC-B as compared to the MFC-A. Dominance of *Geobacter* population, bacteria primarily responsible for electricity generation in MFC was observed in both the setups. Fig. 5 also confirms that *Geobacter* population was more abundant in MFC-B than MFC-A resulting in the better performance of the former.

Electrochemical study of microbial fuel cell The electrochemical behaviour of the two identical MFCs treating TCP under different conditions was studied using cyclic voltammetry. The voltammograms were plotted at the scan rate of 10 mV/s using the three electrode system once the stable voltage output was observed for both the setups. The presence of redox loop for both the MFC systems confirmed the active microbial communities performing the electron transfer. Fig. 6A, B shows the voltammograms for MFC-A and MFC-B with respective controls. In both the cases, the voltammograms of samples were found similar to those of the control which could be the indication of similar microbial communities acting in respective controls and samples (40). However, shifting of the oxidation peak in the sample curve with respect to controls could be due to the environmental change on the addition of TCP. On the addition of sample (TCP) to the anodic chamber in MFC-A, the microbial activity could have been affected which shifted the curve to lower current as can be seen in the voltammogram. As for MFC-B since TCP has been added to different chamber, the electron producing microbes are not

affected considerably and the voltammogram peaks are observed to be more prominent. The almost similar cathodic backward scan in case of MFC-A indicates almost similar reduction reaction occurring in both the cases however a prominent difference in the voltammogram in case of MFC-B indicates the change in the reduction reaction occurring due to the change in the environment upon addition of TCP (41). The more distinguishable peaks in case of MFC-B as compared to MFC-A also indicates better performance of MFC-B.

Conclusion For the sustainable production of energy, wastewater is increasingly being considered as a potential resource. Environmental and economically feasible methods are needed for the removal of toxic chemicals from the wastewater and MFC can serve as one such technology in the coming future. TCP was treated in the DCMFC under different operational conditions. The results of the study predicted that MFC treating TCP under aerobic condition (MFC-B) performed better as compared to the treatment of TCP under anaerobic conditions (MFC-A). MFC-B showed higher COD and TCP removal efficiency of 89.64% and 79.49%, respectively, as opposed to 72.49% COD and 60.39% TCP removal efficiency in MFC-A. The PD was also better for MFC-B (1059.58 mW/m^2) as compared to MFC-A (446.76 mW/m^2). The results also depicted that the increase in TCP concentration had derogatory effect on the performance of both the setups. The results conclude that the toxic TCP can be better treated under aerobic conditions in MFC. However, the influent concentration must be monitored before treatment to obtain better performance.

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jbiosc.2018.08.012>.

ACKNOWLEDGMENTS

The authors are thankful to Aligarh Muslim University, Aligarh for providing necessary research facilities. Authors are also thankful to the Council of Science and Technology, Uttar Pradesh (CST, UP) for funding this work.

References

- Hameed, B. H., Tan, I. A. W., and Ahmad, A. L.: Adsorption isotherm, kinetic modeling and mechanism of 2,4,6-trichlorophenol on coconut husk-based activated carbon, *Chem. Eng. J.*, **144**, 235–244 (2008).
- Salmerón-Alcocer, A., Ruiz-Ordaz, N., Juárez-Ramírez, C., and Galíndez-Mayer, J.: Continuous biodegradation of single and mixed chlorophenols by a mixed microbial culture constituted by *Burkholderia* sp., *Microbacterium phyllosphaerae*, and *Candida tropicalis*, *Biochem. Eng. J.*, **37**, 201–211 (2007).
- Hameed, B. H.: Equilibrium and kinetics studies of 2,4,6-trichlorophenol adsorption onto activated clay, *Colloids Surf. A Physicochem. Eng. Asp.*, **307**, 45–52 (2007).
- Ji, H., Chang, F., Hu, X., Qin, W., and Shen, J.: Photocatalytic degradation of 2,4,6-Trichlorophenol over $g\text{-C}_3\text{N}_4$ under visible light irradiation, *Chem. Eng. J.*, **218**, 183–190 (2013).
- Yin, L., Shen, Z., Niu, J., Chen, J., and Duan, Y.: Degradation of pentachlorophenol and 2,4-dichlorophenol by sequential visible-light driven photocatalysis and laccase catalysis, *Environ. Sci. Technol.*, **44**, 9117–9122 (2010).
- Olaniran, A. O. and Igbinsola, E. O.: Chlorophenols and other related derivatives of environmental concern: properties, distribution and microbial degradation processes, *Chemosphere*, **83**, 1297–1306 (2011).
- Choi, J. H., Kim, Y. H., and Choi, S. J.: Reductive dechlorination and biodegradation of 2,4,6-trichlorophenol using sequential permeable reactive barriers: laboratory studies, *Chemosphere*, **67**, 1551–1557 (2007).
- Huang, L., Shi, Y., Wang, N., and Dong, Y.: Anaerobic/aerobic conditions and biostimulation for enhanced chlorophenols degradation in biocathode microbial fuel cells, *Biodegradation*, **25**, 615–632 (2014).
- Jesus, A. G.-D., Romano-Baez, F. J., Leyva-Amezcuca, L., Juárez-Ramírez, C., Ruiz-Ordaz, N., and Galíndez-Mayer, J.: Biodegradation of 2,4,6-trichlorophenol in a packed-bed biofilm reactor equipped with an internal net draft tube riser for aeration and liquid circulation, *J. Hazard. Mater. J.*, **161**, 1140–1149 (2009).

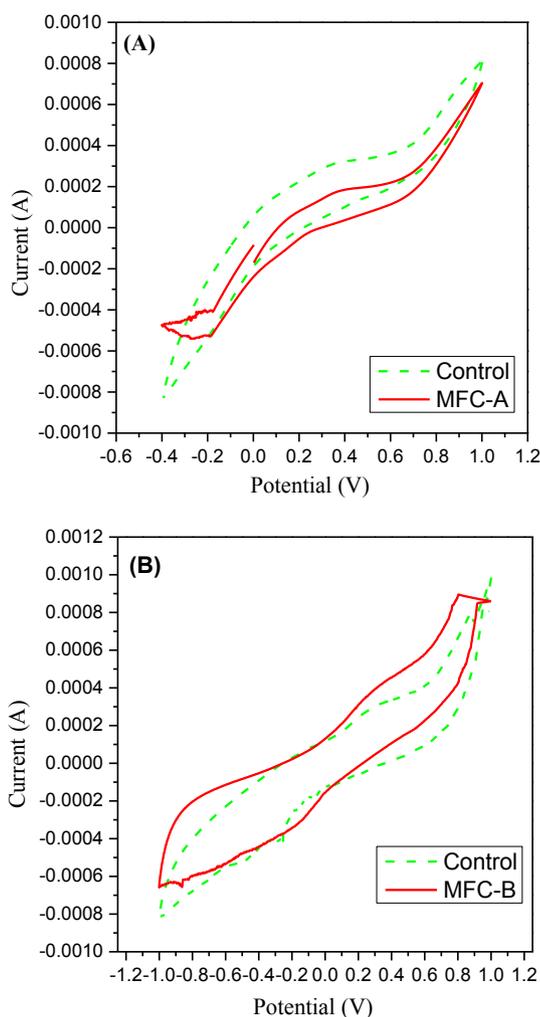


FIG. 6. Cyclic voltammograms for (A) MFC-A and (B) MFC-B during the study.

10. Aifei, Y., Yali, F., Zhuwei, D., and Haoran, L.: Mechanism of 2,4,6-trichlorophenol degradation in microbial fuel cells system with microbe isolated from submarine sediment, *Int. J. Electrochem. Sci.*, **10**, 1459–1468 (2015).
11. Eker, S. and Kargi, F.: COD, 2,4,6-trichlorophenol (TCP) and toxicity removal from synthetic wastewater in a rotating perforated-tubes biofilm reactor, *J. Hazard. Mater.*, **159**, 306–312 (2008).
12. Miao, M., Zhang, Y., Shu, L., Zhang, J., Kong, Q., and Li, N.: Development and characterization of the 2,4,6-trichlorophenol (TCP) aerobic degrading granules in sequencing batch airlift reactor, *Int. Biodeterior. Biodegradation*, **95**, 61–66 (2014).
13. Ruiz, C., Mena, E., Can, P., Villasen, J., and Rodrigo, M. A.: Removal of 2,4,6-trichlorophenol from spiked clay soils by electrokinetic soil flushing assisted with granular activated carbon permeable reactive barrier, *Ind. Eng. Chem. Res.*, **53**, 840–846 (2014).
14. Khan, M. Z., Mondal, P. K., Sabir, S., and Tare, V.: Degradation pathway, toxicity and kinetics of 2,4,6-trichlorophenol with different co-substrate by aerobic granules in SBR, *Bioresour. Technol.*, **102**, 7016–7021 (2011).
15. Collins, G., Foy, C., McHugh, S., and O'Flaherty, V.: Anaerobic treatment of 2,4,6-trichlorophenol in an expanded granular sludge bed-anaerobic filter (EGSB-AF) bioreactor at 15°C, *FEMS Microbiol Ecol.*, **53**, 167–178 (2005).
16. Gardin, H., Lebeault, J. M., and Pauss, A.: Degradation of 2,4,6-trichlorophenol (TCP) by co-immobilization of anaerobic and aerobic microbial communities in an upflow reactor under air-limited conditions, *Appl. Microbiol. Biotechnol.*, **56**, 524–530 (2001).
17. Zhao, J., Chen, X., Zhao, J., Lin, F., Bao, Z., He, Y., Wang, L., and Shi, Z.: Toxicity in different molecular-weight fractions of sludge treating synthetic wastewater containing 4-chlorophenol, *Int. Biodeterior. Biodegradation*, **104**, 251–257 (2015).
18. Huang, L., Chai, X., Quan, X., Logan, B. E., and Chen, G.: Reductive dechlorination and mineralization of pentachlorophenol in biocathode microbial fuel cells, *Bioresour. Technol.*, **111**, 167–174 (2012).
19. Liu, S. H., Wu, C. H., and Lin, C. W.: Enhancement of bioelectricity generation for an air-cathode microbial fuel cell using polyvinyl alcohol-membrane electrode assemblies, *Biochem. Eng. J.*, **128**, 210–217 (2017).
20. Logan, B. E., Hamelers, B., Rozendal, R., Schroder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., and Rabaey, K.: Microbial fuel cells: methodology and technology, *Environ. Sci. Technol.*, **40**, 5181–5192 (2006).
21. Khan, M. D., Khan, N., Sultana, S., Joshi, R., Ahmed, S., Yu, E., Scott, K., Ahmad, A., and Khan, M. Z.: Bioelectrochemical conversion of waste to energy using microbial fuel cell technology, *Process Biochem.*, **57**, 141–158 (2017).
22. Khan, M. D., Abdulateif, H., Ismail, I. M., Sabir, S., and Khan, M. Z.: Bioelectricity generation and bioremediation of an azo-dye in a microbial fuel cell coupled activated sludge process, *PLoS One*, **10**, e0138448 (2015).
23. Luo, H., Liua, G., Zhang, R., and Jin, S.: Phenol degradation in microbial fuel cells, *Chem. Eng. J.*, **147**, 259–264 (2009).
24. Cai, H., Wang, J., Bu, Y., and Zhong, Q.: Treatment of carbon cloth anodes for improving power generation in a dual-chamber microbial fuel cell, *J. Chem. Technol. Biotechnol.*, **88**, 623–628 (2013).
25. Liu, H. and Logan, B. E.: Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane, *Environ. Sci. Technol.*, **38**, 4040–4046 (2004).
26. Damiano, L., Jambeck, J. R., and Ringelberg, D. B.: Municipal solid waste landfill leachate treatment and electricity production using microbial fuel cells, *Appl. Biochem. Biotechnol.*, **173**, 472–485 (2014).
27. Khan, M. Z., Singh, S., Sreekrishnan, T. R., and Ahammad, S. Z.: Feasibility study on anaerobic biodegradation of azo dye reactive orange 16, *RSC Adv.*, **4**, 46851–46859 (2014).
28. **American Society for Testing and Materials**: Standard test methods for chemical oxygen demand (dichromate oxygen demand) of water, D1252-95. ASTM Annual Book of Standards, American Society for Testing and Materials, Philadelphia, PA (1995).
29. **Bureau of Indian Standards**: Indian standard method for sampling and test (physical and chemical) for water and wastewater, IS-3025, part 32, first revision, New Delhi, India (1988).
30. Sultana, S., Khan, M. D., Sabir, S., Gani, K. M., Oves, M., and Khan, M. Z.: Bio-electro degradation of azo-dye in a combined anaerobic-aerobic process along with energy recovery, *New J. Chem.*, **39**, 9461–9470 (2015).
31. Khan, M. Z., Singh, S., Sultana, S., Sreekrishnan, T. R., and Ahammad, S. Z.: Studies on the biodegradation of two different azo dyes in bioelectrochemical systems, *New J. Chem.*, **39**, 5597–5604 (2015).
32. Khan, M. D., Khan, N., Nizami, A.-S., Rehan, M., Sabir, S., and Khan, M. Z.: Effect of co-substrates on biogas production and anaerobic decomposition of pentachlorophenol, *Bioresour. Technol.*, **238**, 492–501 (2017).
33. Rikame, S. S., Mungray, A. A., and Mungray, A. K.: Electricity generation from acidogenic food waste leachate using dual chamber mediator less microbial fuel cell, *Int. Biodeterior. Biodegrad.*, **75**, 131–137 (2012).
34. Cheng, S. and Logan, B. E.: Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells, *Electrochem. Commun.*, **9**, 492–496 (2007).
35. Feng, Y., Yang, Q., Wang, X., and Logan, B. E.: Treatment of carbon fiber brush anodes for improving power generation in air-cathode microbial fuel cells, *J. Power Sources*, **195**, 1841–1844 (2010).
36. Reimers, C. E., Stecher, H. A., III, Westall, J. C., Alleau, Y., Howell, K. A., Soule, L., White, H. K., and Girguis, P. R.: Substrate degradation kinetics, microbial diversity, and current efficiency of microbial fuel cells supplied with marine plankton, *Appl. Environ. Microbiol.*, **73**, 7029–7040 (2007).
37. Jia, J., Tang, Y., Liu, B., Wu, D., Ren, N., and Xing, D.: Electricity generation from food wastes and microbial community structure in microbial fuel cells, *Bioresour. Technol.*, **144**, 94–99 (2013).
38. Rismani-Yazdi, H., Carver, S. M., Christy, A. D., Yu, Z., Bibby, K., Peccia, J., and Tuovinen, O. H.: Suppression of methanogenesis in cellulose-fed microbial fuel cells in relation to performance, metabolite formation, and microbial population, *Bioresour. Technol.*, **129**, 281–288 (2013).
39. Raskin, L., Rittmann, B. E., and Stahl, D. A.: Competition and coexistence of sulfate-reducing and methanogenic populations in anaerobic biofilms, *Appl. Environ. Microbiol.*, **10**, 3847–3857 (1996).
40. Yuan, Y., Zhou, S., Xu, N., and Zhuang, L.: Electrochemical characterization of anodic biofilms enriched with glucose and acetate in single-chamber microbial fuel cells, *Colloids Surf. B Biointerfaces*, **82**, 641–646 (2011).
41. Elmekawy, A., Srikanth, S., Vanbroekhoven, K., Wever, H. D., and Pant, D.: Bioelectro-catalytic valorization of dark fermentation effluents by acetate oxidizing bacteria in bioelectrochemical system (BES), *J. Power Sources*, **262**, 183–191 (2014).