Effect of Cathode Biofilm and Non-feeding condition on the Performance of Membrane-less Microbial Fuel Cell Operated under Different Organic Loading Rates

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***Abstract*- The novelty of this study is evaluating the performance of membrane-less microbial fuel cell (ML-MFC) after non-feeding conditions for a few days. Results showed that there was no effect of non-feeding condition up to two weeks on organic matter removal as well as electricity production upon restart. Moreover, effect of formation of cathode biofilm at organic loading rate (OLR) ranging from 0.33 to 1.25 kg COD/m^3 d on performance of ML-MFC was evaluated. Although biocathodes are reported to improve performance of the MFC, formation of thicker biofilm on the cathode was observed to reduce power output. However, it contributed to increasing organic matter removal. At OLR of 1.25 kg COD/m^3 d the COD removal efficiency was greater than 92%. Maximum TKN and NH_4^+-N removal of 96.89% and 97.38%, respectively, was observed at this loading. The maximum power density and current density of 3.76 mW/m^2 and 14.79 mA/m^2, respectively, was observed at OLR of 1.25 kg COD/m^3 d, demonstrating the utility of ML-MFC for wastewater treatment and simultaneous electricity harvesting.**

***Keywords*- MFC; Wastewater Treatment; Electricity Generation; Biofilm; Shutdown**

I. INTRODUCTION

Biological wastewater treatment, such as activated sludge process, trickling filters and lagoons are implemented for the treatment of organic wastewaters to reduce organic load on the receiving water bodies. However, the aerobic processes (conventional activated sludge process and lagoons) require high oxygen in order to maintain and grow microorganisms for degrading the organic matter. The energy required for aeration is approximately 60% of the energy used at wastewater treatment facilities [1]. In addition, the excess sludge generated from these processes requires further treatment, demanding additional infrastructure and energy for operating sludge treatment unit. In the United States, it has been estimated that the activated sludge process owned by public treatment works requires 0.349 kWh/m^3 of wastewater treated [2]. Moreover, estimated electricity consumption for the movement and treatment of water and wastewater is around 3% to 4% of the total energy consumption [3]. Therefore, wastewater treatment can be made sustainable if energy and other resources are recovered during treatment. Hence, there is a need for paradigm shift in the concept of wastewater treatment; i.e., instead of treatment it can be considered as recovery process of valuable resources.

In recent years, microbial fuel cells (MFCs) have gained popularity due to their ability to simultaneously treat wastewater and generate renewable electricity. MFC is a bioreactor that converts biochemical energy to electrical energy during substrate oxidation using bacteria as a catalyst [4-7]. This concept has been significantly developed for application as a renewable electricity generation [5], however, full-scale implementation of MFCs in wastewater treatment facilities is constrained due to different factors affecting the net power yield of this technology [5]. The various parameters that affect the yield of electricity are biofilm growth on the anode and the cathode, organic loading rate (OLR), spacing between the electrodes, electrode materials, internal and external resistance of the MFC, specific surface area of the electrodes required with respect to reactor volume, and area of proton exchange membrane (PEM), proton permeability, etc. [4-8].

Membranes are used in MFC to separate anodic and cathodic chambers. However, the commercially available polymeric cation exchange membranes are extremely costly and increase production cost of this device. Hence, scientists have worked on development of membrane-less microbial fuel cell (ML-MFC), to reduce it production cost [9]. In this study following objectives are investigated (i) the effect of organic loading rate ranging from 0.33 to 1.25 kg of chemical oxygen demand (COD)/m^3 d on the performance of a lab-scale ML-MFC, (ii) The effect of biofilm growth on cathode on COD removal efficiency and energy generation, (iii) The effect of non-feeding of substrate on the performance of lab-scale ML-MFC after restart under different organic loading rates.

II. EXPERINMNETAL

A. Start-up of MFC

The schematic diagram and the lab scale ML-MFC used in this study are shown in Fig. (1). The anode chamber was at the bottom, and the cathode chamber was at the top of cylinder-shaped reactor, made of polyacrylic plastic having internal diameter of 15 cm. Glass wool (4 cm depth) and glass bead (4 cm depth) were placed in between anode chamber and cathode chamber to separate them. Three graphite rods (GF series, GEE Graphite Limited, Dewsbury, West Yorkshire, UK), were used as the anode and the cathode electrodes. The total height of the reactor was 60 cm and the distance between the respective anode and cathode electrodes was 20 cm, 24 cm, and 28 cm. The surface area of each graphite electrodes was 70.21 cm^2. The total working volume of this ML-MFC was 10.4 L. ML-MFC was aerated at a rate of 60 mL/min in the cathode chamber so that protons (H^+) coming along the influent were getting reacted with oxygen at the surface of cathode electrode, where the electrons, protons and oxygen combined to form water.

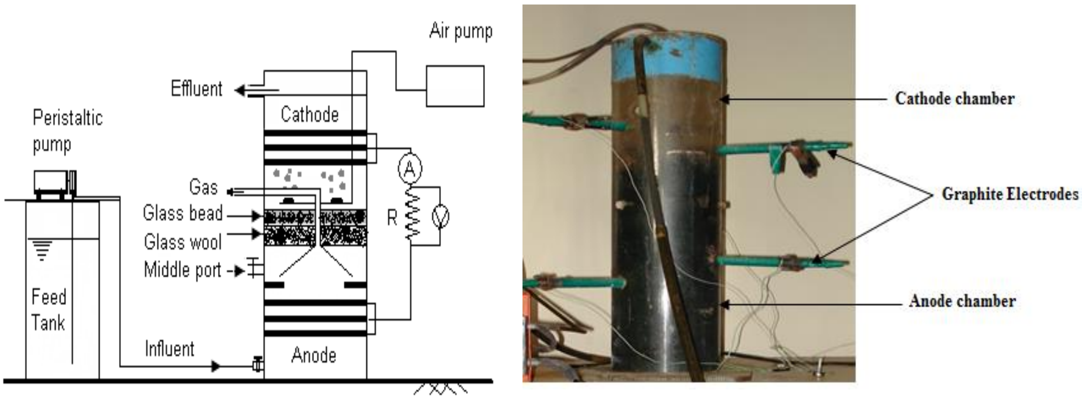


Fig. 1 (a) Schematic and (b) photograph of membrane-less microbial fuel cell (ML-MFC) used in the study

The anode chamber was inoculated with anaerobic sludge (1L) collected from a septic tank to enrich electrochemically active microbes. The sludge having solids concentration of 72.25 g SS/L and 18.2 g VSS/L was preheated at 105°C for 15 min to suppress methanogens. This method was found effective to obtain an enriched culture of electrogens [9]. With continuous flow, the cathode chamber was filled with treated effluent from the anode chamber. Cathode chamber was aerated externally to neutralise H^+ ions (to form H_2O) coming from the anode chamber. External inoculation was not done in the cathode chamber.

B. Operating Conditions of ML-MFC

The operating conditions used during this study are given in the Table 1. The ML-MFC was operated under different OLRs with respect to volume of anode chamber. Synthetic wastewater containing sucrose as carbon source, having average COD in the range of 267-1252 mg/L was used as organic carbon with composition as reported by Ghangrekar and Shinde [9]. The influent pH was maintained in the range of 7.2 to 7.9 and the ML-MFC was operated at ambient temperature in the range of 18-30 °C. Effect of non-feeding of substrate was evaluated by not feeding the reactor starting from 87th to 91th, 180th to 193rd and 217th to 224th days, and the performance of the reactor was evaluated after restart under same organic loading rate. Biofilm growth on the cathode was observed, and its effect on performance of ML-MFC was studied.

TABLE 1 OPERATING CONDITIONS OF ML-MFC

Exp. No.	Days	Flow rate (L/d)	COD inlet (mg/L)	OLR (Kg COD/ m^3.d)	HRT	Inlet Alkalinity (mg/L)	Inlet TDS (mg/L)
1	00-25	6.00	266 (±30)	0.33	19.60	320 (±36)	537 (±33)
2	26-54	8.99	305 (±24)	0.56	13.08	366 (±15)	538 (±13)
3	55-86	8.99	438 (±35)	0.80	13.08	507 (±23)	752 (±25)
4	92-138	8.99	441 (±29)	0.81	13.08	315 (±39)	490 (±55)
5	139-228	6.74	531 (±44)	0.73	17.45	543 (±30)	881 (±71)
6	229-268	5.44	866 (±71)	0.96	21.60	720 (±65)	1268 (±48)
7	269-291	4.90	1252 (±306)	1.25	24.00	1043 (±165)	1669 (±298)

(±) Standard deviations

C. Analysis

Analysis of the parameters such as COD, alkalinity, total dissolved solids (TDS), Biochemical Oxygen Demand (BOD), total Kjeldahl nitrogen (TKN), ammonical nitrogen, nitrate nitrogen, suspended solids (SS) and volatile suspended solids (VSS) was done as per the Standard Methods [10]. The potential and current were measured daily using a digital multi meter (RISH Multi 15S, India) and converted to power according to P = I*V, where, P = power (W), I = current (A), and V = voltage (V).

III. RESULTS

Performance of ML-MFC was evaluated for contribution of organic matter removal in the anode chamber and overall voltage and current produced. With active aeration, residual organic matter entering the cathode chamber from the anode chamber is utilized by the biofilm eventually grown on the cathode electrodes and on the wall of cathode compartment with time of operation. The excessive biofilm growth was observed in the cathode chamber after three weeks of operation, leading to substantial drop in working voltage from 0.2 V to 0.13 V. Similarly, biofilm growth was observed under different OLRs and an attempt was made to evaluate its effect on overall reactor performance based on performance observations only.

A. Performance of ML-MFC at Different OLRs and Effect of Cathodic Biofilm

The ML-MFC was operated under different OLRs with respect to volume of anode chamber, and different influent COD concentrations as shown in the Table 1. The efficiency of the substrate removal in the anode and cathode chamber is given in the Table 2. The overall efficiency of the substrate removal in the ML-MFC was observed to be on the higher side (>90%) of the reported values in literature [9, 11]. Active aerobic conditions were maintained in cathode chamber by aerating at the rate of 60 mL/min. Due to aerobic conditions in cathode chamber biofilm growth was observed which substantially removed the COD leaving the anode chamber. Initially at OLR of 0.326 and 0.56 kg COD/m^3 day and hydraulic retention time (HRT) of 19.6 and 13.08 h, respectively, the COD removal efficiency in the cathode chamber was more than anode chamber. This is due to effective aerobic biofilm growth in the cathode chamber. The growth of biofilm started after a week of operation, and it was quite visible after 22 days of continuous operation. On 31 day of operation, the cathode chamber was drained from the middle port to remove the biomass accumulated in the cathode chamber and the biofilm was removed by physical cleaning the electrodes in the cathode chamber. With the removal of biofilm in the cathode chamber the removal efficiency of substrate was dropped down (COD outlet before biofilm removal = 11 mg/L and after removal the COD outlet = 41 mg/L). Similar biofilm growth was observed in the cathode chamber under other OLRs but the removal efficiency of the anode chamber increased with increase in the biomass and granulation of the sludge in the anode chamber. The COD removal efficiency of anode and cathode chamber under different OLRs of ML-MFC is presented in Fig. 2.

When the organic loading rate was 0.73 kg COD/m^{3}d, maximum average COD removal efficiency of 97.27% was observed with influent COD concentration around 531 mg/L. At a higher COD concentration, higher COD removal efficiency was observed. This higher COD removal efficiency was due to increased biofilm formation in cathode chamber. When COD was increased from 305 to 438 mg/L, it was observed that in anode chamber COD removal was 62.4% and in the cathode chamber it was 32.16%. Higher organic matter removal in cathode chamber showed higher growth of aerobic biofilm in cathode chamber. Increase in OLR increased the COD removal efficiency of the anode chamber. The effluent BOD under all the loading rates was less than 20 mg/L (Table 2). Substrate removal was on the higher side but, further pilot scale studies, mass-energy balance, and cost estimation are required for ML-MFC technique to consider as a possible treatment technology for wastewater treatment plants.

TABLE 2 PERFORMANCE OF THE ML-MFC FOR COD AND BOD REMOVAL

Days	COD			Total COD removal in reactor (%)	COD removal in cathode (%)	BOD (mg/L)		BOD removal efficiency (%)
	Inlet (mg/L)	Anode effluent (mg/L)	COD removal in anode chamber (%)			Inlet	Outlet	
0-25	267 (±23)	151(±22)	43.40	92.33	48.93	202(±22)	17(±2)	91.36
26-54	305(±24)	197(±18)	35.59	94.56	58.97	162(±8)	13(±12)	91.74
55-86	438 (±35)	134(±11)	69.49	95.03	25.54	216(±36)	11(±9)	94.94
92-138*	441(±29)	129(±34)	70.84	94.38	23.54	212(±8)	4.5(±2)	97.87
139-228	531(±45)	166(±42)	68.71	97.27	28.56	222(±8.2)	4(±2)	98.3
229-262	866(±78)	147(±34)	83.06	95.84	12.78	322(±25)	5(±1)	98.57
263-291	1251(±105)	136(±27)	89.1	96.08	6.98	552(±33.9)	5(±3)	98.97

* The alkalinity was reduced (Table 1); External resistance of 800 -1200 Ω was used in the experiments.
(±) standard deviation. Half of the sludge was withdrawn on the 100th day

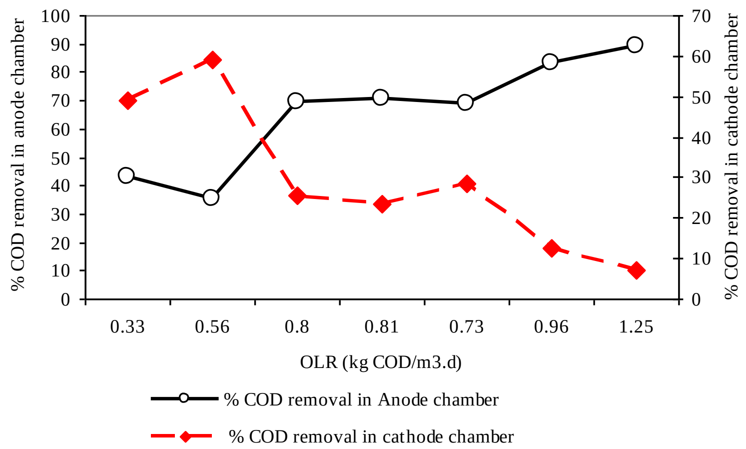


Fig. 2 Performance of anode and cathode compartment at different OLRs

B. Nitrogen Removal in ML-MFC and Effect of Nitrogen

Nitrogen was supplemented in the form of ammonium nitrogen to fulfill the nutrient requirement in the synthetic wastewater. During performance evaluation of the MFCs it was observed that they are capable of removing nitrogen from the wastewater. Different forms of nitrogen maintained in influent and observed in the effluent are presented in Table 3. The maximum TKN and ammonium nitrogen removal in ML-MFC was 96.89%, 97.38%, respectively. Oxidation of nitrogen in anode chamber suggests that nitrification was occurring, likely as a result of oxygen diffusion through the cathode. Oxygen diffusion through the cathode is known to result in a loss of carbonaceous substrates for aerobic degradation, as evidenced by low Coulombic efficiency in MFCs [12]. In the ML-MFC there was no significant effect of alkalinity and TDS on TKN and ammonium nitrogen removal. Major TKN reduction is observed in the cathode chamber. This could be attributed to the ammonia stripping in the cathode chamber due to aeration or simultaneous nitrification and denitrification occurring in the cathode chamber. The nitrate nitrogen concentration in the effluent of ML-MFC was less than 10 mg/L, due to nitrification and denitrification occurring in the cathode chamber. The nitrate nitrogen concentration in the effluent was well within the acceptable limit for discharge of effluent. During treatment of swine wastewater, Min et al. [13] reported that MFC can remove ammonium nitrogen up to 83% after 100 h of operation with increase in nitrite and nitrate concentrations from 0.4 to 2.9 mg NO^-_2-N/L and 3.8 to 7.5 mg NO^-_3-N/L, respectively. Whereas, in this study higher nitrogen removal is observed under higher applied OLRs and the nitrate concentration in the effluent was lower.

TABLE 3 DIFFERENT FORM OF NITROGEN OBSERVED IN ML-MFC

	Exp. No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	OLR (Kg COD/m^3.d)	**0.33**	**0.56**	**0.80**	**0.81***	**0.73****	**0.96**	**1.25**
TKN (mg/L)	Inlet	25.68 (±0.28)	25.39 (±0.36)	36.66 (±1.80)	19.41 (±04.53)	43.41 (±2.59)	58.49 (±2.98)	106.4 (±1.58)
	Anode outlet	22.4 (±0.28)	22.28 (±3.40)	29.48 (±19.27)	22.4 (±5.60)	38.42 (±0.98)	37.11 (±14.27)	62.72 (±9.50)
	Cathode outlet	8.68 (±0.19)	15.68 (±0.04)	6.71 (±4.49)	5.49 (±1.90)	5.41 (±0.64)	5.32 (±1.91)	3.34 (±1.61)
	Efficiency (%)	65.28	38.23	81.66	71.76	87.51	90.9	96.8
Ammonical Nitrogen (mg/L)	Inlet	20.34 (±0.23)	20.18 (±0.11)	31.93 (±1.35)	17.67 (±3.44)	38.35 (±2.87)	55.23 (±2.34)	81.55 (±1.24)
	Anode outlet	17.12 (±0.79)	16.4 (±3.59)	28.06 (±1.05)	9.24 (±1.08)	28.36 (±4.45)	46.48 (±1.63)	63.24 (±1.89)
	Cathode outlet	6.54 (±1.29)	6.82 (±0.08)	4.39 (±1.67)	1.48 (±00.13)	1.36 (±0.52)	2.05 (±0.098)	2.18 (±0.38)
	Efficiency (%)	67.84	68.38	86.25	91.62	96.45	96.28	97.38
Nitrate Nitrogen (mg/L)	Cathode outlet	ND	2.42 (±0.14)	2.35 (±0.12)	1.23 (±0.11)	2.24 (±0.11)	1.62 (±0.23)	2.94 (±0.38)

* The alkalinity is reduced to half, ** Increasing hydraulic retention time (±) standard deviation

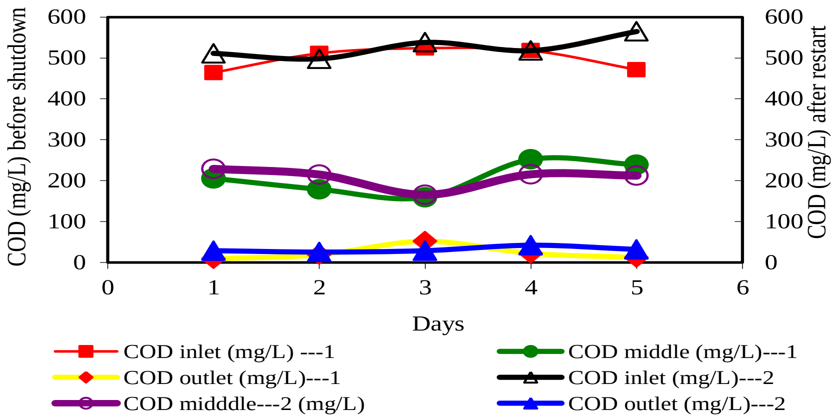
C. Effect of Non-feeding on the Performance of ML-MFC After Restart

The wastewater feeding was stopped for 5 days (87-91) to see the performance of the ML-MFCs during restart after shutdown. Similarly the feeding was stopped for 180-193 days and 217-224 days to see the ML-MFC performance after shutdown period. Due to shut down there was no change in COD removal efficiency after the restart. Potential drop was observed on the 3rd day after restart (0.207 V to 0.181 V), but it picked up on the next day (for all non-feeding condition). After different shutdown periods the COD removal efficiency observed is shown in Table 4. The performance of the reactor before and after shutdown is shown in the Fig. 3. It was observed that there was no effect on COD removal due to the short duration of the non-feed condition because of shutdown and the trend of COD for anode effluent (middle port samples) and outlet before non-feeding and after a restart of ML-MFC were similar.

TABLE 4 PERFORMANCE OF ML-MFC DURING RESTART AFTER SHUTDOWN PERIOD

Shut down period, days	5-days before shutdown			5-days after restart		
	COD inlet	**COD outlet**	**% removal**	**COD inlet**	**COD outlet**	**% removal**
87-91	438 (±35)	29.56(±10)	93,25	441 (±29)	34(±8)	92.29
180-193	498 (±28)	23 (±22)	95.38	526 (±26)	32 (±6)	93.99
217-224	531 (±13)	30 (±26)	94.41	531 (±24)	33 (±9)	93.78

(±) standard deviation



COD inlet---1- before shut down; COD inlet ---2 - after restart

Fig. 3 Observations of COD before and after shutdown period of 180th -193rd day in ML-MFC

Observations were made to find out the effect of the shutdown period on the electricity generation by stopping the feed to the ML-MFC for few days. The feeding was stopped for 180-193 days and 217 - 224 days. It was observed that even with no feed for more than 13 days, there was potential between anode and cathode. This potential difference was mainly due to endogenous decay of microorganisms. Without feeding on the 13th day the potential of 0.397 V (open circuit) and a current of 0.41 mA were observed in ML-MFC. Table 5 presents the observations made before shutdown and after a restart of the ML-MFC. Further, after restarting on 224th day observations were made just after feeding at hourly interval to see the performance of a reactor. Before feeding the open circuit voltage between the anode and cathode of ML-MFC was 0.621 V, and soon after feeding the voltage was increased to 0.705 V.

TABLE 5 CURRENT AND VOLTAGE BEFORE AND AFTER SHUTDOWN PERIOD IN ML-MFC

Days	**Open circuit voltage**	**Voltage (V)**	**Current (mA)**
5 days before shut down (180-192)	0.712	0.208	0.260
	0.739	0.210	0.263
	0.674	0.168	0.210
	0.668	0.183	0.229
	0.69	0.166	0.208
5 days after restart (194-198)	**0.397***	**0.012***	**0.015***
	0.684	0.135	0.169
	0.796	0.21	0.263
	0.801	0.209	0.261
	0.76	0.207	0.259
	0.749	0.181	0.226

* The values are for 193rd day before addition of fresh feed after 13 days of shutdown.

Due to the shutdown of the reactor, there was no time loss for the reactor to restore normal performance. As no adverse effect on the performance of the ML-MFC was noticed during restart after shut down, therefore MFCs can withstand if any sudden breakdown occurs in the treatment plant or in the industry hence not producing any wastewater. To the best of our knowledge this effect has not been studied so far for the MFC. This capability of MFC to maintain its performance even for the non-feed condition of short duration is very important for practical application of this technology as a wastewater treatment process.

D. The Effect of Cathode Bioflim Growth on Electricity Production in ML-MFC

ML-MFC took one week to get acclimatized with necessary microbial culture development to produce steady current. A continuous external load of 800 Ohms was used in the experiments 1 to 7. The electricity generated in ML-MFC at different organic loading rates is presented in Table 6. It was observed that at higher OLR and higher HRT the current production increased. In experiment no 5, with increasing HRT to 17.45 h under similar OLR, as compared to earlier experiment no. 4, the current density increased from 9.31 mA/m^2 to 11.97 mA/m^2 and power density increased from 2.3 mW/m^2 to 3.50 mW/m^2. Thus, indicating that, higher HRT is favourable for obtaining higher power density. It was reported that the power density increased from 790 mW/m^2 at HRT = 4.2 h to 1320 mW/m^2 at HRT of 15.6 h [14].

TABLE 6 ELECTRICITY GENERATION OBSERVED IN THE ML-MFC UNDER DIFFERENT OLR

Exp. No.	**Days**	**OLR (kgCOD/ m^3.d)**	**Resistance (Ω)**	**OCV (V)**	**Voltage (V)**	**Current (mA)**	**Power density (mW/m^2)**	**Current density (mA/m^2)**
1	0-25	0.33	800	0.382	0.115 (±0.05)	0.144 (±0.03)	0.87 (±0.59)	6.84 (±0.08)
2	26-54	0.56	800	0.387	0.112 (±0.04)	0.140 (±0.02)	0.66 (±0.29)	6.65 (±0.04)
3	55-86	0.80	800	0.581	0.110 (±0.09)	0.138 (±0.01)	0.73 (±1.04)	6.55 (±0.42)
4	92-138*	0.81	800	0.687	0.157 (±0.05)	0.196 (±0.02)	1.46 (±0.63)	9.31 (±0.03)
5	139-228**	0.73	800	0.749	0.202 (±0.04)	0.252 (±0.03)	2.31 (±0.78)	11.97 (±2.01)
6	229-262	0.96	800	0.751	0.209 (±0.04)	0.261 (±0.02)	3.50 (±1.19)	12.39 (±0.06)
7	263-291	1.25	800	0.729	0.248 (±0.05)	0.310 (±0.01)	3.76 (±1.27)	14.72 (±0.12)

OLR- with respect to anode compartment *- the alkalinity is reduced to half, ** Increasing hydraulic retention time; OCV – open circuit voltage

Due to low COD removal in the anode chamber during initial days, substantial COD fraction from the influent was entering into the cathode chamber. Because of continuous aeration in the cathode chamber, built-up of aerobic microorganisms was observed. After a few days from start-up, when sufficient microbial growth occurred in cathode chamber, considerable COD reduction was observed in the cathode chamber. Therefore, the effect of biofilm in the electricity production is studied in the ML-MFC at different OLRs. Substantial drop in voltage was observed on the 9th day of continuous operation, from 0.18 V (8thday) to 0.1 V (9thday) and current dropping from 0.33 mA to 0.18 mA. After removing the biofilm from the cathode chamber the voltage and current increased gradually. Similarly, the current was dropped from 0.78 mA to 0.42 mA in experiment no. 3, and in experiment no. 7 the potential was dropped from 0.80 V to 0.63 V. Thus excessive biofilm in cathode chamber is a limiting parameter for electricity production in ML-MFC. Biofilm growth is beneficial for removal of COD, but further investigations are needed to find out the optimum growth of biofilm that can give maximum current and removal efficiency. Quantification of biofilm and identification of microbial community of the biofilm is required to estimate the efficiency of COD and nitrogen removal.

IV. CONCLUSION

Within the OLR range tested in these experiments (0.33 to 1.25 kg COD/m^3.d) the higher OLRs favour higher COD removal efficiency. Even the COD removal efficiency of anode chamber was also increased with increase in OLR (43% to 89%). This performance shows the ability of ML-MFCs for effective wastewater treatment. Removal of nitrogen was also observed along with COD removal. The maximum TKN and NH_4^+-N removal in ML-MFC was 96.89% and 97.38%, respectively. High growth of biofilm in the cathode chamber decreases the production of current, and hence, after certain interval removal of biofilm is required from the cathode chamber to restore power production. There was no adverse effect on the ML-MFC performance upon restart after a shutdown period of two weeks, demonstrating ability of ML-MFC to give a steady performance without any time lapse, if any shutdown occurs in the treatment plant.

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