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Short Communication

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Performance assessment of aeration and *radial oxygen* loss assisted cathodes based integrated constructed wetland-microbial fuel cell systems

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Abstract

The present study explores low-cost cathode development possibility using radial oxygen loss (ROL) of *Canna indica* plants and intermittent aeration for wastewater treatment and electricity generation in constructed wetland-microbial fuel cell (CW-MFC) system. Two CW-MFC microcosms were developed. Amongst them, one microcosm was planted with *Canna indica* plants for evaluating the ROL dependent cathode reaction (CW-MFC dependent on ROL) and another microcosm was equipped with intermittent aeration for evaluating the intermittent aeration dependent cathode reaction (CW-MFC with additional IA). The CW-MFC with additional IA has achieved 78.71 % and 53.23%, and CW-MFC dependent on ROL has achieved 72.17 % and 46.77 % COD removal from synthetic wastewater containing glucose loads of 0.7 g $L^{-1}$and 2.0 gL^{-1}, respectively. The maximum power density of 31.04 mW $m^{-3}$and 19.60 mW m^{-3} was achieved in CW-MFC with additional IA and CW-MFC dependent on ROL, respectively.

Keywords: Radial oxygen loss, Constructed wetland-microbial fuel cell, Intermittent aeration, cathode reaction, Microbial fuel cell

1. Introduction

Integrated constructed wetland-microbial fuel cell (CW-MFC) system is emerging as a new hybrid technology for bioelectricity generation from wastewater treatment (Yadav 2010). This is essentially a merger of best features of two conventional technologies namely constructed wetlands (CWs) and microbial fuel cell (MFC). A characteristic MFC consists of an aerobic cathodic zone and an anaerobic anodic zone parted by a proton exchange membrane and connected with an external electric circuit consists of an external resistance. In MFC, during wastewater treatment electricity is also generated as a byproduct. Most of the studies on MFCs so far have been limited to lab scale only (Santoro et al., 2017). CWs run with relative low external energy requirements and are easy to operate and maintain for wastewater treatment. The upper section of the CW maintains relatively aerobic zone because of its close contact with the atmosphere. However, CWs also experience slow rate of organic matter decomposition which is due to the existence of the anaerobic conditions in the lower sections (Tanner et al., 1998) resulting in scant availability of favorable electron acceptors. The anode in the MFC technology works as a temporary electron acceptor and thus, can help in enhancement of the anaerobic treatment rate of CWs. The CW and the MFC have similar aerobic and anaerobic zones which promotes the integration of these two technologies to make an integrated CW-MFC technology. For the advancement of the CW-MFC technology for bioelectricity generation and efficient wastewater treatment, a low-cost, efficient cathode development is highly required. It is well established that wetland plants release oxygen through their root systems (root zone) and influences biogeochemical cycles in the sediments through the effects of redox status of the sediments (Brix, 1997). In the same context, there is a possibility of developing a cathode assisted with oxygen release from root system or radial oxygen loss and using them for cathodes reaction in CW–MFCs. In the CW–MFCs, radial oxygen based cathode development is not yet studied in details. Oon et al., (2017) has done primary work on role of submerged plant (Elodea nuttallii) on cathode development of CW-MFC. The said plant cannot be used for most popular type of subsurface flow CWs. As far authors' knowledge, this is the first work which focuses on a low-cost cathode development for vertical subsurface flow CW-MFC technology. In this study, ROL dependent and intermitted aeration dependent cathodes were established for the first time in CW-MFCs and tested for power generations in CW-MFCs along with wastewater treatment.

2. Materials and methods

2.1 Synthetic wastewater composition

A synthetic wastewater containing the desirable amount of glucose as a carbon source was utilized throughout the study. The wastewater composition was adopted and modified from *Wang et al.* (2004) ,briefly, varying amount of glucose (0.35 g L^{-1}; 0.7 g L^{-1}, 1.2 g $L^{-1}$and 2.0 g L^{-1}); 0.4 g L-1 peptone; 0.25 g L^{-1} meat extract; 0.2 g L^{-1} NH_4Cl; 0.025 g L^{-1}, $MgSO_4.7H_2O$; 0.02 g L^{-1} $FeSO_4.7H_2O$; 0.045 g L^{-1} $K_2HPO_4.3H_2O$; 0.03 g L^{-1} KNO_3, 1 ml L^{-1} of trace nutrient solution (0.15 g L^{-1} H_3BO_3; 0.15 g L^{-1} $CaCl_2.6H_2O$; 0.03 g L-1 $CuSO_45H_2O$; 1.5 g L^{-1} $FeCl_3.6H_2O$; 0.12 g L^{-1} $ZnSO_4.7H_2O$; 0.03 g L^{-1} KI).

2.2 Integrated CW-MFC design and configuration

Two CW-MFC microcosm reactors were designed and built using PVC pipe (Length 60 cm; Ø-15 cm), having bottom end of the reactor sealed using a PVC cap. Each CW-MFC microcosm was also equipped with three sampling ports along the microcosm length at 5, 22, and 42 cm from the bottom. A MudWatt™ (MudWatt, USA) made of graphite felt, with 80 cm diameter and 0.5 cm thickness was used as an anode at a height of 14 cm from the bottom of the microcosm. Platinum (Pt) coated (0.8 mg cm^{-2}) carbon cloth (13.5 cm length, 7.5 cm width) placed at 46 cm from the bottom of the microcosm was used s cathode. A circuit was made by joining anode and cathode through electric wire and external resistance. With this setup, the distance between cathode and anode was kept at 32 cm, and the gap was filled with stone gravel of size 0.2-0.5 cm up to the height of 55.0 cm from the bottom surface of the microcosm. In the middle of the gravel layer an 8.0 cm thick layer of glass wool was also placed for stopping the oxygen diffusion. In the first microcosm, there was no plants and aeration was performed using a small aquarium air pump (3.5 L min^{-1} aeration capacity, Venus Auqa make pump, China) equipped with a circular porous stone ring, which was placed just below the cathode. The cathode zone was aerated for 20 minutes every 6 hours. This cyclic aeration was maintained using a programmable electrical stopper. The second microcosm was similar to first but it was not equipped with aeration arrangement and planted with *Canna indica* plants. The second microcosm was relied on radial oxygen loss of the root system of *Canna indica* plants to complete the cathode reaction. A schematic diagram of the reactors design and setup is shown in Fig. 1. (**Insert Fig.1 here)**

2.3 Experimental details and analysis

CW–MFCs were investigated in fed batch mode for wastewater treatment and bioelectricity generation from the synthetic wastewater using fed batch. The void volume of the CW–MFCs was 1800 ml. 50 ml of anaerobic digested slurry of a running bioreactor was used as inoculum in each microcosm. Each microcosm was allowed acclimatized until stable voltage reading. During this period, synthetic wastewater was fed to each of the microcosms on a daily basis to support the microbial community. For the artificial intermittent aeration, one microcosm was aerated for 20 min every 6 hours. For sampling, wastewater of the CW–MFC was taken out and homogenized, and then 50 ml of sample was taken for Chemical Oxygen Demands (COD) analysis. The remaining wastewater was poured again into the same CW–MFC. A 20 ml sample of wastewater from the bottom (near to cathode) or upper portion (near to anode) of CW–MFCs were taken out for measuring various parameters. pH was measured using a pH meter (Eutech Instruments CyberScan pH 1500). The voltage (V) was measured at least three times in a day using a digital handheld multimeter (Sanwa CD772) on a fixed resistance. The COD and Dissolved Oxygen (DO) were determined according to standard methods (APHA, 2005). Once CW–MFC was established, the polarization curve was obtained by using different resistances between 660,000 Ω and 4.7 Ω for each CW–MFC systems. The current (I) and power (P) were determined through basic electrical calculations using standard relations. The power density and the current density were then calculated by dividing the power and current by the volume (m^3) of the CW–MFC. For electron balance and carbon balance only glucose part of wastewater is considered. Reported MFC net biomass yields range from 0.07 and 0.22 g biomass COD per g substrate COD (Rabaey and Verstrate 2005). In this study, average of two value from the said study (i.e 0.14 g biomass COD per g of substrate COD) is taken for carbon balance. Carbon balance was estimated by using following two equations:

$$Y = \frac{X}{\Delta COD} \qquad (1)$$

$$\varphi = 1 - CE - \mathrm{Y} \qquad (2)$$

Where Y is cell yield, X is the biomass (g COD) produced over time, φ is the COD balance and CE is the columbic efficiency.

In order to estimate the electron balance, columbic efficiency were determined using following formula (Oh et al., 2004).

$$CE = \frac{C_{Ex}}{C_{Th}} \times 100 \quad (3)$$

Where C_{Ex} is the total coulombs calculated by integrating the current measured at each time interval (i) over time as

$$C_{Ex} = \sum_{i=1}^{T} \frac{V_i t_i}{R} \quad (4)$$

Where R is the external resistance, T is the duration of the entire experiment; Vi is the average voltage in a time.

$\boldsymbol{C_{Th}}$ the theoretical amount of Coulombs that is available from glucose oxidation was calculated as $C_{Th} = \frac{b(C_i - C_t)VF}{M}$ (5)

Where M is a relative molar mass; b is electronic mole number produced by per mole substrate; Ci, Ct are COD initial and final in every batch respectively; V effective volume of anode region; F is Faraday constant. By using equation 5, carbon (biomass, removed carbon etc) is converted into coulombs.

3. Results and discussion

3.1. Wastewater treatment

3.1.1 Dissolved Oxygen, pH and treatment efficiency

The DO conditions in cathode portion of the CW-MFC with additional IA varies 2.5 ($\pm$0.33) to 4.8 ($\pm$0.27) mg L^{-1} with 2.0 and 0.35 g L^{-1} dose of glucose in wastewater respectively. In case of CW-MFC dependent on ROL, it varies 0.81 ($\pm$0.17) to 2.4 ($\pm$0.23) mg L^{-1} with 2.0 and 0.35 g L^{-1} dose of glucose in wastewater respectively. Cathode shows variation in DO value with different glucose loading in different CW-MFCs. With increasing glucose loads, the DO decreased but remained relatively higher in aerated CW-MFC, most likely due to artificial aeration was applied in cathode portion. The decrease in DO with increased dose of glucose can be explained as DO is consumed by increased glucose loads for their oxidation. The pH values measured at anode portion of the reactor remained acidic which shifted towards more acidic on increasing glucose loading in both the CW-MFC microcosms, but the cathode portion of both the CW-MFC microcosms showed less acidic pH. In this study anode zone pH varied 4.37 (±0.20) to 6.12 (±0.21) and cathode zone pH varied 4.52 (±0.23) to 6.48 (±0.25). The shift in pH in cathode region is likely due to the proton consumption by the cathodic reactions as shown in following cathode reaction. The probable anode and cathode reactions for this case are:

$$Anode:\ C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (6)$$

$$Cathode:\ 6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \quad (7)$$

The percentage COD removal measured from different microcosm reflects the wastewater treatment efficiency of these microcosms. COD removal decreased at higher glucose loading in both systems as initial organic loads increases in wastewater with increased glucose loads. However, COD removal efficiency was consistently better in CW-MFC with additional IA than the CW-MFC dependent on ROL due to aerobic microbial oxidation in aerobic situation. The highest COD removal of 78.71 % and 53.23% were achieved in CW-MFC with additional IA in glucose loads of 0.7 g L^{-1} and 2.0 g L^{-1}, respectively. Similarly, the highest COD removal of 72.17 % and 46.77% were recorded in CW-MFC dependent on ROL at glucose loads of 0.7 g L^{-1} and 2.0 g L^{-1}, respectively. The highest COD removal was achieved in the bottom portion of CW-MFCs. This might be due to MFC arrangement that provided extra electron acceptor in the form of anode to enhance the anaerobic reaction. Previously, such type of electron transfer mechanism in the anaerobic reaction has been proposed to enhance the bioremediation of organic contaminants in subsurface environments (Lovely, 2008 ; Zhao et al., 2013).

The study also demonstrates the open circuit and closed circuit performances of both the CW-MFCs and COD removal contribution of different zones of the CW-MFCs. A clear difference between the percentage COD removal performance (8.7% bottom zone; 4.2 middle zone and 7.9 in surface zone) of closed circuit and open circuit operations of CW-MFC dependent ROL was observed. This difference was not clearly observed in CW-MFC with additional IA. The reason may be the diffusion of air that might reach the anode zone. In the case of CW-MFC dependent ROL, there was no aeration which might have helped in establishing the redox gradient. DO levels in CW-MFCs in present study also indicate the different in redox gradient in both systems. Electron balance for both the systems were provided in supplementary information section, it also indicates that more numbers of electron passed through electric circuit in the CW-MFC dependent ROL than the CW-MFC with additional IA and may have helped in improvement in COD removal. Carbon balance information provided in supplementary information section indicates the more carbon is used in biomass generation in CW-MFC with additional IA as compared to CW-MFC dependent ROL. Overall high removal of COD in CW-MFC with additional IA can be explained on the basis of this fact as aerobic reaction synthesized more biomass and removes more COD as compared to anaerobic reaction.

In CW-MFC dependent ROL, a more sharp redox gradient develop due to anaerobic regime, but more numbers of electron transfers via external circuit which helps in COD removal. Srivastava et al., (2015) also reported that the closed circuit operations of CW–MFCs performed 12–20% better than open circuit operation and 27–49% better than Normal-CW for COD removal.

3.2. Bioelectricity generation

Power density and current density productions are shown in Fig. 2. The CW-MFC with additional IA and CW-MFC dependent on ROL indicated a positive correlation between glucose loads and power produced. Power The CW-MFC with additional IA shows lower power density production than CW-MFC dependent on ROL. These results also indicate that with platinum containing carbon cloth as cathode, the amount of rhizospheric oxygen released by *Canna indica* plants in cathode zone of CW-MFC was sufficient to run cathode reaction. Besides this, artificial aeration has not shown any significant impact on electricity generation. The poor power production observed in CW-MFC with additional IA might be due to air diffusion to anode zone in the process of aeration. The high removal of substrate might also be a reason, as it would have reduced the food substrate for electricity generating bacteria. For the case of CW-MFC dependent on ROL , electron and carbon balance also supports the findings where more numbers of electron are passed through external circuit and and less number of electrons are consumed in biomass synthesis. Gill et al (2003) reported that there was not much increment in power production at a high rate of aeration and suspects that higher rate of gassing might disturb the immobilization of microbes on electrodes. The maximum current density achieved was at 181.36 mAm^{-3} in CW-MFC dependent on ROL (6 hr retention time and 2.0 g L^{-1} glucose loading) and132.72 mAm^{-3} l (36 hr retention time and 1.2 g L^{-1} glucose loading) in CW-MFC with additional IA. Results reveal that at higher loads of glucose, aeration helped in producing more voltage but overall voltage in CW-MFC with additional IA is not appreciable compared to CW-MFC dependent on ROL. This might be due to diffusion of air into the lower anaerobic zone of CW-MFC with additional IA as DO level in middle and bottom zones of the reactor were found to be 1.22-1.29 and 0.4- 0.7 mg L^{-1}, respectively in initial one hour after aeration with lower glucose loads. Another reported CW-MFC study with intermittent aeration also observed that higher aeration (more than 600 ml min^{-1}) does not generate high electricity due to diffusion of air into the lower compartment (Oon et al., 2017). Liu et al. (2014) also observed that there was not any proportional relationship between substrate concentration and current generations. There might be many reasons for such behavior of MFCs, as substrate concentration not only regulates DO level but also other factors such as pH and redox gradient which are important for the generation of voltage in MFC. In this study, anaerobic culture was used as inoculums which might also contribute to lower power production in both the CW-MFCs as anaerobic bacteria easily dominate the eletrogen for organic matter utilization. Logan et al. (2006) also reported similar competitive process for low-columbic efficiency.

Polarization curves were drawn and provide as supplementary information. From the curve, it is evident that current density increased with decrease in resistance and reached a maximum at the lowest resistance whereas voltage decreases with increasing current density. This observation is similar to any classical trend which has been observed in many other studies where voltage drops with a fall in applied resistor loads (Srivastava et al., 2015; Logan et al., 2006). In the case of CW-MFC with additional IA, the maximum current density observed to 236.40 mA m^{-3} at an external resistance of 4.7 Ω. The maximum power density achieved was 31.04 mWm^{-3} at an external resistance load of 5.5K Ω. In CW-MFC with additional IA, maximum voltage was 0.695 *V*. Similarly, in the case of CW-MFC dependent on ROL, the maximum current density achieved was 225.58 mA m^{-3} at an external resistance of 33 Ω. In the case of CW-MFC dependent on ROL, maximum power density observed was 19.60mWm^{-3} at the external resistance of 560Ω at maximum voltage was 0.632 V. The curve also indicates that internal resistance developed in CW-MFC with additional IA was many times higher than the internal resistance developed in CW-MFC dependent on ROL. Important information on major losses such as activation loss, ohmic loss and concentration loss which hampers the current production can be obtained from a polarization curve (Srivastava et al., 2015; Logan et al., 2006). Logan et al. (2006) described the characteristic features of polarization curve as first initial drop in voltage indicates the activation losses, second slow linear like fall in voltage reflects the ohmic losses and the third fast drop in the voltage at higher current shows the concentration losses. All the above described major losses can also be observed in the current study.

4. Conclusion

This study demonstrated that cathode reaction of CW-MFC could be efficiently performed by radial oxygen loss using a Pt-containing cathode. Excessive aeration for performing cathode reaction of CW-MFC does not have significant impact on bioelectricity generation, but it improves the treatment performance of CW-MFC. However, the comparative study indicates that artificial aeration enhances the wastewater treatment performance. Maximum power density of 31.04 mW $m^{-3}$and 78.71 % COD removal were achieved in CW-MFC with additional IA. Similarly, maximum power density of 19.60 mW $m^{-3}$and 53.23% COD removal were also achieved in CW-MFC dependent on ROL.

Appendix A. Supplementary

The tables containing carbon balance and electron balance appear in the electronic supplementary file Table S1 and Table S 2. Polarization curves are appears as Fig. S1

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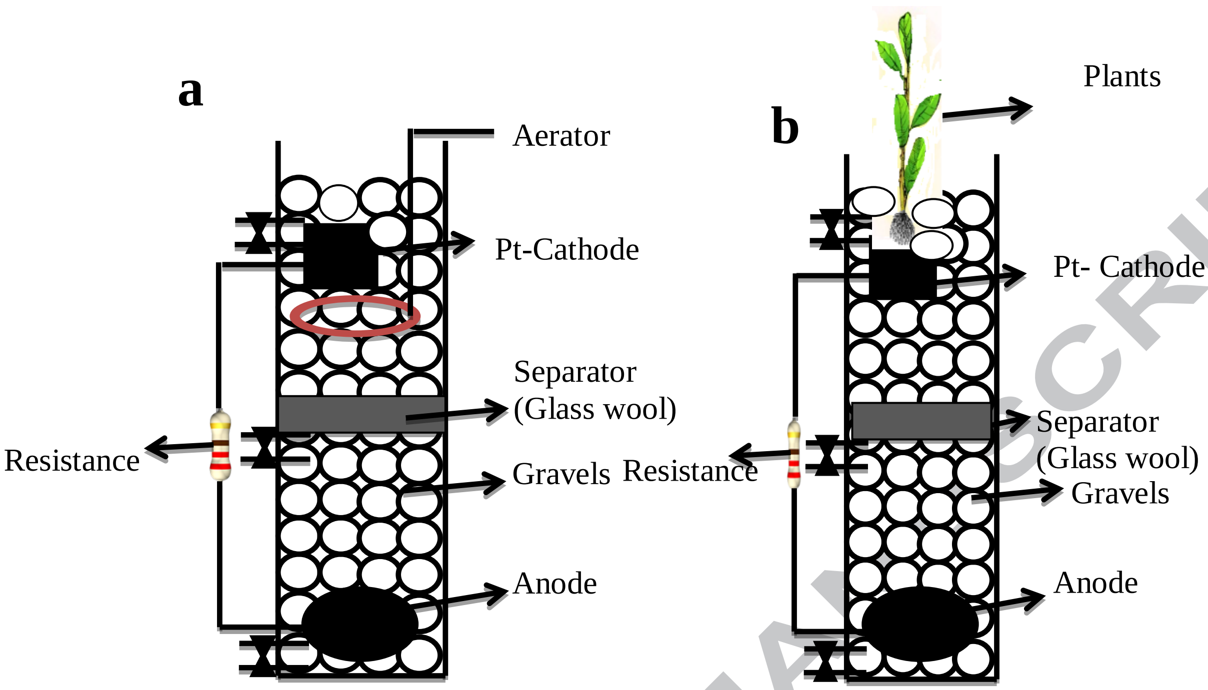

Fig. 1 (a) CW-MFC with additional IA **(b)** CW-MFC dependent on R

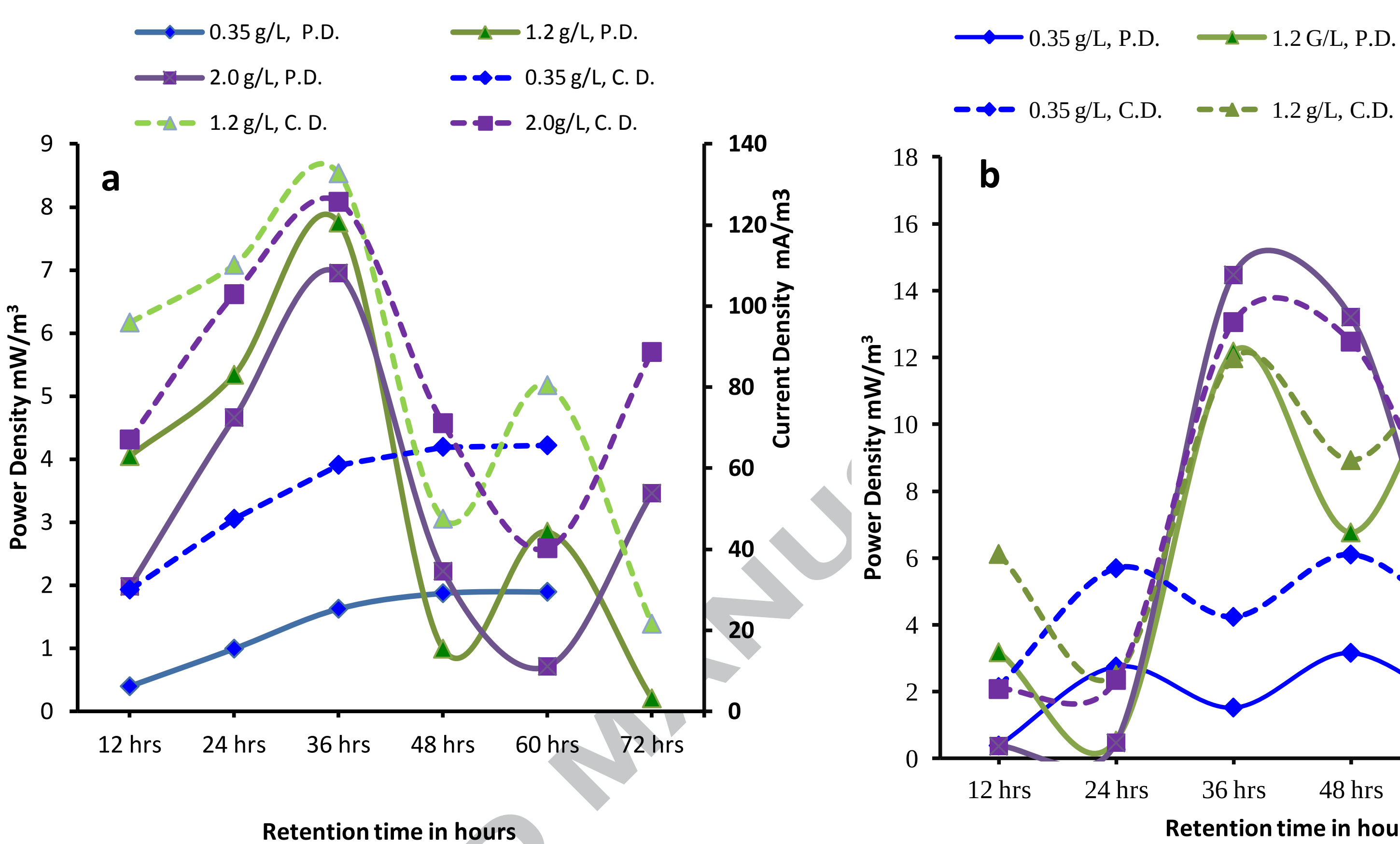

Fig. 2 Power density & Current density production in of CW-MFC with additional IA (a); CW-MFC dependent on RO(b) and

Highlights

- Radial oxygen loss from the wetland plants can help in the development of cathode
- High rate aeration does not promote electricity generation
- MFC integration into CW enhances the treatment efficiency
- *Simultaneous wastewater treatment and* electricity generation in CW-MFC