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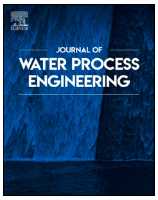

Influence of evapotranspiration on wastewater treatment and electricity generation performance of constructed wetland integrated microbial fuel cell

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ABSTRACT

Evapotranspiration (ET) is a natural phenomenon of water loss through plants, which can influence the treatment performance of constructed wetlands integrated with microbial fuel cells (CW-MFC). The influence of ET on the electrochemical performance and treatment performance of CW-MFC in an actual field with real wastewater has never been studied in detail. Thus, this research aims to explore the effects of water loss ascribed by ET on different performance indicators of a CW-MFC fed with municipal wastewater in actual field conditions, for instance, voltage and power generation, internal resistance, and wastewater treatment. During the first 48 h of the operation of CW-MFC, a significant drop in the water level by 336 mm (from 15 mm to 351 mm from the top surface) in the cathode electrode zone due to ET was recorded. This water loss at the cathode enhanced cathodic reduction kinetics by increasing the oxygen saturation at the cathode, thereby improving the voltage generation from 182.5 ± 12.5 mV (first 6 h when the cathode was more water-saturated) to 800 ± 13.47 mV (after 48 h when the cathode was air-saturated due to loss of water), which corresponds to the current and power density of 85.71 mA/m^3 and 25.71 mW/m^3, respectively. Together with this significant coulombic efficiency (CE) and net energy recovery (NER) of 11.95 % and 2.44 Wh/kg·COD was achieved. The internal resistance of CW-MFC was also noted to decrease from 1000 Ω in the first 6 h to 700 Ω at the end of 48 h. After 48 h of the observation period, the CW-MFC could achieve chemical oxygen demand (COD), ammonium, and phosphate removal efficiency of 80 ± 7.98 %, 73.17 ± 5.01 %, and 75.60 ± 1.65 %, respectively. This study demonstrates a substantial effect on the performance of CW-MFC due to ET water loss in the actual field. Thus, ET is a critical aspect and worth considering for large-scale implementation of CW-MFCs, especially in tropical regions or with dense plantations.

1. Introduction

Rapid population growth and intensive industrialization have elevated a growing need for new resources and thus forced society to consider wastewater as one of the potential sources of water, energy, and nutrients [1]. Annually, around 1000 km^3 of wastewater is generated around the world, in which municipal wastewater contributes to >300 km^3, mainly containing organics and some nutrients. Thus, municipal wastewater can be considered a futuristic source of fresh water [2]. However, present wastewater treatment technologies are usually cost and energy-intensive, thus, there is an imperative need to consider an approach that is less complex, cost-effective, eco-friendly, and sustainable [1].

In this context, constructed wetlands (CWs) have been widely used as wastewater treatment systems due to their intrinsic properties to remove a wide range of organic matter and nutrients [3]. CWs have also been acknowledged as recreational, aesthetic, and eco-friendly technology that can promote ecological balance [4]. Among different types of CWs, the subsurface flow CWs are renowned mainly for treating municipal wastewater [5]. However, large land area requirement by CWs for

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wastewater treatment is still a bottleneck of the technology resulting from low treatment efficiency by largely dominated slow anaerobic processes [6]. Thus, to achieve a higher treatment efficiency, CWs need an extensive surface area [6]. Meanwhile, microbial fuel cells (MFCs) have drawn increasing attention among researchers during the last decade to adopt as a sustainable device for wastewater treatment with a low carbon footprint and energy recovery. MFCs utilize electroactive microorganisms as a catalyst at solid conductive material-based anodes as electron acceptors for the oxidation of organic and inorganic pollutants in an anaerobic environment with the generation of bioelectricity [7]. Nevertheless, MFCs are still struggling to be scaled up due to several challenges, among which the electrode redox kinetics, reactor configurations, and process parameters are the persisting challenges to be addressed [6].

Integration of MFCs with CWs creates a new synergy between these two complementary technologies and thus divulges higher probabilities towards practical applications in wastewater treatment sectors [6,8]. For instance, an amalgamated technology *i.e.* CW-MFC, was first introduced in 2010 and demonstrated its promising approach for updating the traditional CWs containing both the aerobic and anaerobic zones suitable to maintain the cathodic and the anodic environment, respectively [9]. This new approach not only found the potential to enhance pollutant removal efficiency but also revealed an ability to extract waste energy within the flux of wastewater in the form of electricity [10–13,52]. As far as the design is concerned, a typical CW-MFC consists of an anode, which is buried in a deeper zone (anaerobic) of the treatment bed, whereas the cathode is placed at air-water interface. At the anode and the cathode electrode, graphite granules are utilized, which can assist i) vegetation support, ii) high biofilm formation due to its porous nature, and iii) filtration of pollutants [14]. Furthermore, the presence of electrically conductive material *i.e.*, anode (granular graphite), in the anaerobic region improves the availability of electron acceptor, thereby enhancing redox kinetics [6]. CW-MFCs has been explored widely in the past few years for wastewater treatment efficiency but only a handful of studies have tested CW-MFC with municipal wastewater in scaled-up/pilot scale project as listed in Table 1.

In MFCs, the performance of the cathode is generally considered a bottleneck due to sluggish oxygen reduction reaction (ORR). Therefore, several researchers have tried to develop sustainable catalysts to improve the ORR in MFCs [15,16]. However, in the case of CW-MFCs, the water column thickness at the cathode is highly important for oxygen diffusion. A thin water column could be enriched with diffused oxygen, hence, avoiding substrate limitation to achieve high ORR kinetics. For example, in 2016, Corbella et al. [5] explored the effect of water level variation at the cathode by changing the position of the cathode on the performance of *Phragmites australis* planted CW-MFC. The study revealed 40 % lower electricity generation at high water levels (lesser air diffusion due to higher water saturation) in comparison to lower water lever (higher diffusion due to lesser water saturation) [5]. However, there is no study in the actual field with real municipal wastewater in tropical conditions where ET is a major factor. ET majorly changes the air diffusion level in the cathode by changing the water level inside the CW-MFC.

Moreover, in contrast with unplanted CW-MFCs, planted CW-MFCs can naturally exhibit higher variation in water column thickness through ET. ET is the aggregate sum of evaporation and transpiration, where evaporation takes place from the substrate (soil/gravel *etc.*), and transpiration occurs by the plant system *via* the plant leaf, stem, and root substrate system [17]. The ET allows movement of water from the soil system to plant, then plant to the atmosphere, eventually assisting biomass production in plants. At the same time, the process of ET leads to water loss in the water column, although daily water loss could not be the same since ET is influenced by several environmental factors, mainly temperature and humidity [18,19]. In planted CW-MFCs, it has been reported that when the water loss due to evapotranspiration exceeds 2.5 mm/d, then it can significantly affect the treatment efficiency [20]. The daily water loss estimated by Yang et al. [21] in planted CW-MFC was about 13.73 %, 10.82 %, and 1.71 % higher than unplanted CW-MFC with *Iris pseudacorus*, *Lythrum salicaria*, and *Phragmites australis* planted systems, respectively. Whereas in another CW-MFC study by Yang et al. [22] with similar vegetation, it was reported to 4.4 % for *Iris pseudacorus* and 1.78 % for *Phragmites australis* higher compared to unplanted control CW-MFC. These studies indicate that in planted CW-MFC, ET could play a crucial role in changing the water level at the cathode zone and,

Table 1

Represents recent pilot scale studies on CW-MFC with different vegetation types.

CW-MFC configuration	Flow type	Working volume (L)	Vegetation type	Wastewater type	Internal resistance (Ω)	Power density	CE (%)	COD removal	References
Vertical	Continuous upflow	12.4	*Ipomoea aquatica*	Real wastewater	156	12.42 mW/m^2	–	94.8 %	[35]
Vertical	Continuous upflow	12.4	*Ipomoea aquatica*	Synthetic dye wastewater	217.7	302 mW/m^3	–	85.65 %	[38]
Horizontal subsurface flow	Continuous	96	*Phragmites australis*	Synthetic wastewater	120	43 mW/m^2	0.45	90–95 %	[39]
Simultaneous upflow-downflow regime	Continuous	8.1 ± 0.12	*Phragmites australis*	Swine wastewater	300	268 mW/m^3	0.1–0.36	64 %	[10]
Vertical	Continuous upflow	–	*Ipomoea aquatic*	Synthetic dye wastewater	53.37	880 mW/m^3	2.5	312.17 mg/L	[40]
Vertical subsurface flow	Continuous upflow	12.4	*Phragmites australis*	Synthetic wastewater	339.80	200 mW/m^3	0.31	90.45 %	[41]
Vertical	Continuous upflow	30	–	Synthetic wastewater	196	7.99 mW/m^2	0.36	91.7 %	[42]
Horizontal subsurface flow	Continuous	54.6	*Phragmites australis*	Real domestic wastewater	1000	14.5 mW/m^2	–	61 ± 19 %	[5]
Vertical subsurface flow	Continuous upflow	19.5	Unplanted	Domestic wastewater	220	–	<2 %	56 ± 9 %	[43]
Vertical subsurface flow stacked CW-MFC cells	Continuous upflow	12.0	Unplanted	Synthetic wastewater	15,000	30.85 mW/m^3	4.6	98.5 %	[44]
Pilot scale horizontal subsurface flow	Continuous	65	*Canna indica*	Synthetic wastewater	1200	0.11 mW/m^3	1.86	98–99 %	[45]
horizontal subsurface flow	Batch	60	*Fimbristylis ferruginea*	Real textile dye wastewater	–	197.94 mW/m^2	–	74.10 ± 1.75 %	[46]
Pilot scale	Batch	316	*Myriophyllum aquaticum*	Real pond wastewater	1000	–	–	66.7 %	[47]
Vertical subsurface	**Batch**	**16.7**	***Canna indica***	**Real municipal wastewater**	**700**	**25.71 mW/m^3**	**11.95**	**80 ± 7.98 %**	**Present study**

thus, the availability of diffused oxygen. For instance, higher ET can lower the water level at the cathode. Therefore, higher oxygen saturation at the cathode can be expected, which can enhance the redox kinetics, eventually enhancing the electricity generation [5,23,24]. In fact, water loss due to ET can also dramatically change the concentration of contaminants in the wastewater, which will also have a certain effect on the treatment efficiency [3]. Altogether, ET is a critical aspect to be considered, particularly for large-scale CW-MFCs with dense plantations. [25].

So far, most of the CW-MFC studies were investigated in the lab environment and utilized synthetic wastewater instead of real domestic or municipal wastewater. Until now, no study has collectively investigated the effect of ET on the treatment and electrochemical performance of scaled-up CW-MFC in an real field setting with real wastewater treatment. Therefore, for the first time, this study investigated the performance of *Canna indica* planted scaled-up CW-MFC fed with real municipal wastewater. The aim of this study was to find out the influence and correlation of different parameters such as temperature, relative humidity, water level, air cathode volume, and ET on the overall performance of CW-MFC in terms of bioelectricity generation and wastewater treatment. Best of our knowledge, this is the first study that explores the effects of ET on different treatment and electrochemical performance indicators of a scaled-up CW-MFC system (16.7 L) with real municipal wastewater in the actual field for advancing the CW-MFC towards realistic application in the field.

2. Materials and methods

2.1. Construction of CW-MFC mesocosm

A cylindrical shape CW-MFC was fabricated using a linear low-density polyethylene (LLDPE) container measuring 60 cm high and 32.5 cm in diameter (Lisa Plastics, Patia, Bhubaneswar, India). The CW-MFC had three sampling ports; the first port was placed at 5.5 cm from the water freeboard, the second port at 33 cm, and the bottom port at 57 cm. The bottom-most region of CW-MFC was bedded with boulders of an average diameter of 20–25 mm up to 10 cm height to serve as a supporting layer and avoid clogging. Just over the boulder layer, a PVC pipe of 20 mm Ø was placed to serve as i) gas vent, ii) water level measurement due to ET loss, and iii) running of continuous up-flow systems if required later. A 15 cm thick layer of graphite granules (5–8 mm Ø) to act as an anodic layer was placed on top of the boulder bed. A cylindrical graphite rod (15 cm × 1 cm = diameter × height) of 400.55 cm^2 area was inserted in this layer to function as electron/charge collector. Thereafter, gravel separation (gravel Ø 5–8 mm) of 20 cm was given through double lining of a perforated (average 10 holes of 5.0 mm Ø) high-density polyethylene (HDPE) liner at both ends of gravel layers. On top of the upper HDPE layer of the liner, a 15 cm of granular graphite layer is placed for working as the cathode. A sapling of *Canna indica* was planted in the cathodic zone of CW-MFC, as shown in Fig. 1(a). At the cathode, a cylindrical graphite rod was placed in cathode zone as electron/charge dispenser (15 cm × 1 cm = diameter × height) [3]. *Canna indica* sapling was rinsed five times with running tap water to remove the dirt. Its root length, shoot length, and weight was measured as 14 cm, 77 cm, and 130 g, respectively. Uprooted *C. indica* had an average of 3 leaves before transplantation to CW-MFC. Graphite granules were washed with diluted acid and later with tap water to remove dirt and unwanted chemicals and introduce exfoliation to enhance the surface area. Both anode and cathode charge collector/dispenser was connected with insulated copper wire of 1.5 mm diameter and sealed with epoxy at one end. Another end is left open for continuous voltage and current measurement, as shown in Fig. 1(a). The net void volume of scaled-up CW-MFC was 16.7 L.

In Fig. 1**(a)**, (a) represents a boulder of average size 20–25 mm; (b) is

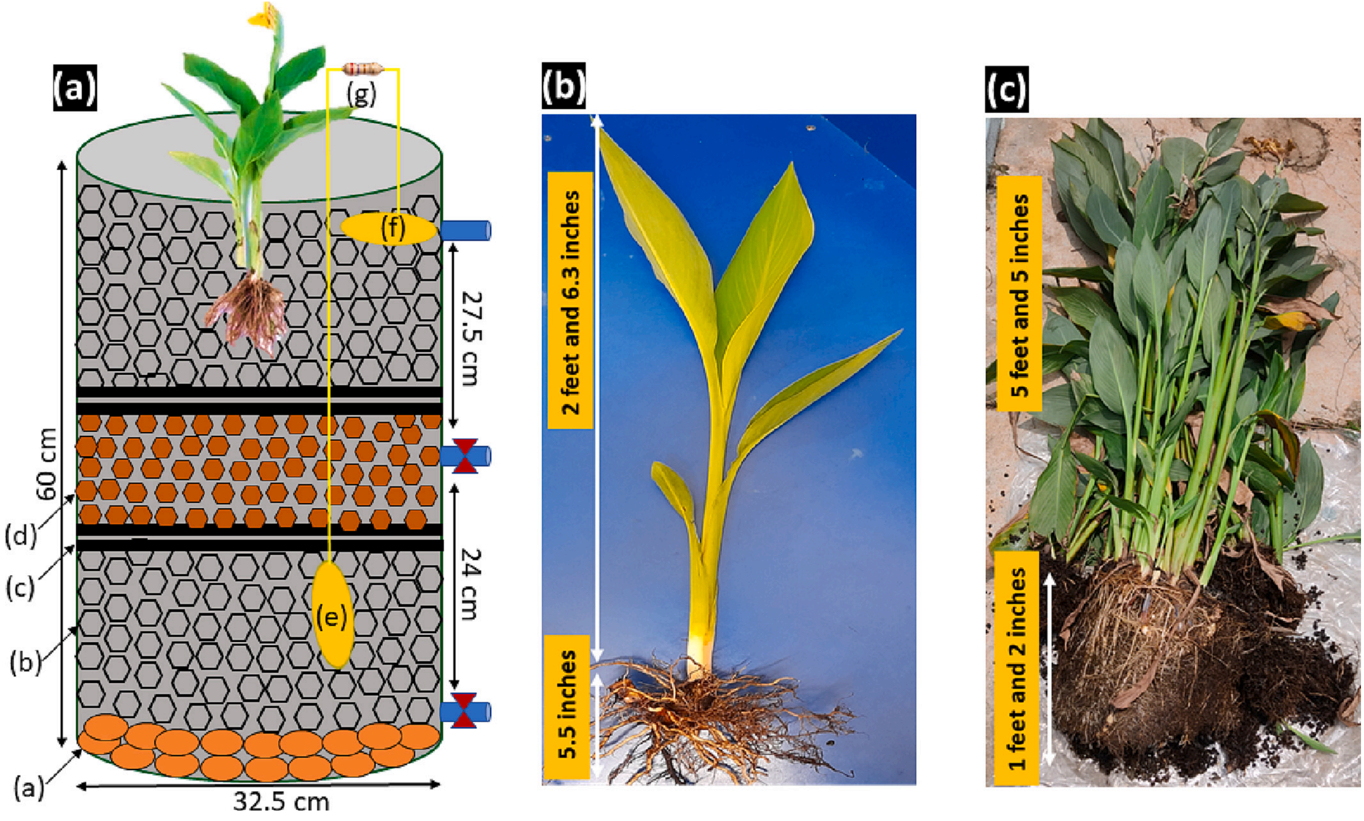

Fig. 1. **(a)** Schematic diagram of *Canna indica* planted scaled up Constructed wetland *cum* microbial fuel cell (CW-MFC); **(b)** one of the representative *Canna indica* plants planted in CW-MFC before the start of the experiment, and **(c)** representative above-ground and underground biomass of harvested *Canna indica* at the end of the experiment.

an anodic layer of granular graphite (5–8 mm Ø); (c) double layered high-density perforated polyethylene liner (HDPE); (d) gravel (5–8 mm Ø) separation; (e) and (f) are the anode and cathode charge collectors/exchangers inserted in anode and cathode regions, respectively, and (g) resistance load connected between anode and cathode.

2.2. *Experimental setup, water quality monitoring, and measurements*

CW-MFC was fed with real municipal wastewater, thus possessing fluctuating characteristic condition with COD, ammonium and phosphate lying in the average range of 125.35 ± 77.93 mg/L, 16.79 ± 6.08 mg/L and 5.12 ± 2.43 mg/L, respectively, throughout the course of the experiment. Initially, CW-MFC was operated for 180 days in batch mode with municipal wastewater for acclimation to stable voltage generation and *Canna indica* vegetation growth. During the acclimatization period, wastewater was changed every alternate day, and voltage was recorded to determine the steady state condition of CW-MFC. Once the steady performance was achieved, CW-MFC was connected with external resistance of 1000 Ω equivalent to internal resistance evaluated through polarization. Further, the same CW-MFC with already matured biofilm was used to test the effects of ET on the performance.

During the entire experiment, wastewater was collected from Institute's (CSIR-Institute Minerals and Materials Technology) sewage chamber into a 100 L LLPDE tank and mixed well manually with bamboo sticks; approximately 50 mL was collected as influent wastewater in a polyethylene bottle. Thereafter, collected wastewater was fed to the CW-MFC system. The initial working volume of the system was 16.7 L. This time CW-MFC filling was considered as 0 h of batch loading. Wastewater was fed to CW-MFC for various contact times such as 8 h, 24 h, and 48 h. Collection of treated sample and feeding of new loads of wastewater was done using the following procedure: (i) CW-MFC was entirely decanted in LLPDE tank after each contact time, (ii) decanted wastewater was mixed thoroughly before sample collection (volume collected: 25 mL), (iii) decanted volume was also measured for evaluation of evapotranspiration (ET) and (iv) CW-MFC was refilled again with the same decanted wastewater. This entire cycle of 48 h was carried out once in a week after end of rainy season and continued for a period of 6 months from September to February. Further, collected samples at each 6 h, 24 h, and 48 h were stored at 4 °C in 50 mL polyethylene bottle and analyzed on the day, followed by sample collection for chemical oxygen demand (COD), ammonium (NH_4^+) and phosphate (PO_4^{3-}) using APHA standard protocols *i.e.*, 5220D-Closed Reflux Colorimetric Method, 4500F-phenate method, and 4500-P D stannous chloride method, respectively [26]. Thereafter, the percentage removal of ammonium, COD, and phosphate was estimated.

The performance parameters, including water level measurement (mm), voltage generation (mV), temperature (°C), relative humidity (%), internal resistance (Ω), and ET (L), were also measured. Temperature (°C) and relative humidity (RH%) were measured by means of a hygrometer (CT-THMO, H11J-029160, Digital temperature humidity meter, India). Voltage generation was recorded on a daily basis with a digital handheld multimeter (Fluke 17B, USA). The polarization experiment was performed at different points of time, *viz.* at 0 h, 24 h, and 48 h, using variable external resistances ranging from 10 kΩ to 100 Ω. The internal resistances were determined using Ohm's law, measuring the tangent value of the voltage *vs.* current curve. Current density and power density were calculated by dividing generated current and power with an anodic volume of CW-MFC. Further, coulombic efficiency (CE) was calculated using Eq. (1) which represents amount of organic matter converted to electrical current [27].

$$CE = \frac{8\int I dt}{F\, V_{an} \Delta COD} \tag{1}$$

where ΔCOD is the change in COD concentration over the fed-batch cycle (g/L), I denote output current (in A), F is Faraday's constant (96,485 C/e^-mol), and V_{an} is the working volume of liquid in the anodic region (L).

Another parameter was computed to access the performance of pilot scale CW-MFC *i.e.*, net energy recovery (NER) in term of Wh/kg·COD, which reflects ability of CW-MFC to convert organics present in wastewater into bioelectricity by Eq. (2) [27].

$$NER = \frac{P}{\frac{V}{HRT} \times \Delta COD} \tag{2}$$

where P is power in W, V is the volume of mesocosms in L, HRT is hydraulic retention time and ΔCOD is the change in COD concentration over the fed-batch cycle (kg/L).

At the end of the experiment, *Canna indica* plant biomass was collected and separated into above-ground and underground biomass and then oven-dried at 80 °C till no longer weight reduction was observed. Dry weight (DW) plant biomass production was measured.

Further, it is very important to note that the entire experiment was carried out in winter season, from September to February in the east region of India *i.e.*, Bhubaneswar, Odisha which experiences a typical tropical climate and remains hot and humid throughout the year and winters are often marked by hot sunshine and persistent humidity (lying >65 % throughout the year).

For a better understanding of the approaches involved in the experimentation for influence of ET on municipal wastewater treatment efficiency and simultaneous electricity generation performance of upscaled CW-MFC in a tropical region, a detailed flow chart is presented in Supplementary file, Fig. S1.

2.3. *ET and treatment efficiency assessment*

Most of the reported CW-MFC studies have not considered ET during the calculation of treatment efficiency. Large loss of water can be experienced by the CW-MFC systems operating in the actual field due to ET and can significantly influence the removal efficiency of the system. To determine treatment efficiency influenced by ET, the following Eqs. (3), (4), and (5) were used for calculating percentage water loss, effluent pollutant concentration after removing water loss (Q), and total percentage removal efficiency after considering ET (RE), respectively.

$$Loss\ of\ water\ volume\ (\%) = \frac{Influent\ volume\ (\mathrm{L}) - effluent\ volume\ (\mathrm{L})}{Influent\ volume\ (L)} \times 100 \tag{3}$$

$$Q\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) = Effluent\ pollutant\ concentration\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) \times \left(1 - \frac{loss\ of\ water\ volume\ (\%)}{100}\right) \tag{4}$$

$$RE\ (\%) = \frac{Influent\ pollutant\ concentration\ \left(\frac{mg}{L}\right) - Q\ \left(\frac{mg}{L}\right)}{Influent\ pollutant\ concentration\ \left(\frac{\mathrm{mg}}{\mathrm{L}}\right)} \tag{5}$$

3. Result and discussion

3.1. *Plant growth assessment*

Canna indica was chosen as the model plant for CW-MFC due to its water-loving feature and versatile growing pattern in the Indian environment. We have noted with the visual observation that there was continuous growth in the plants throughout the experimental period. The summer season was reported to be optimum for the growth of this plant, whereas in winter, a few leaves turned yellow may be due to lower atmospheric temperature. At the time of harvest, *Canna indica* roots were found to be tightly bound to the graphite granules with significantly dense root system compared to initially planted sapling at the cathodic region, revealing graphite granules as a plant-compatible material capable of supporting vegetation growth (Fig. 1(c)) [28]. At the end of the experiment (*i.e.*, 180 days), significant growth in the plant in terms of stem and root was observed. During this period, the stem grew up from 77.0 cm to 131.06 cm and the root length from 14.0 cm to 36.57 cm. The dry biomass of *Canna indica* planted in CW-MFC above ground (stem and leaves), and underground (roots) was noted to be 392.8 g and 105.9 g, respectively. Accordingly, the net total dry biomass production was found to be 48.89 kg/m^2 of planted area in CW-MFC at the end of the experimental period, which was significantly higher than other vegetation types used in CW-MFC such as *Iris pseudacorus* (3.60 kg/m^2), *Lythrum salicaria* (2.37 kg/m^2), and *Phragmites australis* (0.49 kg/m^2) [21]. Higher *Canna indica* biomass production in the CW-MFC matrix could improve the overall performance *via* the following possible mechanisms i) high ET due to higher growth of the plants and higher leave surface area of *Canna indica*, ii) enhanced oxygen reduction reaction by root aeration due to high root biomass growth, thereby enhancing bioelectricity production [3], iii) supporting the growth of electroactive bacteria in the root zone helping to improve bio-catalytic reaction [1], iv) reduce the internal resistance of the reactor by enhancing biodegradation potential and current flux [1] and v) assimilate high nutrients matter, overall improving the pollutant removing efficiency [3]. Besides, the presence of vegetation in CW-MFCs also provides an additional advantage as it is a CO_2-neutral process due to the ability of plants to sequester atmospheric CO_2 into biomass [29].

3.2. Effect of temperature and humidity on evapotranspiration

Evapotranspiration (ET) is a combination of two processes *viz* evaporation -water loss from open water bodies, and transpiration water loss from living plant surface. ET process is dependent on several important factors, such as net solar radiation, wind speed, the surface area of the water body, type and density of vegetation cover, root depth, moisture availability, and season of the year [30]. In this study, wastewater volume was estimated at different time intervals of 0 h, 6 h, 24 h, 30 h, and 48 h for determination of water loss through ET, starting at 12:00 pm (noon as 0 h). Since environmental factors like RH% and temperature influence ET, water volume loss differed for different time intervals. At 0 h, 16.7 L effluent was fed to the CW-MFC system, which gradually decreased by 3.1 ± 0.26 L during the first 6 h, another 1.3 ± 0.3 L during 12–24 h, another 1.95 ± 0.29 L during 24–30 h and another 1.1 ± 0.25 L during 42–48 h. This means that, during 48 h, the total ET volume loss was 7.45 L, which decreased the actual effluent volume from 16.7 L to 9.25 L. From Fig. 2, it can be noted that the highest ET loss took place in 0–6 h (*i.e.*, 12:00 h to 18:00 h), but the highest relative humidity and temperature were recorded between 42 and 48 h and 24 and 30 h of operation, respectively. At the same time, the second highest ET loss occurred at the highest temperature hours (24–30 h). Corbella et al. [5] also reported similar ET loss from 12:00 h to 18:00 h in a pilot scale CW-MFC planted with *Phragmites australis* vegetation. In addition, Pedescoll et al. [24] even reported a shift in ET peak in CWs planted with different vegetation while considering day/night variations. Where ET increased from 0 mm/day in the early morning and reached the peak of 80 mm/day and 60 mm/day in mid-day and decreased progressively to 40 mm/day and 15 mm/day in the night-time with *Typha augustifolia* and *Phragmites australis* planted hydroponic CW. Also, ET rates could vary depending on the water use efficiency of different vegetation for above-ground biomass production. The highest ET loss in the initial hours (0–6 h) could be due to high surface evaporation along with transpiration since the water level was high. However, with time, surface evaporation decreased with a gradual decrease in water level, which may have resulted in low ET during the final hours of the experiment. Similar results of decreased ET with an increase in water table depth were also reported in a field-scale ET study [31]. In Fig. 2, it can also be seen that high RH% hours (42–48 h) enforced the lowest ET loss. This could be due to the lower and negative correlation of ET with RH%, as reported by Papaevangelou et al., [32].

3.3. Effect of evapotranspiration on cathode exposed to air

Fig. 3 depicts the increment in the depth of air-exposed cathode with water loss due to ET from the system from 0 h to 48 h. It is observed that the highest water loss due to ET was observed at the end of 6 h but surprisingly highest cathode water column height reduction of 99 mm was noticed in 24–30 h. This is possible due to the high environment temperature recorded around 36.83 ± 1.06 °C during 24–30 h, which caused in evaporation of water. The least reduction in the water column

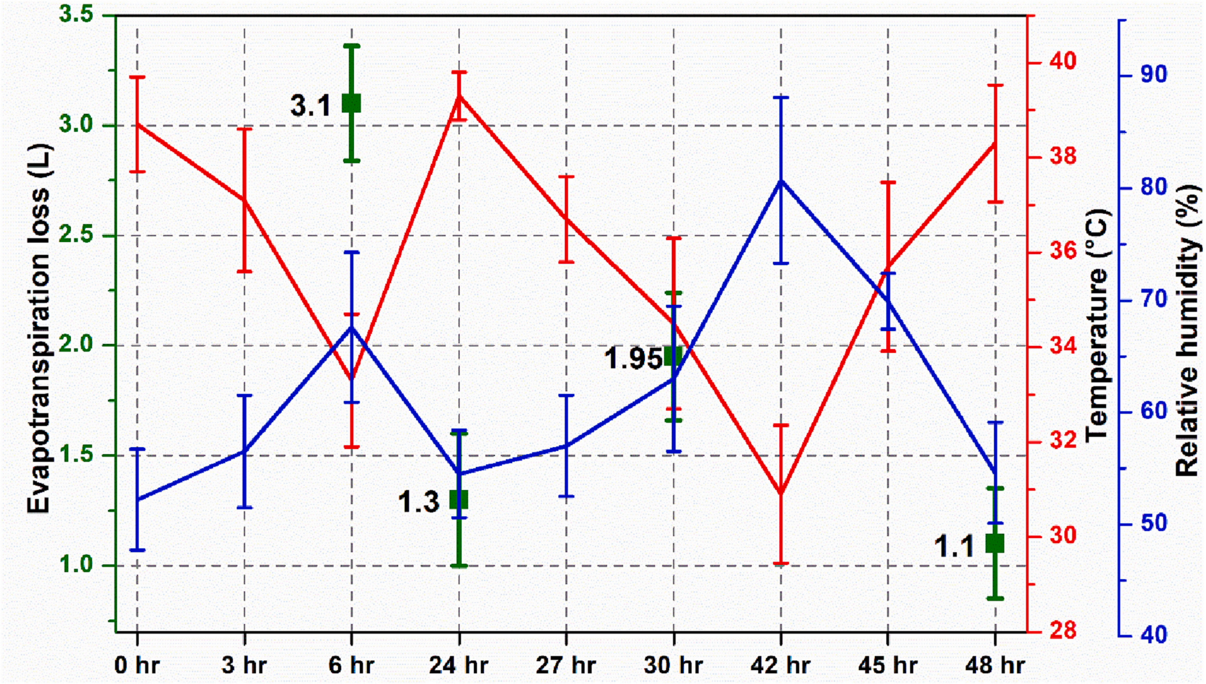

Fig. 2. Water loss due to evapotranspiration in different time duration with temperature and RH%.

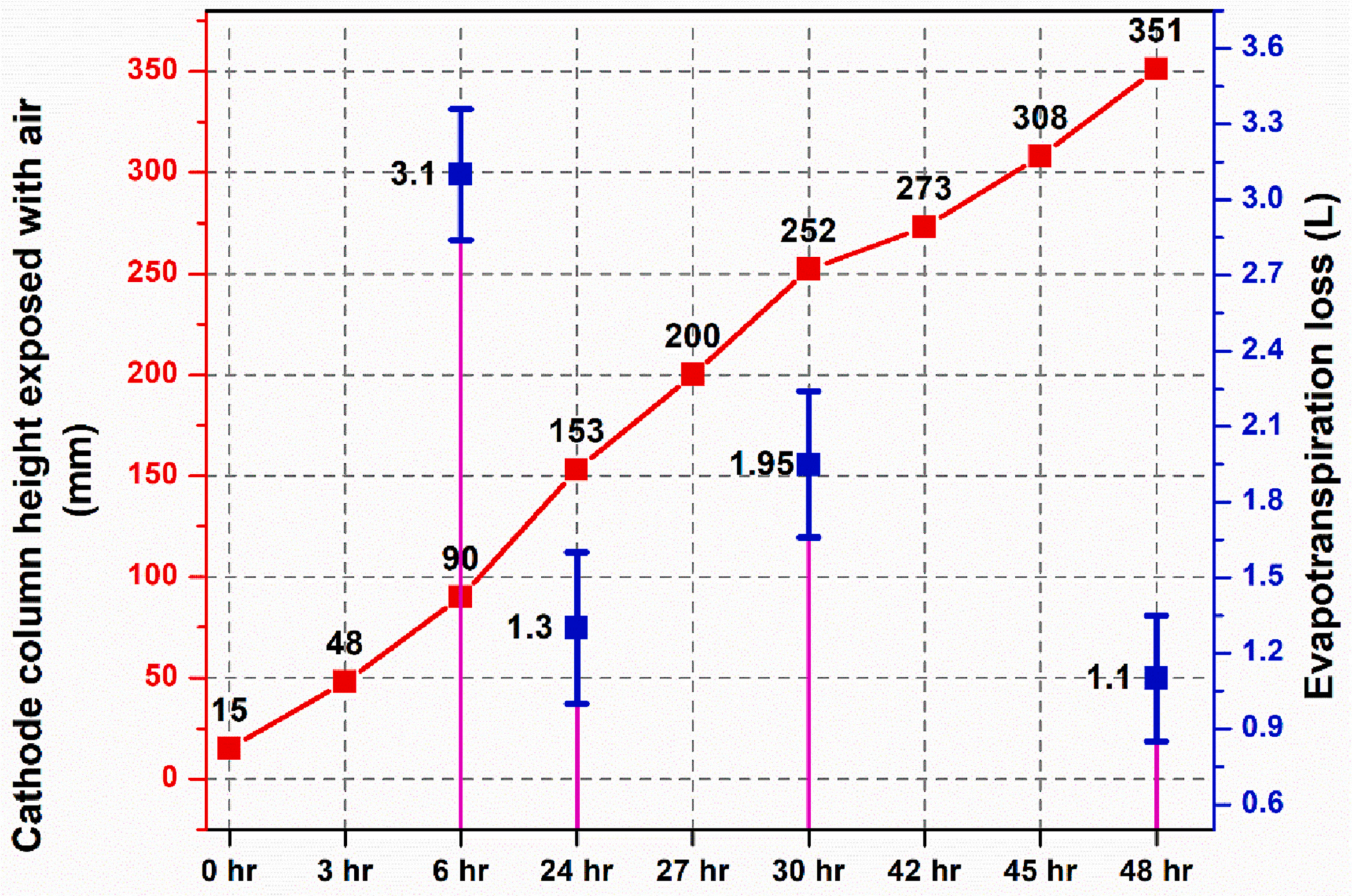

Fig. 3. Relation of water loss due to ET and cathode exposure with air in CW-MFC.

was observed between 42 and 48 h, corresponding to the least water loss through ET due to comparatively lower environmental temperature (34.96 ± 1.48 °C) and water level reaching far from the root system. However, the water column was significantly reduced up to 336 mm at the end of 48 h, which enhanced the availability of oxygen (dissolved oxygen) at the cathode due to the high diffusion of atmospheric air into the cathode zone.

Water loss due to ET in *Canna indica* planted CW-MFC system has induced notable variation in water level in the cathode zone, and therefore it can influence redox conditions of the anode and cathode zones of the CW-MFC system. The decreased water level at the cathode in CW-MFC implies an increase of cathode exposure in air, thus, could promote oxygen diffusion in the empty cathode, suggesting improvement in cathode kinetics which generally act as a limitation in CW-MFC due to slow kinetics resulting from oxygen scarcity [5,23,33]. These results are in agreement with the findings of Pedescoll et al. [24], revealing a positive relationship with linear regression between ET and the redox state of horizontal CWs planted with different vegetation, which promoted the natural aeration of filter medium due to ET. Thus, favorable oxidized conditions confirm the influence of vegetation not only on ET rates but also on the redox state of CWs.

3.4. Effect of cathode exposed to air on voltage generation

CW-MFC voltage was noted to increase with the decrease in water level owing to an increase in exposure of cathode with air in CW-MFC (Fig. 4). During the first 6 h of the study (after decanting and restarting the CW-MFC for ET test experiments), steady cell voltage was not observed, which is possibly due to the time required to induce the natural mediators for microbial growth or overcoming the air diffusion shock during decanting. It is generally considered a lag phase period [34]. This lag phase can also be favorable for maintaining the redox potential gradient between the anodic and cathodic region of CW-MFC, which is lost due to easy diffusion of oxygen while decanting and restarting the reactor for ET experiments at 0 h [7].

At the start of 24 h, cell voltage increased to 182.5 ± 12.5 mV, which gradually increased to 540 ± 22.31 mV by the end of 30 h, to 625 mV ± 24.12 on 42 h, and finally up to 800 mV ± 13.47 at the end of 48 h. It can be noted that the output cell voltages were higher in the daytime as compared to night-time. This could be possible because in the daytime, wetland plants release oxygen from root exudates, creating more suitable aerobic conditions at the cathode, which enhance of electrical output [35]. Another possible reason could be a substantial decrement in water level at the daytime resulting from ET loss which enhanced oxygen diffusion in the cathodic region and hence enhanced the oxygen reduction kinetics. This voltage generation at 48 h was significantly higher compared to a similar kind of recent study in which CW-MFC planted with *Canna indica* could produce an average voltage of 231 ± 53 mV during 48 h of hydraulic retention time [36].

In addition, an increase in cathode column exposure to air played a vital role. This study noted a linear increase in cell voltage after 24 h followed by a reduction of the water column in the cathode side that apparently increased dissolved oxygen concentration due to air diffusion in this zone. The achieved results are in agreement with Corbella et al. [5], displaying the major governing factor for enhanced voltage generation as the availability of oxygen at the cathode, which was related to water level variation caused by the plant's evapotranspiration. Although, after the end of the experimental period, *i.e.*, 180 days when plants were harvested, a significant drop in cell voltage from 800 ± 13.47 mV to 80 ± 50.45 mV was observed in 48 h of the experimental

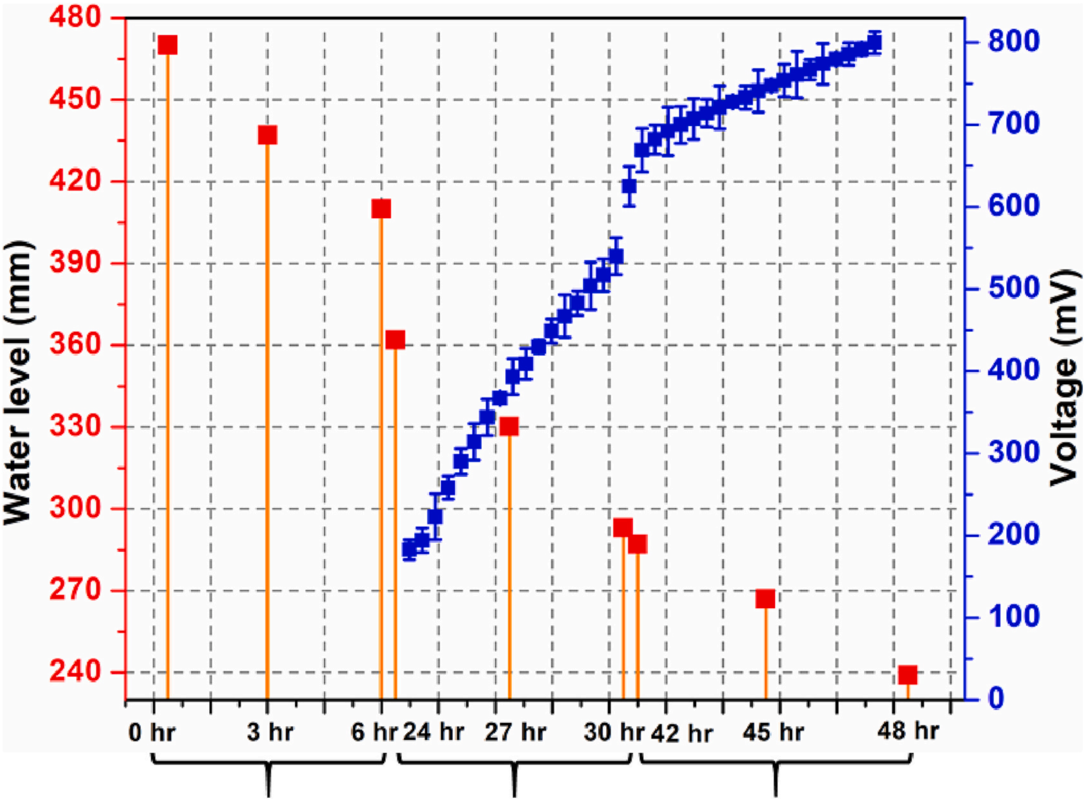

Fig. 4. Water level variation with voltage generation.

period. This could be possibly due to (i) disturbance in the cathodic region since roots were tightly bound to graphite granules and were passively aerating the cathode; after their removal (*i.e.*, after harvesting), oxygen was heavily depleted, which reduced the oxygen reduction rate considerably and thereby the overall decrease in cell voltage was observed and (ii) low diffusion of atmospheric oxygen in the cathode column due to significant reduction in water level drop in cathode region from 336 mm (with planted CW-MFC) to just 25 ± 10 mm (after harvesting) in 48 h. A very lower water level drop in the CW-MFC system after the harvesting of plants is because water loss can occur only through evaporation which was also very less as the water surface was not directly exposed to the atmosphere due to subsurface constructed wetlands conditions in current CW-MFC.

3.5. *Influence of cathode exposure to air on internal resistance and electricity generation*

As discussed earlier, the water level profile was noted to be decreased with time at the cathode side due to ET-based water loss, which in turn affected the electrical performance of CW-MFC on the positive side as a result of more oxygen availability due to air diffusion to the spaces which was vacated by water. Polarization was conducted to study the power generation profile of CW-MFC at different times, *viz.* 0 h, 24 h, and 48 h, in association with the ET losses similar to those performed by other researchers [15,16]. In general, due to irreversible losses (*i.e.*, overpotentials), the actual output cell voltage is always less than the theoretically calculated voltage of the redox reactions occurring at the cathode and anode [37]. Overpotentials can arise in both the cathode and anode. However, the cathodic overpotential losses primarily limit the performance of CW-MFC or MFC. These overpotentials are defined as activation losses, concentration overpotential, and ohmic losses, which can be identified in polarization curves [37]. In particular, cathodic activation losses are attributed to reaction interface area, temperature, and oxidant concentration, whereas cathodic ohmic losses are due to the charge transfer resistance of the system, while concentration and activation overpotential is related to the energy required for the supply of oxygen in the reaction interface followed by the energy requirement for its reduction [5,23]. Thus, both activation and concentration overpotential rely on oxygen concentration.

Results revealed a high potential of 800 ± 13.47 mV at 48 h followed by 540 ± 22.31 for 24 h and 182.5 ± 12.5 mV for 0 h (Fig. 5). Fig. 5 also revealed that the internal resistance decreased from 1000 Ω (0 h) to 900 Ω (24 h) and eventually to 700 Ω at the end of 48 h, which is clearly indicating that the CW-MFC in 48 h had lowest ohmic overpotential losses. With a gradual decrease in the internal resistances, enhancement in current density was found and noted as 85.71 mA/m^3, 65.71 mA/m^3, and 8.28 mA/m^3 for 48 h, 24 h, and 0 h, respectively. Following a similar trend, at 48 h, CW-MFC could achieve a maximum power density of 25.71 mW/m^3, which significantly surpassed the value of 15.11 mW/m^3 and 0.480 mW/m^3 for 24 h and 0 h. It is confirmed from the results that water losses with time by ET provide vacant space for more oxygen diffusion at the cathode with time, which eventually increases the oxygen saturation, thereby enhancing cathode performance. Furthermore, the columbic efficiency (CE) of CW-MFC was also computed at 24 h and 48 h. This study noted an increase in the CE from 3.8 % at 24 h to 11.95 % at 48 h of the experimental period. Three times increase in CE also confirmed enhanced cathodic performance, which resulted in a high current recovery. In addition to this, the NER of CW-MFC mesocosm at 48 h was calculated as 2.44 Wh/kg·COD. Moreover, the achieved CE% in the present research work is significantly higher than other pilot scales/scaled-up CW-MFC studies undertaken, as listed in Table 1.

3.6. *Pollutant removal efficiency*

COD, ammonium, and phosphate removal efficiencies were estimated at 6 h, 24 h, and 48 h in comparison to the initial concentration of pollutants (0 h) in municipal wastewater. This study estimated pollutant concentration considering the ET losses using Eqs. (3), (4), and (5).

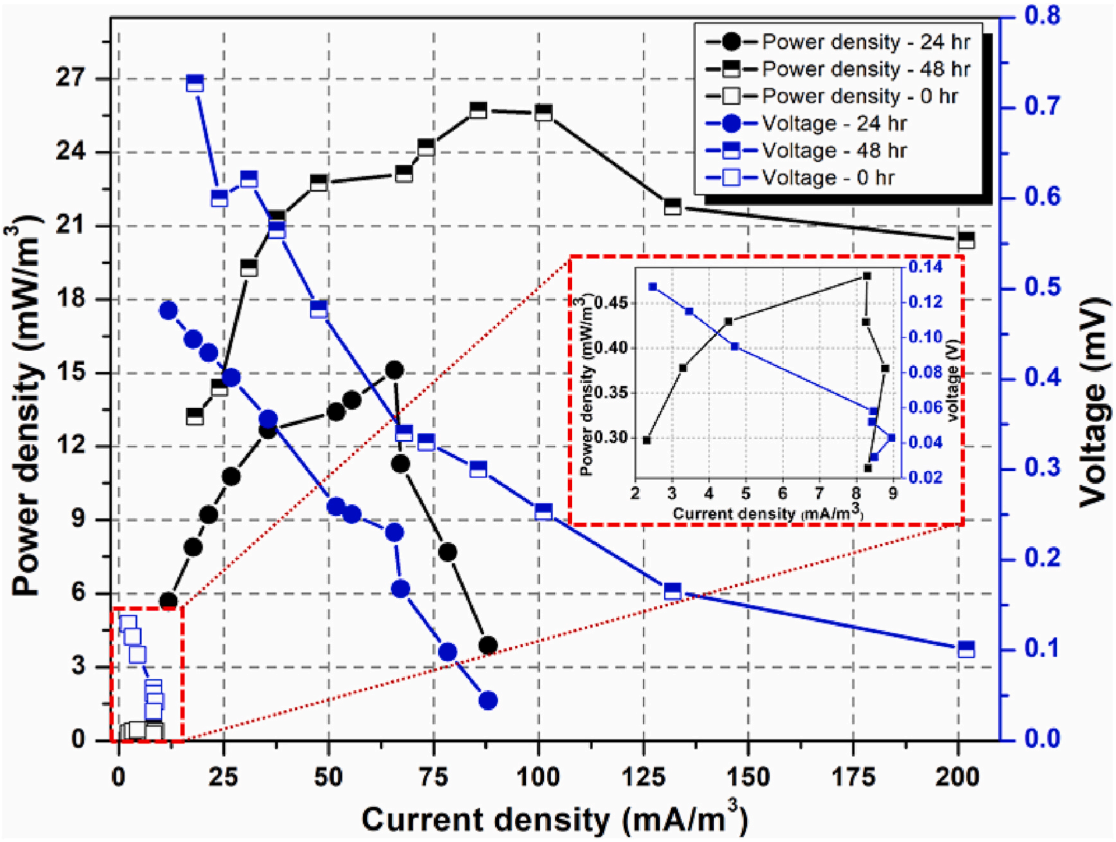

Fig. 5. Polarization curves at different time intervals of 0 h, 24 h, and 48 h.

Table S2 represents pollutant removal efficiencies with and without considering ET (in the Supplementary file). The overall removal efficiency of COD, ammonium, and phosphate was noted to be 80 ± 7.98 %, 73.17 ± 5.01 %, and 75.60 ± 1.65 %, respectively in 48 h duration (Fig. 6). In particular, the effluent COD removal efficiency of 57.06 ± 5.11 %, 73.30 ± 2.87 % and 80 ± 1.98 % was observed in 6 h, 24 h, and 48 h, respectively, with the corresponding final concentration of 85 ± 11.2 mg/L, 80 ± 4.70 mg/L and 39 ± 2.29 mg/l, respectively. The results are in accordance with a recently published study, where a high COD removal of 88.1 % was achieved in a batch mode operated *Canna indica* planted vertical CW-MFC [18]. Likewise, Corbella et al. [5] reported COD removal of 61 ± 19 % when the cathode was positioned 5 cm below the water level and 60 ± 10 % when the cathode was placed at the same level of water in *Phragmites australis* planted pilot scale CW-

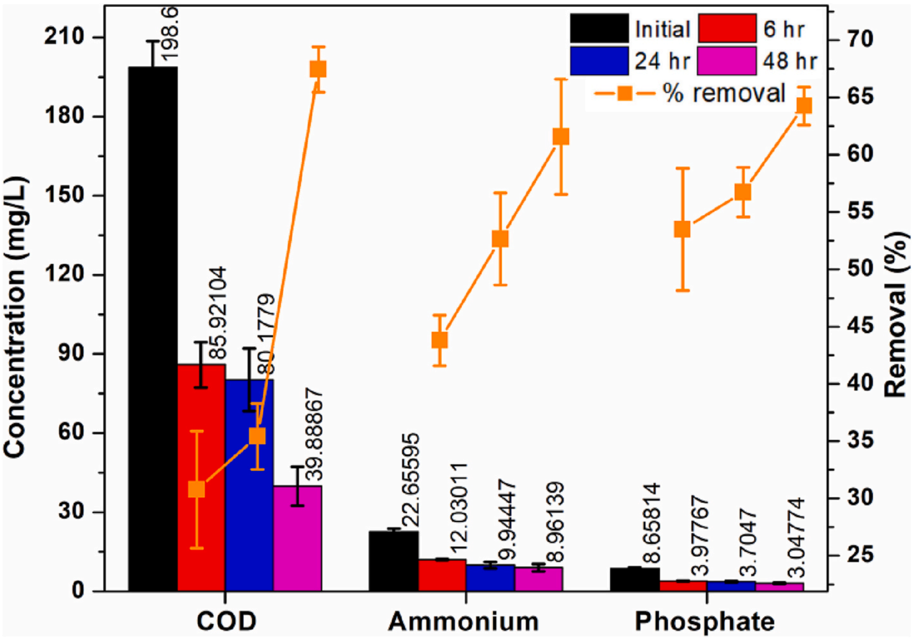

Fig. 6. Removal and concentration of COD, ammonium and phosphate at initial, 6 h, 24 h and 48 h contact time.

MFC. In the case of COD removal, plant roots might have played a role by providing a microaerobic zone for more organic degradation along with the availability of a high surface area for microbial growth and activity [18,48]. This might have led to direct COD reduction by heterotrophic and electroactive bacteria available in the root zone area [3]. In addition, the high biomass of *Canna indica* assisted the availability of more carbon sources as fuel for microorganisms to support microbial activity [1]. Similar results were demonstrated in a study where the impact on different plant species of a saline environment was investigated in CWs. *Canna indica* provided a large surface area for microbial growth along with high average root activity [49]. Moreover, the presence of conductive materials (both charge collector and graphite granules) supported high microbial attachment for biofilm growth owing to its porous nature and act as artificial electron acceptors in the anodic zone of CW-MFC; thus promoting the degradation of organic materials [6].

Ammonium removal efficiency in contact time of 48 h was noted to be 73.13 ± 5.01 % which was relatively higher than what was obtained in 6 h and 24 h, *i.e.*, 59.39 ± 2.21 %, 68.73 ± 4.01 % and respectively. These results are also in agreement with other findings, wherein the highest ammonium removal of 88.7 ± 2.5 % was achieved with *Iris pseudacorus* planted CW-MFC and least with unplanted CW-MFC (*i.e.*, 43.5 ± 9.1 %); also *Iris pseudacorus* accounted highest biomass growth along with the highest uptake of nutrients [21]. Similarly, other researchers reported average ammonium removal efficiency of 60 ± 40 % with variations in cathode position respective to the water level in planted CW-MFC [5]. Generally, due to the absence of electron acceptors, anaerobic reactions are usually slow in CWs [45]. However, the reasonable explanation for high ammonium removal in *Canna indica* planted CW-MFC could be i) conductive material acting as an electron acceptor in absent or oxygen-deficient regions, thus, assisting electron transport mechanism for the microbes [50]; ii) better environment for the growth of nitrifying bacteria (*i.e.*, *Nitrobacter*, *Nitrosomonas*) in high biomass *Canna indica* rhizosphere, where oxidation of ammonium to nitrate could take place in the aerobic microsites, adjacent to roots and rhizomes [3]; iii) high oxygen diffusion in the CW-MFC column due to increase in exposure of cathode with air because of high ET [33]; iv) uptake of ammonium as a nutrient by highly grown *Canna indica* [1], and v) adsorption on high biomass produced in the wetland matrix by *Canna indica* vegetation [21]. Ammonium removal is also advantageous for easy assimilation in the microbial and plant biomass due to its less mobile and biologically amenable nature, among several other forms of nitrogen [29].

Phosphate removal was estimated as 67.16 ± 5.33 %, 71.10 ± 2.17 %, and 75.60 ± 1.65 % for 6 h, 24 h, and 48 h, respectively. Phosphorus uptake through *Canna indica* could be the probable reason for high phosphate removal along with adsorption on filter media. Various studies also support this phenomenon due to the well-known fact that water-loving macrophytes (aquatic plants like *Canna indica*) have a binding capacity to uptake phosphorous from wastewater to their tissues [51].

4. Conclusion

Considerable bio-electricity enhancement and efficient pollutant removal efficiency were observed with *Canna indica* planted CW-MFC. Water losses *via* ET exposed cathode with air due to water level drop in cathode affects the performance of *Canna indica* planted CW-MFC in a positive direction. High water loss from the cathode zone creates vacant spaces which were filled by atmospheric air by diffusion and expose the cathode to atmospheric oxygen. This situation has developed air cathode-like conditions in *Canna indica* planted CW-MFC, producing 25.71 mW/m^3 power density and 85.71 mA/m^3 current density with a CE of 11.95 %. In contact time of 48 h, in *Canna indica* planted CW-MFC, a drop in the water level by 336 mm in the cathode electrode zone was recorded. This water loss at the cathode improved cathodic reduction kinetics. It improves the voltage generation from 182.5 ± 12.5 mV to 800 ± 13.47 mV. At the same time, pollutant concentration in terms of COD, ammonium, and phosphate substantially reduced to 80 ± 7.98 %, 73.17 ± 5.01 %, and 75.60 ± 1.65 %, respectively. This study suggests an important role of ET in the performance of CW-MFC, which should be considered while designing large-scale systems for real-life applications.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yamini Mittal kindly acknowledges the GATE-SRF fellowship provided by the Council of Scientific and Industrial Research (CSIR), New Delhi, India. She is also thankful to AcSIR, Uttar Pradesh, India for helping her to carry out her Ph.D. work. AKY is grateful to CSIR-IMMT and CSIR-INDIA for providing all the facilities and infrastructure through Major Laboratory Project Grant (MLP-059) for carrying out the work. AKY also acknowledge the grant under TMD Scheme (DST, New Delhi) [DST/TMD-EWO/WTI/2K19/EWFH/2019/109 (G2)].

Data availability

The authors do not have permission to share data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2023.103580.

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