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Effect of vegetation type on treatment performance and bioelectric production of constructed wetland modules combined with microbial fuel cell (CW-MFC) treating synthetic wastewater

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Abstract

An operation of microcosm-constructed wetland modules combined with microbial fuel cell device (CW-MFC) was assessed for wastewater treatment and bioelectric generation. One of the crucial aims of the present experiment is also to determine effect of vegetation on wastewater treatment process and bioelectric production in wetland matrix with microbial fuel cell. Accordingly, CW-MFC modules with vegetation had higher treatment efficiency compared to unplanted wetland module, and average COD, NH_4^+, and TP removal efficiency in vegetated wetland modules were ranged from 85 to 88%, 95 to 97%, and 95 to 97%, respectively. However, the highest NO_3^- removal (63%) was achieved by unplanted control module during the experiment period. The maximum average output voltage, power density, and Coulombic efficiency were obtained in wetland module vegetated with *Typha angustifolia* for 1.01 ± 0.14 V, 7.47 ± 13.7 mWatt/m^2, and $8.28 \pm 10.4\%$, respectively. The results suggest that the presence of *Typha angustifolia* vegetation in the CW-MFC matrix provides the benefits for treatment efficiency and bioelectric production; thus, it increases microbial activities which are responsible for biodegradation of organic compounds and catalyzed to electron flow from anode to cathode. Consequently, we suggest that engineers can use vegetated wetland matrix with *Typha angustifolia* in CW-MFC module in order to maximize treatment efficiency and bioelectric production.

Keywords Constructed wetlands · Microbial fuel cell · Vegetation · Wastewater treatment · Bioelectric production

Introduction

Nowadays, intensive industrialization together with rapid population growth and economic improvements have raised a growing demand on water sources, and thus wastewater is now being considered as one of the important resources of water, energy, and plant fertilizing nutrients (Mudakkar et al. 2013; Stuermer 2017). In this respect, effective wastewater

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management should include a holistic approach based on how the wastewater treats as cost-effective, less complex, and eco-friendly in an ecological systems and how the people can obtain the secondary benefits while treating wastewater (Rasilainen et al. 2010; Türker et al. 2016b, c).

Ecological wastewater treatment methodologies (EWTM) are engineered and man-made modules that mimic the natural purification mechanisms such as nitrification, denitrification, ammonification, and adsorption in order to treat wastewater and increase water quality (Türker et al. 2014; Türker and Yakar 2017; Vymazal and Březinová 2015). Among the ecological treatment technologies, constructed wetlands (CWs) are one of the attractive, cost-effective, eco-friendly, less complex, easy to operate, and alternative ecological treatment systems to remove organic and inorganic pollutants from the wastewater; thus, investigation and use of CWs for wastewater treatment have grown in popularity in recent years worldwide (Türker et al. 2017; Vymazal and Kröpfelová 2009). In this respect, more than 100,000 CW treatment systems worldwide currently treat over billion liters of wastewater per day,

and so they represent the unique ecosystem services to human societies through treating wastewater and improving water quality (Chen et al. 2012; Türker et al. 2014; Vymazal 2007).

Bio-electrochemical wastewater treatment methodologies (BWTM) demonstrated as an innovative and promising strategy in addressing energy production and environmental conservation issues associated with landscape lighting, low-power sensors, and bioremediation (Logan et al. 2006). In this technology, the biodegradable organic compounds in wastewater are defined as an energy source instead of undesirable waste, and bioelectric is generated while treating wastewater according to principles of microbial fuel cell (MFC) (Li et al. 2011; Mohan et al. 2011). Typically, a MFC device has an anode and a cathode chamber with a separator, as well as an external circuit (Li et al. 2011; Logan et al. 2006). In a MFC device, electrogenic active bacteria are capable of converting biodegradable substrate in wastewater into bioelectricity (Oon et al. 2015; Yadav et al. 2012). While biodegradable substrate at the anode chamber is oxidized and removed by microorganisms, proton (H^+) and electrons (e^-) are released and then they travel to cathode chamber (Li et al. 2011). As a result of this process, MFC devices produce a bioelectric current through the transfer of electrons from anode chamber to cathode chamber and electrode reactions are occurred.

The combination of ecological wastewater treatment methodologies (EWTM) with bio-electrochemical wastewater treatment methodologies (BWTM) such as CW-MFC is a new, innovative, sustainable, and intriguing concept in order to treat wastewater and generate electricity at the same time (Oon et al. 2015; Türker and Yakar 2017). In a CW-MFC module, the chemical energy is harvested both organic matter in wastewater and various rhizodeposition products including root exudates and secretion by electrogenic bacteria in the wetland matrix (Corbella et al. 2015; Liu et al. 2013). This unique operational characteristic of a CW-MFC may make an ideal approach to treating wastewater by removing organic pollutants, as well as generating electricity in the same systems, especially when instillation, operation, and maintenance costs are limiting factors for applying other treatment technologies such as conventional methods. However, CWs combined with MFC for wastewater treatment are relatively new technologies in terms of the literature and biological, chemical, and physical processes ongoing pollutants purification of which are not well understood yet (Oon et al. 2016; Oon et al. 2017). In particular, knowledge of the roles played by vegetation in CW-MFC units for wastewater treatment and bioelectric production is still lacking, and not much direct research has evaluated effect of vegetation in facilitating to generate bioelectric in CW-MFC modules so far.

Fuel sources in a CW-MFC system come from organic pollutants in wastewater and the plant rhizodeposits in wetland matrix (Ong et al. 2010; and Yakar 2017). Therefore, the purification and bioelectric production performance of the CW-MFC are based on the combined action of associated microorganisms, plant roots, and type of rhizodeposition (Corbella et al. 2015; Oon et al. 2016). Correspondingly, bacteria in the rhizosphere play the most crucial role on purification performance and they break down the organic compounds and release electrons in the CW-MFC, and plant vegetation has been shown to improve the bacterial activity in order to eliminate organic pollutants and generate bioelectric (Corbella et al. 2015; Liu et al. 2013). Besides, due to the presence of vegetation in the CW-MFC units, releasing carbon sources as a rhizodeposit from plant roots supporting the growth and activity of microorganism associated with bioelectric production. In this respect, the active reaction zone of a CW-MFC is its rhizosphere where physical, chemical, and biological processes happen through the complex interactions between microorganisms, plants, rhizodeposits, and contaminants (Chen et al. 2012; Liu et al. 2013; Oon et al. 2016). Accordingly, each plant species used in CW-MFC has its own chemical composition, ecological demands, and physiological characteristic and these properties may directly affect the removal performance of CW treatment systems, as well as bioelectric production in CW-MFC systems. Therefore, the selection of suitable vegetation for rhizosphere plays a crucial role in a CW-MFC matrix to obtain higher pollutant removal and produce more bioelectric by electrogenic bacteria. However, information about the effect of vegetation structure of CW-MFC in organic matter removal simultaneous bioelectric production is currently very limited. Although, some pioneering research showed that plants in wetland matrix may provide potential benefits to improve treatment efficiency and bioelectric production (Fang et al. 2013; Oon et al. 2017), these studies have various limitations in their practices: (1) information about effect of different plant species on treatment and bioelectric production is still insufficient. In this respect, Fang et al. (2013) and Oon et al. (2017) evaluated the plant effect by using only one and uncommon plant species (either *Ipomoea aquatica* or *Elodea nuttallii*) in their CWs; however, they did not address the comparison and effect of popular and common species (e.g., *Typha latifolia* L., *Typha angustifolia* L., *Juncus gerardii*, and *Carex divisa*) on treatment performance and bioelectric production in the CW-MFC systems. (2) Lack of knowledge associated with plant responses (growth, biomass production, and pigment concentrations) in wetland matrix during the treatment and bioelectric production period. (3) There are no results related to the effect of vegetation structure on soil biochemistry during the wastewater treatment simultaneous bioelectric production in CW-MFC matrix. Therefore, we suggest that the results from the present study could be move one step forward to the importance of vegetation in CW-MFC systems which provided some

perspectives in terms of the practical application while remediating wastewater and bioelectric production.

In the present experiment, using the same media, four different and common plant species of *Typha latifolia* L., *Typha angustifolia* L. (Typhaceae), *Juncus gerardii* Loisel. subsp. *gerardii* (Juncaceae), and *Carex divisa* HUDSON (Cyperaceae) were tested as plant vegetation in designing constructed microcosm wetland modules combined with microbial fuel cell devices (CW-MFC), and effect of these different vegetation on pollutant treatment process and bioelectric production in the CW-MFC were studied in terms of the ecotechnological perspective.

As mentioned above, the objectives of the present experiment are the following: (1) to test the feasibility and efficacy of microcosm scale CW-MFC modules with vegetation to remediate wastewater and produce bioelectric simultaneously; (2) to investigate the effect of vegetation type and different plant species on treatment performance and bioelectric production. In this case, a well-designed CW-MFC may combine with the best vegetation structure in order to obtain higher wastewater treatment performance and generate higher bioelectric production; (3) to examine and compare wastewater removal and bioelectric production in vegetated and unplanted wetland modules. (4) To assess the effect of vegetation on some important soil enzyme activities (dehydrogenase, urease, and phosphatase) while remediating wastewater process and generating bioelectric.

Material and methods

Microcosm module design

The study was performed in Anadolu University, Department of Biology, Eskişehir, Turkey. The climate of the research site is semi-arid Mediterranean that is characterized by mean annual precipitation of 373.8 mm, average temperature 10.8 °C.

Ten parallel constructed microcosm wetland (CW-MFC) modules (identified as M_1, M_2, M_3, M_4, and M_5) were fabricated and designed using polyester chambers with 45 cm length, 45 cm width, 40 cm depth, and 0.2 m² total surface area for each module. The CW-MFC modules were placed outside and divided into five groups (with two replicate) employing either *T. latifolia* (M_1), *T. angustifolia* (M_2), *J. gerardii* (M_3), *C. divisa* (M_4), or unplanted control (M_5) according to the presence of main vegetation in their matrix. Selected macrophytes were collected from natural wetlands in Eskişehir, and the rhizomes were immediately transplanted in the related modules at a plant density of about 10 rhizomes/m². In the present experiment, coarse sand was selected as the filtration media for the modules, and 5 cm of gravel (Ø 50–80 mm) was placed on the filtration media and effluent section

of modules. Moreover, each CW-MFC module had a 5-cm deep inlet section of 5–8 mm gravel at its base.

A horizontal rectangular (2×8 cm and 1 cm thickness) magnesium cathode (C) was selected due to high electrical conductivity and it was located 5 cm below the system surface for each CW-MFC module (Yadav et al. 2012). Furthermore, a graphite-based anode (A_1) (2×8 cm and 0.5 cm thickness, 32 cm² total surface area) was also placed into filtration media, 20 cm above of the each CW-MFC module. A glass wool layer (0.01 cm thickness) was located in the each CW-MFC module in order to separate the cathode and anode compartments. In this case, we believed that the separation of cathode and anode chambers in each CW-MFC module was to facilitate the magnesium and graphite electrodes separation being brought to a minimum operational risk of these electrodes coming into contact in MFC devices in the modules (Doherty et al. 2015b). Finally, the cathode and anode were connected with insulated copper wires across a 1000 Ω (Fig. 1).

Culture period, wastewater dosage, and operation of wetland modules

The CW-MFC modules were fed with mixture of Hoagland medium and sludge which was collected from a treatment plant for domestic wastewater for 75 days in order to support the vegetation growth and establish microorganisms (Oon et al. 2015). In this case, the opinion is to this composition of mixing sludge contains both essential minerals such as Ca, Mg, Na, K, N, P, and various micro elements for plants and active associated microorganisms which are related to bioelectric production. Besides, anode of each CW-MFC modules was inoculated with the sludge for a period of 1 month prior to experimental start up.

In the present study, the experiment was carried out with modified synthetic wastewater suggested by Liu et al. (2013). Main composition of this synthetic wastewater are glucose (0.20 g L^{-1}), NH_4Cl (0.15 g L^{-1}), KCl (0.13 g L^{-1}), $NaHCO_3$ (3.13 g L^{-1}), and 1 mL micro elements solution (contained per liter: 5.6 g $(NH_4)_2SO_4$, 2 g $MgSO_4 \cdot 7H_2O$, 200 mg $MnSO_4 \cdot H_2O$, 3 mg H_3BO_3, 2.4 mg $CoCl_2 \cdot 6H_2O$, 1 mg $CuCl_2 \cdot 2H_2O$, 2 mg $NiCl_2 \cdot 6H_2O$, 5 mg $ZnCl_2$, 10 mg $FeCl_3 \cdot 6H2O$, and 0.4 mg $Na_2MoO_4 \cdot 2H_2O$). Furthermore, this synthetic wastewater was modified according to chemical oxygen demand (COD), ammonium (NH_4^+), Nitrate (NO_3^-), and total phosphorus (TP) concentration during the experiment period, and thus the influent concentrations of COD, NH_4^+, NO_3^-, and TP were ranged between 103 and 554 mg L^{-1}, 19.9–225.5 mg L^{-1}, 12.6–191.5 mg L^{-1}, and 10–29.8 mg L^{-1}, respectively.

The synthetic wastewater was stored in a 50-L polyethylene influent tank which was continuously stirred. The wastewater was supplied to each module by using a peristaltic pump which was controlled by a timer. The modules were operated

under the same hydraulic loading rate of 83 mL/h and dosed every 8 h over the period of 24 h, so the hydraulic retention times of the units were to set to 4 days. The wetland modules operated continuously for a period of 88 days until the vegetation showed wilting at the end of October 2016.

Wastewater sampling and analysis

The water samples from influent and effluent were collected in order to evaluate treatment performance of each CW-MFC modules. Wastewater samples were taken according to the hydraulic retention time of wetland modules (4 days), and physicochemical parameters such as pH, electrical conductivity (EC), dissolved oxygen (DO), and redox potential (ORP) were measured with HACH HQ40D multi-parameter meter concurrent with sampling. The concentration of chemical oxygen demand (COD) from different modules was determined with a colorimeter (HACH DR/890). The concentration of ammonium (NH_4^+) and Nitrate (NO_3^-) was measured by using ammonium electrode (INTELLICAL ISE ammonium electrode, 2406549) and nitrate electrode (INTELLICAL ISE Nitrate electrode, 2984790) connected HACH HQ40D multi meter. Total phosphorus (TP) concentration was also determined by a colorimeter (HACH DR/890) following the manufacturer's instructions immediately after sampling.

Plant monitoring, biomass production, and pigment concentrations

Plant height in each module was monitored every 4 days, and any instances of plant disease were recorded. Plant height was calculated as the average height of leaves in the CW-MFC module. Also, all plant biomass both aboveground and belowground were collected from the CW-MFC module to determine dry biomass of plant at the end of the experiment. Dry biomasses of all plant species were calculated by drying the harvested biomass in an oven at 65 °C for 48 h. Photosynthetic pigments (Chlorophyll a and b) concentrations of plants in each module were also measured during the experiment period. For the analysis, fresh tissues from mature leaves of plants were collected every 4 days from modules, and photosynthetic pigments were determined according to Wellburn (1994).

Bioelectric monitoring and measurement

The bioelectric production performance of each module was determined with daily measurements of the voltage drop (V) across the external resistor using a digital handheld multimeter (Fluke 287 TRUE RMS) described before (Zhao et al. 2013). According to this procedure, the cathode and anode were connected with wires and resistance, and then the potential between edges of the resistance were continuously monitored. Moreover, power density was calculated by dividing the

power and current by surface area (m²) of anodes (Oon et al. 2015). After the 88th day of operation, polarization curves were determined by varying the external resistance from 1 to 580.000 Ω finally to infinity at open circuit and measuring the steady state voltage across the resistor within 15 min (Doherty et al. 2015a, b).

Soil enzyme activities

Sediment samples were also collected from each module every 4 days in the experiment period in order to determine some soil enzyme activities (dehydrogenase, urease, and phosphatase). These enzymes have a crucial role associated with the cycling of carbon (dehydrogenase), nitrogen (urease), and phosphorus (phosphatase) in a wetland matrix (Zhang et al. 2010). The collected media samples from each module were sieved and then stored in refrigerator at 4 °C prior to analysis of enzyme activity within one week. The dehydrogenase, urease, and phosphatase enzyme activities in sediment of the modules were determined according to Kong et al. (2009).

Calculation and statistical analysis

The COD, NH_4^+, NO_3^-, and TP removal efficiencies of each module were calculated as:

$$Removal\ performance\ (\%) = [(C_i - C_e)/C_i] \times 100 \qquad (1)$$

where C_i and C_e are the COD, NH_4^+, NO_3^-, and TP concentrations of influent and effluent samples in mg L^{-1}.

Current (I) was determined according to Ohm's law using the formula:

$$I = V/R \qquad (2)$$

where V corresponds to cell voltage and R (Ω) is the external resistance.

Power (W) is determined by using the formula:

$$P = I \times V \qquad (3)$$

where I corresponds to current (A) and V (V) is cell voltage. Moreover, power density was calculated by dividing the power and current with surface (m²) of anode (Oon et al. 2015).

Coulombic efficiency (CE) of each module was determined as:

$$CE = \frac{I \times M}{F \times q \times n \times \Delta COD} \qquad (4)$$

where CE is Coulombic efficiency (%), M is molecular mass of O_2 (32 g O_2/mol O_2), I is current (A) and F is Faraday's constant (C/mol), which is 94,685. q is flow rate (L/s), n is number of electrons donated per mole O_2 (4 mol e$^-$/mol O_2), Finally, ΔCOD represents the change in COD between

influent and effluent (mg/L) (Oon et al. 2016).

In order to analyze the performance of each module, statistical tests were carried out with SPSS version 18.0 of the statistical software package and a statistical confidence of $p < 0.05$. The Shapiro-Wilks test was performed on the normality of the data. The statistical relations between influent COD, NH_4^+, NO_3^-, and TP concentrations and those of in effluent of each module were determined with a one-way ANOVA test (for example, in order to determine if the concentrations in the effluent are lower than those in the influent). Moreover, determination statistical differences among the bioelectric production and soil enzyme activities in different modules were also analyzed by a one-way ANOVA test.

Results and discussion

Plant monitoring and evaluation

As seen in Table 1, the average plant's height in M_1, M_2, M_3, and M_4 were measured as 66.5 ± 8.37 cm for *Typha latifolia*, 142.8 ± 8.08 cm for *Typha angustifolia*, 82.6 ± 6.63 cm for *Juncus gerardii*, and 75.4 ± 8.2 cm for *Carex divisa*. On the other hand, one-way ANOVA statistical analysis indicated significant differences for plant height between all macrophytes growing in wetland modules ($p < 0.05$). This result suggested that plant species which were grown in the same supporting media were shown their own speciation and growth characteristic during the wastewater purification process. Moreover, the plant species in the wetland modules reached the maximum vegetative growth during the summer

months (June, July, and August), whereas some of the leaves of plant turned yellowish due to the decrease of the outside temperature after September.

The photosynthetic pigments in individual species were determined to assess healthy growth trend during the experiment period. The chlorophyll pigment concentrations of plants in CW-MFC units were found relatively similar ($p > 0.05$); however, higher chlorophyll a and b pigment concentrations were determined as 19.2 ± 6.4 and 13.01 ± 3.21 µg L^{-1} for *J. gerardii* and *C. divisa* growing in modules 3 and 4 (Table 1). This may be explained by several causes such as operational conditions or growth condition associated with vegetation structure (Türker et al. 2016a).

The whole biomass content of plant species was calculated as 655.5, 1113.9, 678.6, and 409.7 DW g/m^2 for M_1, M_2, M_3, and M_4, respectively. In this respect, it can be seen that *T. angustifolia* in wetland module produced more biomass compared to those of other plant species. This phenomenon is important because higher biomass in a wetland matrix might also have catalyzed bioelectric production (Fang et al. 2013), so more biomass production in the wetland module could have enhanced the cell voltage, and reduced the internal resistance of system, resulting more bioelectric production (Türker and Yakar 2017).

Treatment performance of the CW-MFC modules

COD monitoring and effect of vegetation on COD removal

Each CW-MFC module was continuously fed with synthetic wastewater which contained glucose as a carbon source for a

Table 1 Plant monitoring, pigment concentrations, and biomass production of individual plants in CW-MFC modules during the experiment period

		Leaves	Stems	Roots	Whole plant
Typha latifolia growing in M_1	Plant height (cm)	66.5 ± 8.37	–	–	–
	Chlorophyll a content (µg L^{-1})	18.5 ± 0.3	–	–	–
	Chlorophyll b content (µg L^{-1})	11.2 ± 0.17	–	–	–
	Biomass production (DW g/m^2)	113.6	439.7	102.2	655.5
Typha angustifolia growing in M_2	Plant height (cm)	142.8 ± 8.08	–	–	–
	Chlorophyll a content (µg L^{-1})	15.2 ± 1.3	–	–	–
	Chlorophyll b content (µg L^{-1})	10.1 ± 0.9	–	–	–
	Biomass production (DW g/m^2)	287.9	706.8	119.1	1113.9
Juncus gerardii growing in M_3	Plant height (cm)	82.6 ± 6.63	–	–	–
	Chlorophyll a content (µg L^{-1})	19.2 ± 6.4	–	–	–
	Chlorophyll b content (µg L^{-1})	11.2 ± 3.76	–	–	–
	Biomass production (DW g/m^2)	334.6	254.2	90.1	678.6
Carex divisa growing in M_4	Plant height (cm)	75.4 ± 8.2	–	–	–
	Chlorophyll a content (µg L^{-1})	18.8 ± 2.5			
	Chlorophyll b content (µg L^{-1})	13.01 ± 3.21			
	Biomass production (DW g/m^2)	118.2	250.3	41.2	409.7

total of 88 days. Correspondingly, the average concentration of COD in the influent and effluent, as well as average COD removal performance of each treatment modules over the entire experiment period are shown in Table 2 and Fig. 2a. It can be seen that average COD concentrations in the influent for the modules was 344 ± 143 mg L^{-1}, and it was ranged from 103 to 554 mg L^{-1} during the experiment period. The effluent COD concentrations taken from all the units were much lower than the influent samples and one-way ANOVA statistical analysis indicated that significant statistical differences between influent COD concentrations in wastewater samples and the effluent from all modules ($p < 0.05$). These results showed that CW-MFC modules with different vegetation types are capable of removing organic matters from synthetic wastewater, and thus such CW-MFC units are clearly developed and used as an alternative, environmental-friendly, and cost-effective treatment option for the removal of organic-based contaminants from wastewater.

Figure 2a, b shows that under the conditions of the same influent load, COD concentrations in the effluent from the vegetated wetland modules were mostly lower than COD concentrations in effluent of unplanted control module; however, no statistical differences were found between vegetated modules and unplanted control ($p > 0.05$). Nevertheless, we determined the strong differences between vegetated and unplanted wetland modules ($p < 0.254$ for M_1, $p < 0.338$ for M_2, $p < 0.201$ for M_3, and $p < 0.192$ for M_4), and these differences in the removal of COD between vegetated and unplanted modules can be an indication associated with lower influent load including organic matter during the experiment period. In this respect, significant relationships associated with the role of plant may be inferred if higher organic matter loading and long-term period were provided during the experiment (Chen et al. 2012; Liu et al. 2013; Oon et al. 2017). Clearly, further studies which have longer study period are needed for deep understanding impact of vegetation on COD removal mechanisms.

As shown in Table 2, COD removal rates were different among five wetland modules as follows: $M_1 > M_4 \approx M_3 > M_2 > M_5$ (unplanted control), and it can be seen that vegetated units have higher removal efficiencies than unplanted control. This result suggested that presence of vegetation in the wetland matrix had a positive effect with a better filtering capacity for organic matter degradation at vegetated modules even under varying initial COD dosage. It was an expected result because vegetated wetlands present more specific surface area for microorganisms associated with organic matter degradation, and thus more carbon sources as fuels were available for microorganisms in order to support microbial activity and bioelectric generation compare to unplanted control module. This is in agreement with various studies in the literature that presence of the vegetation had significant impact on the reduction of organic matter and contributes the biodegradation of organic matter by microorganisms in the wetland matrix (Chen et al. 2012; Vymazal and Kröpfelová 2009; Zurita et al. 2009). Furthermore, the greater microbial attachment on the anodes of vegetated wetland units might enhance the COD removal performance, and thus more electrons were traveled from the anode to the cathode in vegetated modules compare to that of unplanted control.

On the other hand, COD removal was slightly differed to wetland modules during the experiment period; however, no statistical differences were found between modules ($p > 0.05$). This result gives the evidence that COD removal mechanisms are the same as that of all vegetated wetland matrix and plants in the modules that showed similar behavior against organic compounds during the study period. Although we did not determine any statistical differences, it can be also concluded that COD concentrations in treated wastewater might be dependent on the vegetation and plant species type in the wetland matrix. In this respect, the COD removal between different wetland modules can be explained with the different ratio of the mineralization of organic matter in the different wetland matrix. Accordingly, different exudates which release form the plant roots were catalyzed different microbial attachment in the wetland matrix, and different growth conditions for the microorganisms in the wetland matrix result in different microbial activity, and as a results different the decomposition rate of the organic substrate in the wastewater. This could explain the slightly best performance of CW-MFC module with *T. latifolia* vegetation in removing COD from wastewater. In this case, *T. latifolia* shown their own growth conditions to create the biofilm forming in the wetland matrix, and it actually favors microbial attachment associated with COD removal.

Ammonium (NH_4^+) monitoring and effect of vegetation on NH_4^+ removal

Nitrogen removal in the wetland environment is mainly based on a pathway of ammonification (the conversion of organic nitrogen-N to ammonium-NH_4^+), followed nitrification (oxidation of NH_4^+ to NO_2^- followed by the oxidation of the NO_2^- to NO_3^-), and basic denitrification (reduction of NO_3^- to NO_2 to NO to N_2O to N_2) (Zhang et al. 2010). Furthermore, nitrification and denitrification processes have a crucial role that contributed to nitrogen removal in a wetland environment. Specifically, nitrification process does not remove any nitrogen, it only transforms ammonia to nitrate (Vymazal and Březinová 2015). In this respect, ammonium (NH_4^+) and nitrate (NO_3^-) concentrations in the wetland modules with different vegetation types were investigated in order to assess the nitrogen removal behavior in CW-MFC modules.

As seen in Fig. 3a, CW-MFC modules achieved significant NH_4^+ reduction during the study ($p < 0.05$), the average NH_4^+ concentrations were decreased from 45.7 ± 32.7 to $1.97 \pm$

Table 2 Average physicochemical parameters and average COD, NH_4, NO_3, and TP values in influent and effluent, as well as removal performances for CW-MFC modules in the experiment period

Treatment module	Sample	pH[a] ($-\log[H+]$)	EC[a] ($\mu S\ cm^{-1}$)	Temperature[a] (°C)	Dissolved oxygen[a] ($mg\ l^{-1}$)	Redox[a] (mV)	COD[a] ($mg\ l^{-1}$)	NH_4[a] ($mg\ l^{-1}$)	NO_3[a] ($mg\ l^{-1}$)	TP[a] ($mg\ l^{-1}$)
M_1 (*T. latifolia* vegetation)	Influent	7.81 ± 0.25	1181 ± 425	22.5 ± 2.3	7.92 ± 0.83	95.37 ± 31.73	344 ± 143*	45.7 ± 32.7*	25.08 ± 16.5*	20.7 ± 9.84*
	Effluent	7.50 ± 0.23	1803 ± 311	19 ± 6.1	5 ± 0.9	55 ± 17.7	30.06 ± 88*	1.97 ± 1.43*	10.42 ± 4.304*	0.76 ± 1.26*
	Removal (%)	–	–	–	–	–	88.25 ± 11.8	95.33 ± 1.93	52.68 ± 13.81	96.38 ± 5.64
M_2 (*T. angustifolia* vegetation)	Influent	7.81 ± 0.25	1181 ± 425	22.5 ± 2.3	7.92 ± 0.83	95.37 ± 31.73	344 ± 143*	45.7 ± 32.7*	25.08 ± 16.5*	20.7 ± 9.84*
	Effluent	7.47 ± 0.19	1829 ± 398	18.9 ± 6.19	5.12 ± 0.9	43.4 ± 21	34.3 ± 19.3*	1.06 ± 0.68*	11.18 ± 3.15*	0.61 ± 0.95)*
	Removal (%)	–	–	–	–	–	85.4 ± 16.3	97.3 ± 1.57	55.52 ± 18.47	97.05 ± 4.408
M_3 (*J.gerardii* vegetation)	Influent	7.81 ± 0.25	1181 ± 425	22.5 ± 2.3	7.92 ± 0.83	95.37 ± 31.73	344 ± 143*	45.7 ± 32.7*	25.08 ± 16.5*	20.7 ± 9.84*
	Effluent	7.49 ± 0.17	1589 ± 279	19.8 ± 9.9	5.5 ± 0.9	56.9 ± 20.1	30.5 ± 21.8*	1.26 ± 0.57*	11.21 ± 2.07*	0.85 ± 1.28*
	Removal (%)	–	–	–	–	–	87.19 ± 16.01	96.3 ± 2.35	41.99 ± 24.06	95 ± 5.49
M_4 (*C. divisa* vegetation)	Influent	7.81 ± 0.25	1181 ± 425	22.5 ± 2.3	7.92 ± 0.83	95.37 ± 31.73	344 ± 143*	45.7 ± 32.7*	25.08 ± 16.5*	20.7 ± 9.84*
	Effluent	7.56 ± 0.15	1880 ± 361	20.16 ± 4.08	5.6 ± 0.8	60.9 ± 18.5	30.2 ± 17.9*	1.77 ± 0.95*	11.34 ± 0.85*	0.61 ± 0.86*
	Removal (%)	–	–	–	–	–	87.8 ± 13.7	95.4 ± 1.54	38.8 ± 27.78	96.9 ± 4.03
M_5 (unplanted control)	Influent	7.81 ± 0.25	1181 ± 425	22.5 ± 2.3	7.92 ± 0.83	95.37 ± 31.73	344 ± 143*	45.7 ± 32.7*	25.08 ± 16.5*	20.7 ± 9.84*
	Effluent	7.87 ± 0.32	1362 ± 615	19 ± 6.2	4.4 ± 1	57.03 ± 17	67.9 ± 30.3*	6.49 ± 7.68*	9.46 ± 6.68*	6.16 ± 5.74*
	Removal (%)	–	–	–	–	–	75.81 ± 17.1	88.1 ± 7.15	63.6 ± 13.3	71.77

An asterisk (*) denotes significant difference ($p < 0.05$) of the mean value between the samples during the treatment period. Plus-minus sign (±) denotes standard deviation
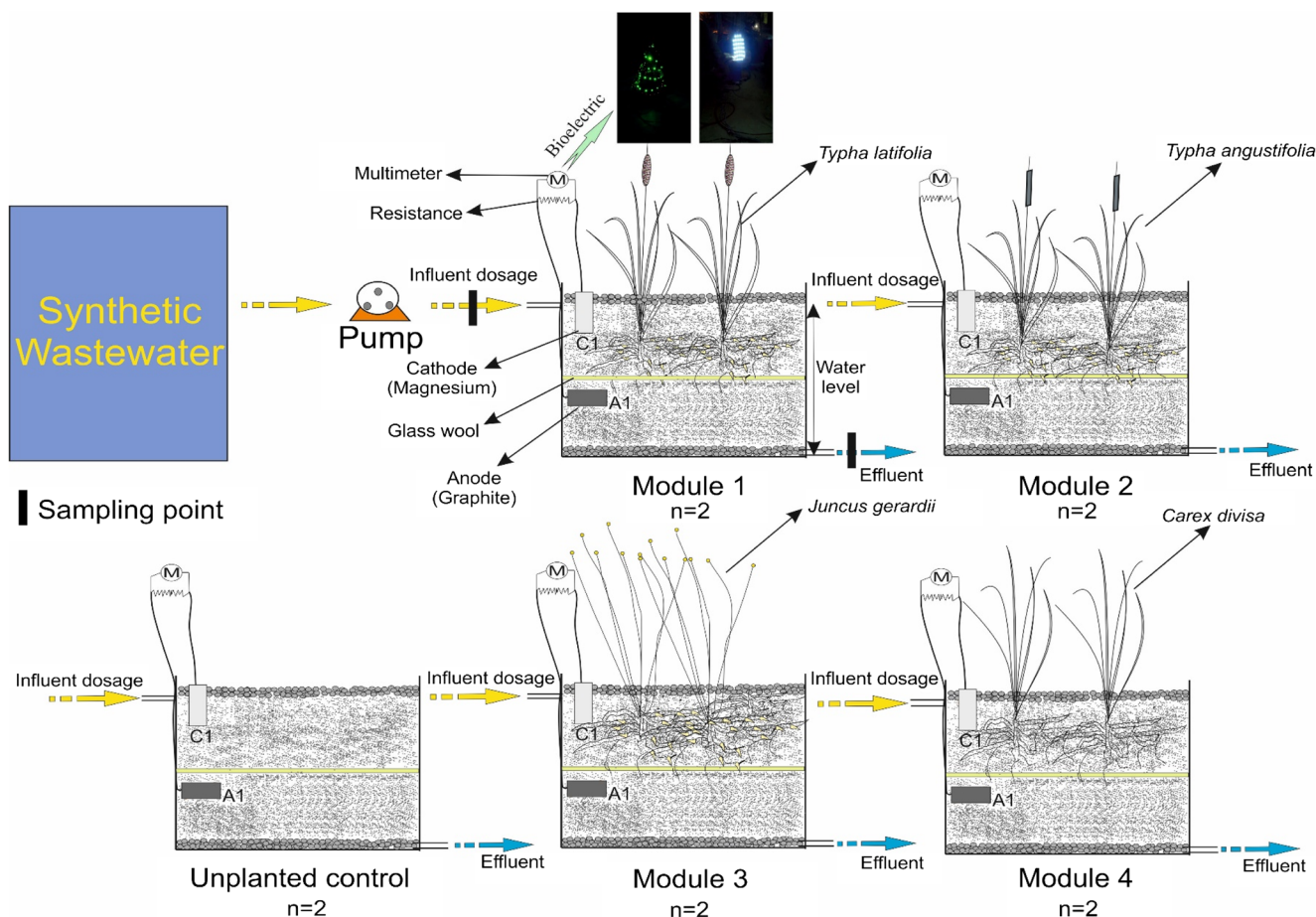
Fig. 1 The schematic demonstration of CW-MFC modules with different vegetation type

1.43, 0.88 ± 0.73, 1.26 ± 0.57, 1.77 ± 0.95, and 6.49 ± 7.68 mg L^{-1} through M_1, M_2, M_3, M_4, and M_5 wetland units, respectively, and suggesting that all modules are capable of

removing NH_4^+ from wastewater. On the other hand, Table 2 shows that NH_4 removal efficiencies improved when the presence of vegetation in the wetland matrix; however, this
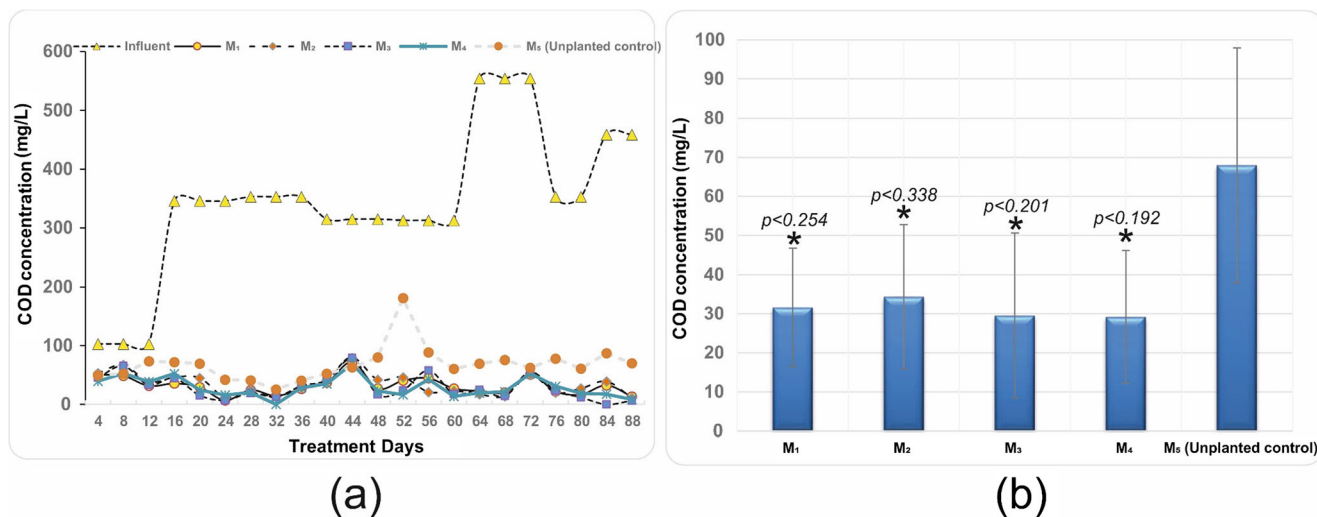

Fig. 2 a Chemical oxygen demand (COD) concentrations in the influent and the effluent of M_1, M_2, M_3, M_4, and M_5 (unplanted control) wetland modules during the experiment. b Average COD concentration in the effluent from treatment modules and control module (M_5), as well as

significance value according to control module in the experiment period. Error bars indicate standard deviation. An asterisk (*) denotes significant difference ($p < 0.05$) between vegetated and unplanted control
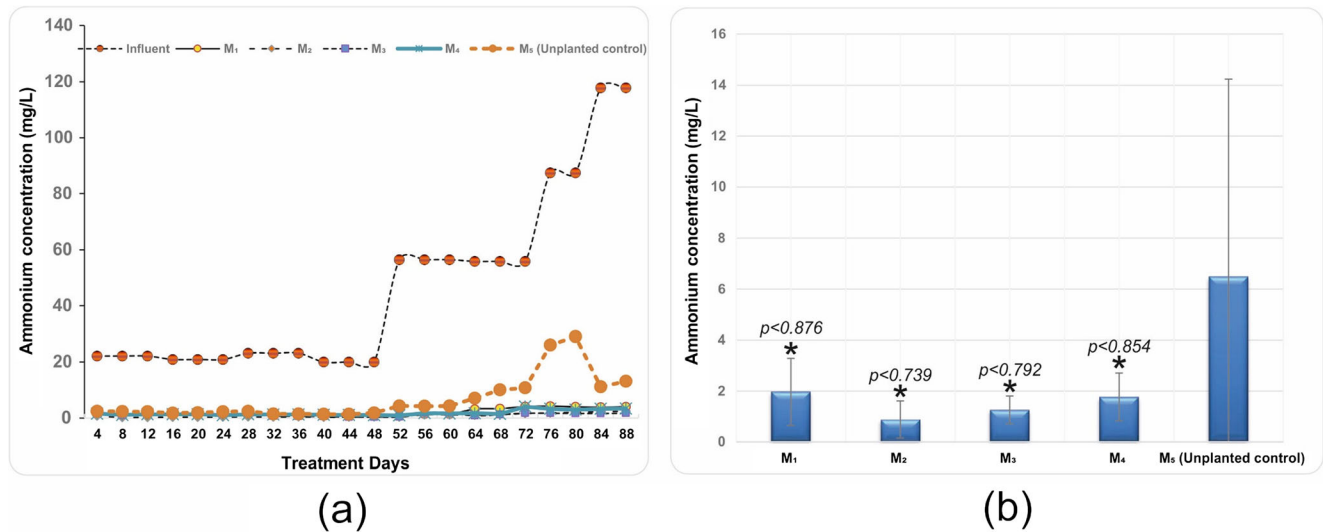
(a)

(b)

Fig. 3 **a** Ammonium (NH_4) concentrations in the influent and the effluent of M_1, M_2, M_3, M_4, and M_5 (unplanted control) wetland modules during the experiment. **b** Average Ammonium (NH_4) concentration in the effluent from treatment modules and control module (M_5), as well as significance value according to control module in the experiment period. Error bars indicate standard deviation. An asterisk (*) denotes significant difference ($p < 0.05$) between vegetated and unplanted control

phenomenon is not significant ($p > 0.05$). Furthermore, the concentration of NH_4 was further decreased at the final effluent and resulted in $95.33 \pm 1.93\%$, $98.1 \pm 0.57\%$, $96.3 \pm 2.35\%$, $95.4 \pm 1.54\%$, and $88.1 \pm 7.15\%$ of average NH_4^+ removal in the M_1, M_2, M_3, M_4, and M_5 wetland modules, respectively. In this respect, the strong statistical differences were found between vegetated and unplanted systems ($p < 0.876$, $p < 0.739$, $p < 0.792$, and $p < 0.854$ for M_1, M_2, M_3, M_4, and M_5 wetland modules, respectively) (Fig. 3b). More NH_4^+ reduction were recorded for vegetated wetland modules compare to unplanted module because the removal of NH_4^+ was mainly depended on nitrification process which happened in the presence of oxygen. The reasonable explanations for these distinctions might be that oxygen provided by vegetation during photosynthesis was used by nitrifying bacteria for nitrification process, biodegradation of organic substrate, and electron acceptor for bioelectric production in the MFC device within wetland units. As a result, a good aerobic environment in the presence of the vegetation was more suitable to full nitrification of NH_4^+; therefore, the NH_4^+ treatment performances of vegetated wetland modules were better than unplanted wetland module.

On the other hand, it can be seen in Table 2 that the best NH_4^+ removal performance is obtained by treatment module with *T. angustifolia* vegetation (M_2) in the experiment period. In this respect, the better NH_4^+ removal efficiency in M_2 module could be also related to the greater root system of *T. angustifolia*. As shown in Table 1, *T. angustifolia* growing in treatment module (M_2) has high biomass than different species grown in other modules, suggesting that stronger stability and larger surface area were created in the M_2 media, and so it presented a good growth environment for nitrifying

bacteria. Accordingly, NH_4^+ removal in CW-MFC greatly increased with presence of *T. angustifolia* vegetation in the wetland matrix, and it can be suggested that the nitrification potential was enhanced through increased nitrifying bacteria colonized on a rhizosphere surface of *T. angustifolia*, which is more adaptive to the growth of nitrifying bacteria compare to other species. Thus, it can be also concluded that high biomass production reinforced efficiency and reliability in NH_4^+ removal in a constructed wetland couple with microbial fuel cell. Various researchers also demonstrated that NH_4^+ removal and adsorption increased with high biomass production in the wetland matrix, and thus wetland plant species which has higher biomass production has higher removal efficiencies than those of the other species (Helder et al. 2010; Zhou et al. 2017).

Nitrate (NO_3^-) monitoring and effect of vegetation type on NO_3^- removal

As seen in Fig. 4a, NO_3^- concentration in effluents of the CW-MFC modules were lower than influent NO_3^- concentrations, and these reductions of NO_3^- in module matrix were significant as well ($p < 0.05$). On the other hand, it can be seen in Table 2 and Fig. 4a that NO_3^- concentrations in effluents of the vegetated wetland modules were higher than unplanted control module, and the average NO_3^- removal efficiencies were determined as $52.68 \pm 13.81\%$, $55.52 \pm 18.47\%$, $41.99 \pm 24.06\%$, $38.8 \pm 27.78\%$, and $63.6 \pm 13.3\%$ for M_1, M_2, M_3, M_4, and M_5 wetland modules, respectively. Moreover, one-way ANOVA statistical analysis results showed significant differences between treatment modules and unplanted system ($p < 0.04$, $p < 0.02$, $p < 0.03$, and $p < 0.04$ for M_1, M_2, M_3, M_4,
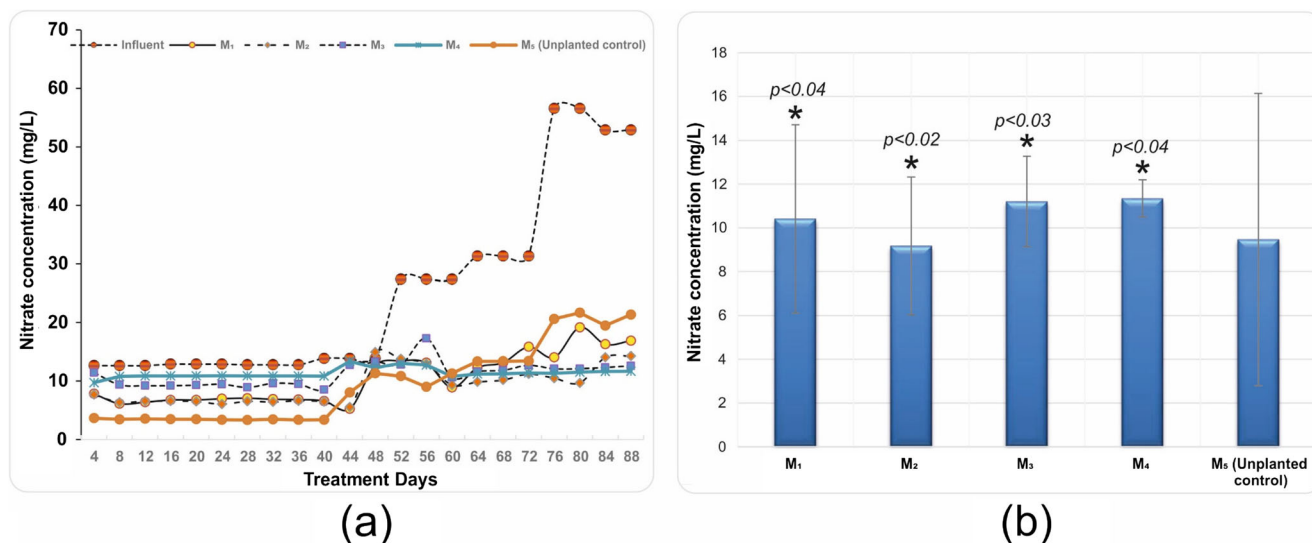
Fig. 4 **a** Nitrate (NO_3^-) concentrations in the influent and the effluent of M_1, M_2, M_3, M_4, and M_5 (unplanted control) wetland modules during the experiment. **b** Average Nitrate (NO_3^-) concentration in the effluent from treatment modules and control module (M_5), as well as significance value according to control module in the experiment period. Error bars indicate standard deviation. An asterisk (*) denotes significant difference ($p < 0.05$) between vegetated and unplanted control

and M_5 wetland modules, respectively) Fig. 4b. These results indicated that the concentration of NO_3^- in the influent was mostly removed through unplanted matrix for a CW-MFC module, and the concentration of NO_3^- in the vegetated wetland modules gradually increased because the diffusion of oxygen and oxygen release from plant roots to wetland matrix inhibited microbial denitrification. Nevertheless, it can be also suggested that the combination of graphite anode as selected electrode and plant's roots in the module matrix provided good specific surface area to microorganism' growth, especially the growth of nitrifiers and denitrifiers in the process of nitrification and denitrification. Therefore, the higher NO_3^- removal efficiencies can achieve using a vegetated wetland module through the use of graphite anode. However, further studies should be investigated on the role of electrodes in the nitrification and denitrification process in the vegetated wetland matrix. All of the results from the NH_4^+ and NO_3^- removal data indicated that NH_4^+ and NO_3^- in wastewater were removed biologically in the module matrix and the higher NH_4^+ and NO_3^- could achieved in a CW-MFC module as long as appropriate operational is provided.

It can be seen in Table 2 that NO_3^- removal in the CW-MFC modules also varied according to the vegetation type despite being not significant ($p > 0.05$). These results indicated that adsorption of NO_3^- is not changed significantly among the vegetation types, and so the number of denitrifying bacteria may be not affected by plant species and vegetation in the wetland matrix. Furthermore, it can be also suggested that plant species and vegetation formation using the present experiment cannot limit the activity of denitrifying bacteria in the wetland modules. However, differences of growth characteristics for microbial attachment came from vegetation

formation can be slightly changed by the removal of NO_3^-, which can be attributed to rapid nitrification in wetland matrix which vegetated by *T. latifolia* during the experiment period.

Total phosphorus monitoring and effect of vegetation on TP removal

The TP removal in a wetland matrix is promoted by physical, chemical, as well as biological transformations, and removal of phosphorus is mainly controlled by the combination effect of filtration media, plants, and bacteria (Lu et al. 2016). Phosphorus removal performance of a CW module could vary with plant species which is used for vegetation purpose, and thus selection of plant could be crucial for phosphorus removal efficiency. As seen in Fig. 5a, the phosphorus removal efficiency of vegetated wetland is higher than that of unplanted control; one-way ANOVA statistical analysis indicated significant difference between vegetated wetland modules and unplanted control ($p < 0.05$). This result suggested that the specific surface area in vegetated wetland modules is relatively large, so more phosphorus is retained in vegetated wetland matrix during the remedial process. Correspondingly, the presence of vegetation in wetland modules reduced the hydraulic conductivity and increased water duration for adsorption of phosphorus from wastewater at vegetated wetland modules hence provided better phosphorus removal. The data from the various studies also support this phenomenon because it is well-known that aquatic plants have the crucial capacity to uptake phosphorus from wastewater to their tissues (Lu et al. 2016; Vymazal 2007).

As seen in Table 2 that TP removal varied from 71 to 97% for treatment modules during the experiment, and the better
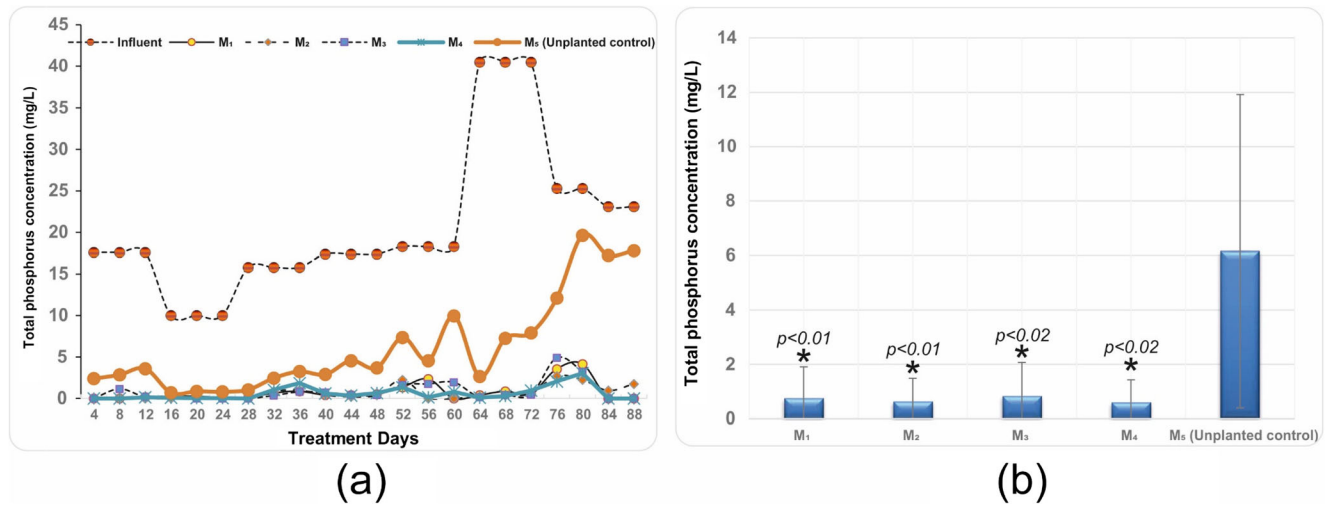
(a)

(b)

Fig. 5 **a** Total phosphorus (TP) concentrations in the influent and the effluent of M_1, M_2, M_3, M_4, and M_5 (unplanted control) wetland modules during the experiment. **b** Average total phosphorus (TP) concentration in the effluent from treatment modules and control module (M_5), as well as significance value according to control module in the experiment period. Error bars indicate standard deviation. An asterisk (*) denotes significant difference ($p < 0.05$) between vegetated and unplanted control

TP removal percent was determined as 97% for treatment module with *T. angustifolia* vegetation (M_2). It is an expected result because *T. angustifolia* produced more biomass than other plant species during the experiment, and thus the presence of more biomass in the wetland matrix also increased the accumulated amount of phosphorus in the plant tissues. Therefore, it can be concluded that the specific surface area related to high biomass content in the M_2 wetland module provides more sorption and accumulation sites in order to take up phosphorus from wastewater compared the other plant species, and this phenomenon is the main reason for obtained higher removal efficiency in the wetland module with *T. angustifolia* vegetation. Unfortunately, there is no information in literature about comparison effect of the plant species type on TP removal while remediating wastewater; thus, it is not possible to make any comparisons.

Physicochemical parameters monitoring and effect of vegetation type on physicochemical parameters

It can be seen in Table 2 that the pH values of the effluent samples from different wetland modules were mostly alkaline during the experiment period, and final effluent samples from M_1, M_2, M_3, M_4, and M_5 were found as 7.50 ± 0.23, 7.47 ± 0.19, 7.49 ± 0.17, 7.56 ± 0.15 and 7.87 ± 0.32, respectively. However, one-way ANOVA statistical analysis concluded no significant differences among the effluent samples from vegetated CW-MFC modules and unplanted control, indicating that pH levels were not significantly affected by vegetation type or presence of plants used in the present experiment ($p > 0.05$).

Electrical conductivity (EC) of the effluent samples obtained from different wetland modules were higher than that of the influent during the experiment period (Table 2), and the average EC values in the final effluent were determined as 1829 ± 398 µS cm^{-1}, 1803 ± 311 µS cm^{-1}, 1589 ± 279 µS cm^{-1}, 1880 ± 361 µS cm^{-1}, and 1362 ± 615 µS cm^{-1} for M_1, M_2, M_3, M_4, and M_5, respectively. Furthermore, there are no significant statistical differences determined between vegetated modules and unplanted control ($p > 0.05$). This result suggests that the EC removal mechanism in CW-MFC is mostly controlled by physical process such as sedimentation, and the vegetation effect on the EC purification process may not be important. However, the highest EC value was determined at the effluent of vegetated wetland modules; probably due to evapotranspiration process for vegetation cover associated with significant water loss and, therefore, increased anion and caution ions in the solution reported before by Leto et al. (2013).

The redox value and DO parameter indicate that the microbial activities in CW wetlands and the anaerobic or aerobic environments can be well defined by means of both redox profile and DO value in the CW-MFC modules. The average redox values and average DO concentrations in the CW-MFC modules during the experiment period are shown in Table 2. Accordingly, redox values and DO concentrations were different between the effluents and influent for each modules, and these distributions were significant as well ($p < 0.05$). The redox values at the vegetated wetland modules ranged from 43.4 ± 21 to 60.9 ± 18.5 mV; whereas, they were in the average 57.03 ± 17 mV in the unplanted module. In addition, average DO concentrations at the vegetated modules were in the ranges of 5 ± 0.9–5.6 ± 0.8 mg L^{-1} and less than 4.5 mg L^{-1} in the unplanted control module. These results indicated that wetland matrix with vegetated by *T. latifolia*, *T. angustifolia*, *J. gerardii*, and *C. divisa* more aerobic

environment than unplanted module and it is an expected result because plant's root systems provide oxygen to wetland matrix during the experiment period. On the other hand, the high DO value in the influent and effluent was attributed to the dissolution of oxygen in the air, and thus this indicated that the wetland matrix of the modules was in aerobic environment. The reasonable explanation might be that the consumption of oxygen in the closed-circuit mode for the CW-MFC modules was due to the low aerobic microbial reactions, so the reaction rate of chemical reaction is higher than biological reaction in the wetland matrix. Therefore, the oxygen consumption rate in wetland matrix was low as suggested relatively that high DO values were detected in the effluent. Moreover, the results also indicated that higher redox value and dissolved oxygen in the vegetated module are related to a decrease in microbial respiration activity due to the depletion of the organic matter, and thus they could be directly related to NH_4^+ and COD concentration in wastewater (Oon et al. 2015). Consequently, experts should consider this phenomenon when they design their own wetland modules.

Bioelectric production in CW-MFC modules and effect of vegetation on bioelectric production

A series of comparative daily measurements of voltage were done to determine the bioelectricity generation from different modules, and investigated impact of the vegetation on bioelectric production of wetland modules. Voltage readings across the 1000 Ω external resistor in the modules were taken during the treatment period and the results are shown in Fig. 6. As seen in Fig. 6, the highest voltage was generated from vegetated wetland module compare to unplanted control, and bioelectric production with graphite anode connected to magnesium cathode in the M_1, M_2, M_3, M_4, and M_5 modules during the experiment period were ranged as 0.7–1.24 V, 0.79–1.34 V, 0.57–1.28 V, 0.62–1.24 V, and 0.37–0.7 V, respectively. One-way ANOVA statistical analysis also indicated that significant differences between generated bioelectric through vegetated modules and unplanted control in the experiment period (Fig. 6) ($p < 0.05$). Therefore, the results clearly demonstrated that the voltage output in a constructed wetland
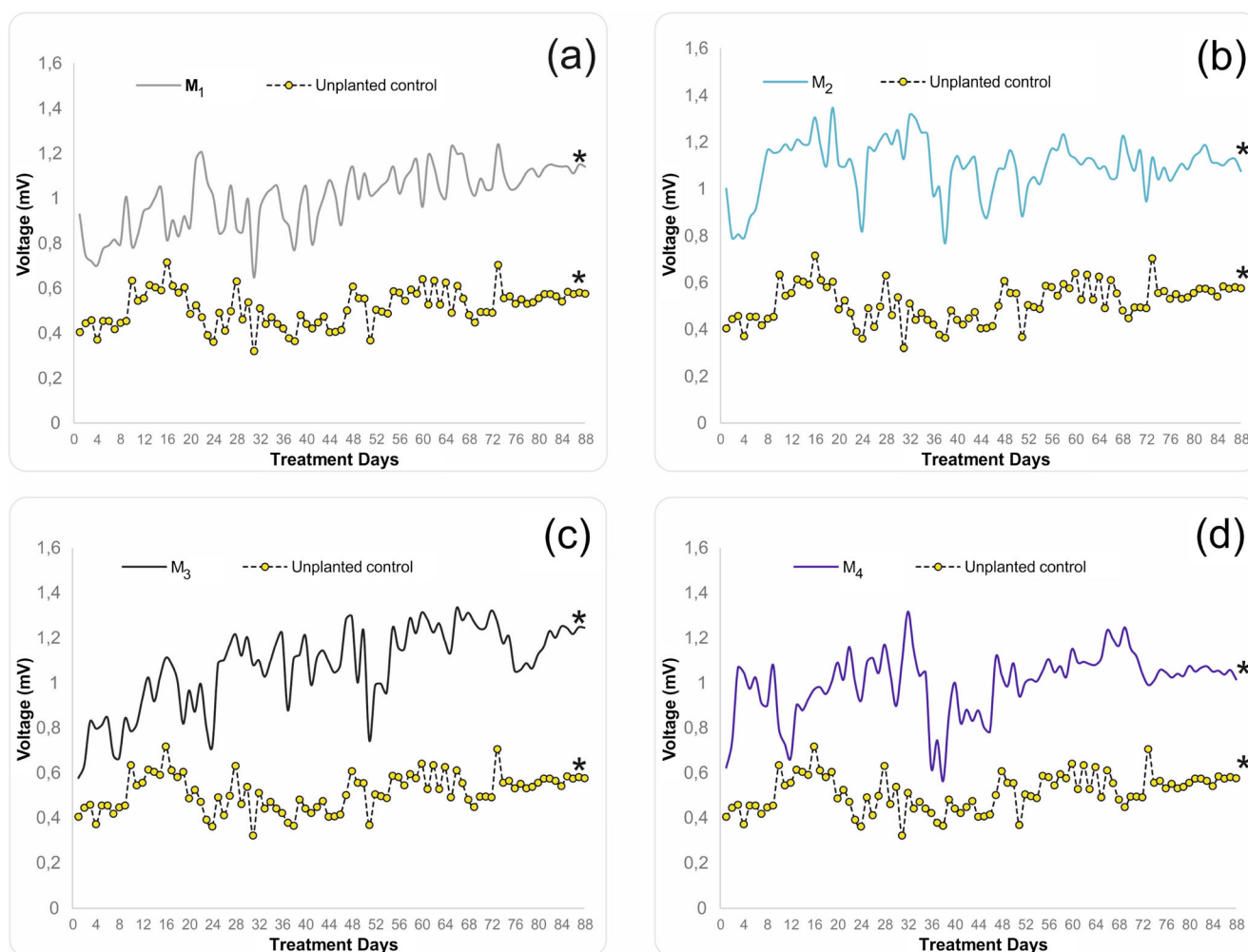

Fig. 6 Bioelectric production of CW-MFC module with *Typha latifolia* (**a**), *Typha angustifolia* (**b**), *Juncus gerardii* (**c**), and *Carex divisa* (**d**) according to unplanted control during the experiment period. An asterisk (*) denotes significant difference ($p < 0.05$) between vegetated and unplanted control

module was significantly higher when the presence of vegetation in the wetland matrix, and thus bioelectric production in the CW-MFC module may be promoted with vegetation or plants. It can be also assumed that vegetated wetland matrix provided more available environment for electrogenic bacteria than unplanted wetland matrix in order to generate bioelectric. Accordingly, the vegetation in the wetland matrix not only increases the availability of biodegradable organic matter arriving at module's anode compartment allowing microbial fuel cell device to generate the bioelectric more efficiently but also increases the electrogenic bacteria' activities which are catalyzed to the electron flow from anode to the cathode as a result of biodegraded nitrogen-based pollutant in wetland matrix. On the other hand, it was very well-known that vegetation produces organic compounds via photosynthesis, part of which is excreted at the roots into in wetland matrix (Helder et al. 2010; Zhou et al. 2017). These organic-based compounds are defined as rhizodeposits, and it can be easily oxidized by microorganism at the rhizosphere. In this respect, another reason associated with high bioelectric production in the vegetated modules comparing unplanted control might be explained that microorganisms in the rhizosphere broke down the organic compounds and release electrons. These electrons harvested by a terminal electron acceptor (graphite anode in this experiment) and then they were used to generate electricity. Correspondingly, more H^+ (protons) and e^- (electrons) were generated from the decomposition of organics in the vegetated wetland modules compared to that of unplanted control, consequently, contributed to increasing of voltage during the experiment period. Similar observations related to some of the chemical and electrochemical reaction involved in this mechanisms are reported by other studies (Helder et al. 2010; Zhou et al. 2017).

The average output voltages of the wetland module with *T. angustifolia* vegetation (M$_2$) were higher than with those of the other vegetated wetland modules, and these differences were significant as well ($p < 0.05$). Therefore, it can be concluded that the vegetation type significantly effects of bioelectric production in the CW-MFC module, and the bioelectric production of the CW-MFC may be mostly promoted with media type used in the wetland systems. More importantly, we found that the highest out voltage in wetland module (M$_2$) among the studied plant species in the present experiment and voltage of the wetland module with *T. angustifolia* vegetation (M$_2$) peaked at 1.34 V with graphite anode connected to magnesium cathode with a 1000 Ω resistor. Hence, it can be also assumed that rhizosphere of *T. angustifolia* provided more available environment for electrogenic bacteria than rhizosphere of other plant species in order to generate bioelectric. Probably, the higher biomass production of *T. angustifolia* compare to other plant species in the wetland matrix provides larger surface area to electrogenic bacteria. In this respect, it can be suggested that electrogenic bacteria colonized more

rapidly on the graphite anode surface in rhizosphere of the *T. angustifolia* compare to those of rhizosphere types, and thus amorphous biofilm easily covered the whole surface of anode in the M$_2$ wetland module. Soil enzyme activities in the wetland module with *T. angustifolia* vegetation also confirmed that more biofilm grow on the anode surface because we found that highest dehydrogenase (22.2 ± 15.5 μg TPF g^{-1} h^{-1}), urease (561 ± 381 μg NH_4^+ g^{-1} 48 h^{-1}), and phosphatase (2.63 ± 1.50 μg p-nitrophenol g^{-1} h^{-1}) activities in CW-MFC module with *T. angustifolia* during the experiment period (Fig. 7). Although soil enzyme activities in the CW-MFC modules were determined in order to explain why bioelectric production was the highest among the studied vegetation types, further investigations such as microbial community structure analysis should be needed to identify the reason behind which bacterial community is dominant in such vegetation types. Consequently, it can be concluded that the formation of electrogenic bacteria—*T. angustifolia* vegetation-graphite surface increase the availability of organic substrate arriving at the module's anode compartment allowing microbial fuel cell device to produce the bioelectric more efficiently and it increases electrogenic bacteria' activities which are catalyzed to the electron flow from the anode to the cathode as a result of biodegraded organic matter in wetland matrix. Therefore, we suggested that the presence of *T. angustifolia* in the wetland matrix positively affects the electron flow from anode to the cathode as a result of increased bioelectric production, so thus engineers should consider the positive effect of *T. angustifolia* on this phenomena in their operational procedure to maximize increasing bioelectric production.

It can be seen in Table 3 that the highest maximum power density of modules was determined as 13.4 mWatt/m^2 (with mean current density of 17.1 ± 16.2 mA/m^2), 18.1 mWatt/m^2 (with mean current density of 25.2 ± 20.01 mA/m^2), 8.1 mWatt/m^2 (with mean current density of 11.4 ± 8.6 mA/m^2), 8.8 mWatt/m^2 (with mean current density of 22.9 ± 19.4 mA/m^2), and 5.39 mWatt/m^2 (with mean current density of 10.6 ± 7.72 mA/m^2) for M$_1$, M$_2$, M$_3$, M$_4$, and M$_5$ modules, respectively. These results indicated that the presence of vegetation in the CW-MFC matrix clearly affects the power density, and thus the maximum power density was obtained for vegetated wetland modules. This may be due to different internal resistance of vegetated wetland modules and unplanted control module. Accordingly, internal resistance is defined as one of the limiting parameter factors in MFC device efficiency, and thus it can be indicated that vegetation could be positively affected to transport limitation, kinetic, and ohmic parameters during the bioelectric production in the wetland modules (Liu et al. 2013; Oon et al. 2015). Furthermore, it can be also concluded that because different organic substrate degradation rate in the vegetated
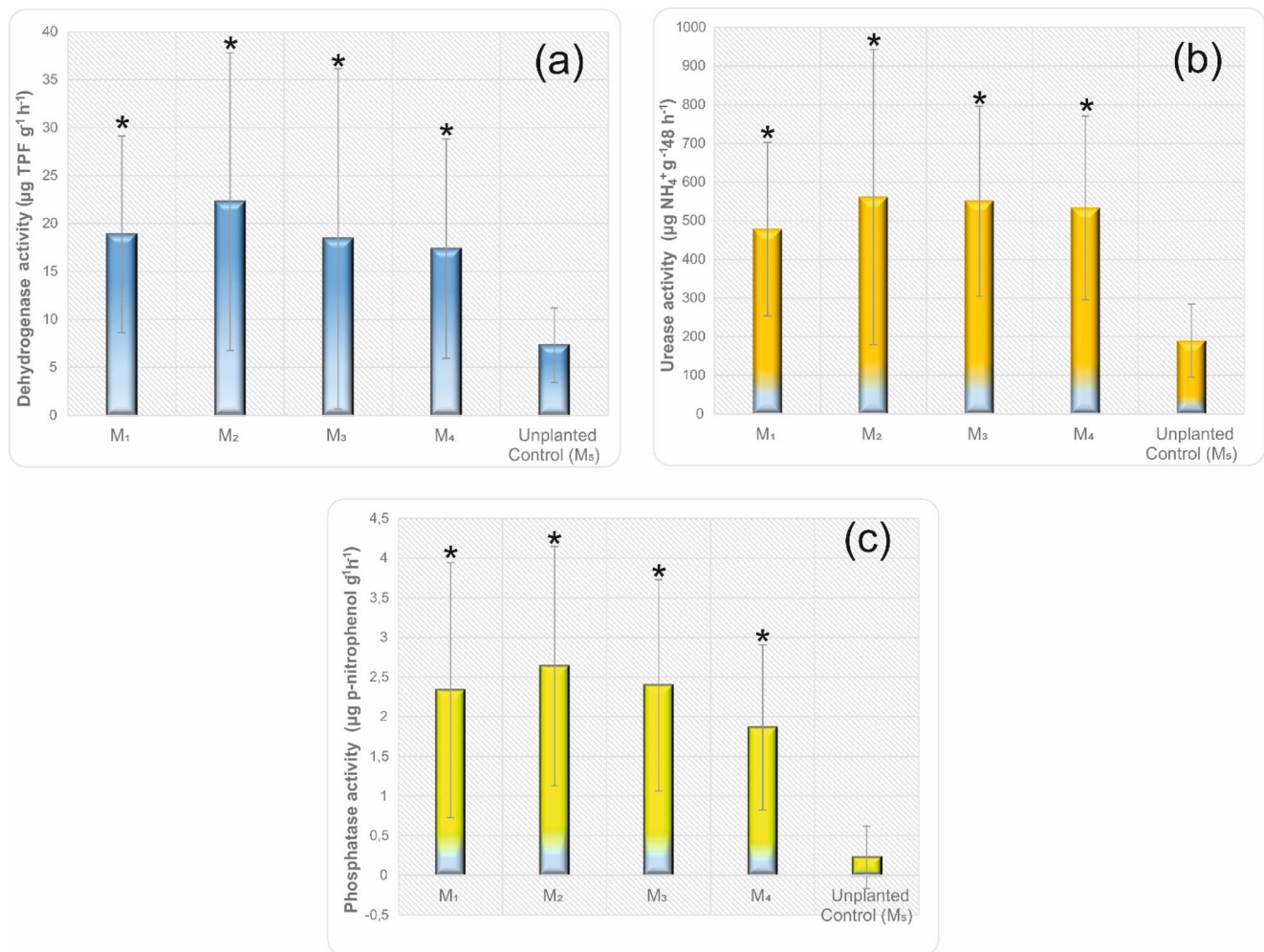
Fig. 7 The activities of dehydrogenase, urease, and phosphatase enzymes in CW-MFC modules in the experiment period. Error bars indicate standard deviation. An asterisk (*) denotes significant difference ($p < 0.05$) between vegetated and unplanted control

and unplanted modules during experiment period. Therefore, it is likely that more organic substrate degradation rate in the vegetated wetland modules positively affects the electron flow from anode to the cathode compare to unplanted control, so thus engineers should consider the positive effect of higher biodegradation potential in vegetated wetland modules in order to maximize increasing bioelectric generation.

The average coulombic efficiencies were calculated for $6.074 \pm 8.42\%$, $8.28 \pm 10.4\%$, $6.57 \pm 6.84\%$, $6.13 \pm 5.68\%$, and $4.64 \pm 5.84\%$ for M_1, M_2, M_3, M_4, and M_5 modules, respectively (Table 3). These results indicated that not all microorganisms both in vegetated modules and unplanted control involve in direct e^- (electron) transfer to cathode, and it can be also suggested that most of the degraded organic compounds in wastewater was not related to power generation during the experiment period.

Table 3 Power density, current density, and Coulombic efficiency of M_1, M_2, M_3, M_4, and M_5 wetland treatment modules in the experiment period. Plus-minus sign (±) denotes standard deviation

Treatment module	Power density (mWatt/m^2)		Current density (mA/m^2)		Coulombic efficiency (%)
	Max	Mean	Max	Mean	Mean
M_1 (*T. latifolia* vegetation)	13.4	4.12 ± 9.06	31.4	17.1 ± 16.2	6.074 ± 8.42
M_2 (*T. angustifolia* vegetation)	18.1	7.47 ± 13.7	33.8	25.2 ± 20.01	8.28 ± 10.4
M_3 (*J. gerardii* vegetation)	8.1	1.91 ± 2.71	21.6	11.4 ± 8.6	6.57 ± 6.84
M_4 (*C. divisa* vegetation)	8.8	4.19 ± 4.49	29.5	22.9 ± 19.4	6.13 ± 5.68
M_5 (unplanted control)	5.39	1.89 ± 2.37	19.8	10.6 ± 7.72	4.64 ± 5.84

The effect of vegetation type on soil enzyme activity in CW-MFC modules

The activity of dehydrogenase, urease, and phosphatase in different wetland modules in the experiment period are shown in Fig. 7. Accordingly, the highest average dehydrogenase activities are found as 18.88 ± 10.2, 22.2 ± 15.5, 18.4 ± 17.7, 17.3 ± 11.4, and 7.31 ± 3.89 µg TPF g^{-1} h^{-1} for M_1, M_2, M_3, M_4, and M_5 wetland modules, respectively. Moreover, the highest average urease enzyme activity was also observed as 478.1 ± 224.7, 561 ± 381.5, 550.8 ± 245.8, 533.3 ± 237.8, and 189.8 ± 94.7 µg NH_4^+ g^{-1} 48 h^{-1} for M_1, M_2, M_3, M_4, and M_5, respectively. The phosphatase activity varied according to the vegetation type, and the highest average enzyme activity was measured as 2.33 ± 1.6, 2.63 ± 1.5, 2.39 ± 1.33, 1.86 ± 1.04, and 0.22 ± 0.39 µg p-nitrophenol g^{-1} h^{-1} for M_1, M_2, M_3, M_4, and M_5 wetland modules, respectively. These results indicate crucial evidence that vegetated wetland matrix had significantly higher microbial activity for biochemical purification reaction and bioelectric production than unplanted module ($p < 0.05$), this phenomenon is explained why we found higher removal efficiency and bioelectric production in the vegetated wetland matrix. Furthermore, it can be seen in Fig. 7 that the soil enzymes had different activities in the different vegetation types and soil enzymes in wetland module with *T. angustifolia* were found to be higher than those of in other CW-MFC modules with different vegetation. It was an expected result because wetland matrix with *T. angustifolia* provides more available environment associated with organic carbon availability and nutrient usage for soil microorganisms as discussed above compared to other module matrix, and thus this phenomena could have increased the rate of microbial growth and soil enzyme activities including dehydrogenase, urease, and phosphatase during the purification process. Unfortunately, there is no information about soil enzyme activities in a CW-MFC module with different vegetation types while remediating wastewater, thus it is not possible to make any comparisons. Nevertheless, we suggested that the monitoring of dehydrogenase, urease, and phosphatase activities in CW-MFC modules could provide information regarding the overall microbial activity associated with treatment process and bioelectric production during the remediation of wastewater.

Conclusion

The present experiment investigates the importance of vegetation in achieving wastewater treatment and bioelectric production. The vegetated module has higher COD (85–88%), NH_4^+ (95–97%), and TP (95–97%) removal efficiency, as well as bioelectric production (0.94–1 V) compare to unplanted control, whereas we obtained the highest NO_3^-

(63%) removal by unplanted control among the treatment modules. Correspondingly, vegetated wetland modules provide more specific area to microorganisms, and so it increases their activities which are responsible for biodegradation of organic compounds and catalyzed to electron flow from anode to cathode. Furthermore, rhizosphere of *T. angustifolia* provided more available environment for microorganisms than rhizosphere of other plant species in order to obtain higher removal efficiency and generate bioelectric during the experiment. Therefore, we suggested that engineers could use vegetated wetland matrix with *T. angustifolia* to maximize treatment efficiency and bioelectric production in CW-MFC modules.

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