A hybrid constructed wetland combined with microbial fuel cell for boron (B) removal and bioelectric production

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Abstract

This study deals with performance assessment of a hybrid constructed wetland-microbial fuel cell (HCW-MFC) for boron (B) treatment and bioelectric production. Our innovative HCW-MFC is first literature report testing for B removal and bioelectric production at the same time in terms of an eco-technological perspective. The results indicated that 63.4 percent B removal was achieved for wastewater containing an average 12.3 mg L⁻¹ inflow of B concentrations. Furthermore, HCW-MFC was able to remove 47.5% and 19.1% nitrate and nitrite from wastewater, respectively. The maximum power and current density was recorded as 78 mWatt/m² and 105 mA/m² for the last unit of the hybrid system, suggesting that the power and the current density were decreased with the increasing B concentration. We found that some soil enzymes such as dehydrogenase and urease in wetland matrix strongly correlated with bioelectric production, thus these enzymes might play important roles in bioelectric production with HCW-MFC.

Keywords: Hybrid constructed wetlands, microbial fuel cell, boron (B) removal, bioelectric production, soil enzymes

1. Introduction

Both natural as well as anthropogenic activities such as using agrochemicals, production of cleaning products, and mining operations lead to contamination of receiving water body and aquatic environments with excessive boron (B) (Hasenmueller and Criss 2013, Türker et al. 2013, Davis et al. 2002). In recent years, the contamination of aquatic ecosystems with elevated concentrations of B have aroused more and more worldwide environmental and health attentions given to the potential toxicity effects of B on crops, plants, animals, and humans (Schoderboeck et al. 2011). Specifically, Drinking-Water Quality Committee recommended a safe limit of B lower than 2.4 mg L^{-1} (Wolska and Bryjak 2013). Hence, it is important to control B in water environment considering the strict environmental production regulation and public health concerns.

Several conventional treatment methods including membrane process, ion exchange, and adsorption techniques have been applied for the removal of B from various types of wastewater (Wolska and Bryjak 2013). However, methods based on conventional strategies are expensive and require costly equipment apparatus or chemicals for B removal (Türker et al. 2014). In comparison to conventional techniques, constructed wetlands (CWs) have experienced significant advancement in recent years to remove pollutants from contaminated water due to their simple operation guide, eco-friendly properties, and cost effectiveness (Vymazal 2013).

It is noted from the literature that some effective researches showed that CWs' potential of removing and controlling B in the wastewater (Türker et al. 2014). Most studies from literature to date have focused on subsurface flow CWs (Allende et al. 2014, Türker et al. 2016, Kröpfelová et al. 2009), with much fewer researches conducted in surface flow CWs associated with different wetland plants, media, climatic conditions, and wastewater characteristic (Ye et al. 2003, Allende et al. 2012). According to these literature data, CW

treatment technologies as both subsurface and surface flow systems are seen as an attractive solution to remove B from various types of wastewater, however, to our knowledge, a hybrid constructed wetland (HCW) has never been used for the removal, and control of B from wastewater sources; so one of the aims of this study was to fill these gaps in literature.

The CW systems combined with microbial fuel cell (CW-MFC) technologies is an innovative technology to achieve the object of both wastewater treatment and bioelectric production at the same time (Oon et al. 2015, Corbella et al. 2015, Doherty et al. 2015a). In this systems, bioelectric is produced with biodegradable substances through bacteria oxidized organic or inorganic matter present in wetland matrix (Oon et al. 2015). Particularly, CW-MFC system is predominantly used to treat sewage, also examples of treatment of different types of wastewater such as azo-dye wastewater (Fang et al. 2015, Yadav et al. 2012) and swine slurry wastewater (Doherty et al. 2015b) have been documented as well. However, there is no information about the use of a constructed wetland system combined with microbial fuel cell (CW-MFC) to treat wastewater which contains both nitrogen substances and excessive B. Accordingly, numerous types of wastewater sources such as domestic, municipal, and industrial contain both nitrogen substances such as nitrate or nitrite and enriched B due to the presence of sodium perborate in detergents or cleaning products, glass production, and mining effluents (Hasenmueller and Criss 2013, Turker et al. 2013, Schoderboeck et al. 2011, Türker et al. 2014). So, these compositions of wastewater sources make a CW-MFC suitable and potentially applicable method for treating wastewater by removing both nitrogen substances and B, as well as generating electricity in an eco-friendly and economical way. It is important to the note that a HCW-MFC was developed for the first by testing B removal and bioelectric production at the same time in terms of an eco-technological perspective. Furthermore, we suggested that the results from the present study

could be meaningful in assessing the potential of HCW-MFC as an effective solution to generate electricity while remediating wastewater sources contaminated with B.

As mentioned above, the objectives of the present study were to reveal information (i) to assess the performance of a HCW system to remediate wastewater containing B and nitrogen substrates such as nitrate and nitrite; (ii) to investigate the feasibility of MFC integrated with HCW in order to produce bioelectric during the B purification process; (iii) to determine the relationship of some important soil enzyme activities such as dehydrogenase, urease, as well as phosphatase with bioelectric production in HCW-MFC.

2. Materials and Methods

2.1. Design of wetland units and culture period

The experiment was carried out in a growth chamber under the following controlled conditions: 24 ± 2 °C, photosynthetic photon flux density $85 \mu\text{mol m}^{-2} \text{s}^{-1}$, and a photoperiod of continuous light. The prototype of HCW-MFC was fabricated and designed using polyester chambers with 45 cm length, 45 cm width, and 40 cm depth. Briefly, the hybrid systems consisted of two constructed wetland units. The first unit was operated according to surface flow design guide based on a flow regime which kept always the water level about 5 cm above the filtration media. The second unit was a typical horizontal flow subsurface unit which employed gravity feed using 1.5° . In this study, organic based peat and sand mixture were selected as the filtration media for HCW-MFC. It was thought that type of filtration media will not only improve retained B in the HCW-MFC due to a ligand exchange mechanism related to *diol* or *cis-diol* complexes but it can also present an ideal environment for microorganisms associated with bioelectric production (Türker et al. 2016, Doherty et al. 2015b). Approximately 5 cm of gravel was also placed on the filtration media and outflow section. Cattail (*Typha latifolia*), a popular wetland macrophyte used as phytoremediation of

B in CWs, was used in this study (Turker et al. 2013, Ye et al. 2003). Rhizomes of *T.latifolia* were collected from a wetland habitat located in Eskişehir, Turkey (39°17' N, 30°30' E), and the rhizomes were immediately transplanted in wetland units at a plant density of about 12 rhizomes/m². A horizontal rectangular (4 cm x 6 cm and 1 cm thickness) magnesium cathode was located above filtration media and in the gravel layer (Yadav et al. 2012). Two graphite anodes (100 cm² surface area) were also placed into organic based peat and sand mixture in the HCW-MFC (Oon et al. 2015). A glass wool layer (0.01 thickness) was placed in the HCW-MFC in order to separate the cathode and anode compartments (Yadav et al. 2012). Finally, the cathode and anode were connected with insulated copper wires across a 1000 Ω , and the distance between the cathode to the anode 1 and the anode 2 were 10 and 20 cm, respectively (Corbella et al. 2015). The detailed schematic demonstration of the HCW-MFC is also given Fig 1.

Prior to the experiment start up, the HCW-MFC was fed with mixture of Hoagland medium and sludge for 45 days in order to support the cattail growth and establish microorganisms (Oon et al. 2015). Furthermore, the sludge was collected from a treatment plant for industrial wastewater, and the sludge was mixed in the Hoagland medium and then poured into the HCW-MFC. In this respect, we believed that this composition of mixing sludge contains both essential minerals for plants and active microorganisms associated with bioelectric production.

2.3. Boron dosage and Operation of wetland units

After cultivation in a period of 45 days, the HCW-MFC unit was fully established for B treatment facility and bioelectric production under the controlled conditions. In the present experiment, the study was carried out with simulated wastewater obtained with Hoagland medium contaminated with various B concentrations of H₃BO₃ dissolution (Merck,

purity>99) arranged in a geometric series: 2, 4, 8, 16, and 32 mg L⁻¹. The simulated wastewater resembling pollutant level in the surface water and some sewage in Western Anatolia, Turkey was prepared (Hasenmueller and Criss 2013, Türker et al. 2016). These types of water sources were selected as references because of their high levels of B, as well as relatively higher concentrations of nitrate and nitrite (data not shown). The simulated wastewater was stored in a 100 L polyethylene inflow tank which was continuously stirred. The wastewater was freshly prepared for each experimental group before the first dosing, and this wastewater sample was kept until the next dosing day on the same week (Allende et al. 2014). The wetland units were operated under the same hydraulic loading rate of 20mL/h and dosed every 8 hours over the periods of 24 h, so the hydraulic retention times of the each unit were to set to 7 days. On the other hand, the outflow water from unit 1 was stored in the outflow tank 1, and then this water was immediately dosed to unit 2. The mean outflow rate was figured out according to Allende et al. (2014), and each dosing event took 30 min. Moreover, the outflow water from unit 2 was collected in outflow tank 2. The wetland operated under a continuous process mode, and the wastewater followed the flow direction in the cathodic compartments both unit 1 and unit 2.

2.4. Bioelectric production monitoring and measurement

The bioelectric production performance of the unit 1 and unit 2 were determined with daily measurements of the voltage drop (V) across the external resistor using a digital handheld multimeter (Fluke CNX 3000 wireless) described before (Zhao et al. 2013). According to this procedure, the cathode and anodes 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 anode (Oon et al. 2015). After the 70th day of operation, polarization

curves were determined by varying the external resistance from 1 Ω to 680.000 Ω finally to infinity at open circuit and measuring the steady state voltage across the resistor within 15 min (Doherty et al. 2015a, Doherty et al. 2015b, Logan et al. 2007).

2.5. Chemical analysis of water, plants, and media from HCW-MFC

2.5.1. Wastewater sampling and analysis

Samples of inflow and outflow water were taken according to the hydraulic retention time (7 days) of wetland units, and physicochemical parameter such as pH, electrical conductivity (EC), temperature, and redox potential were measured with HACH HQ40D multi-parameter meter concurrent with sampling. Boron (B) concentrations was analyzed in the laboratory according to the standard methods (APHA 1998). Nitrate and nitrite in water samples were measured with a colorimetric method using a colorimeter (HACH DR/890) immediately after sampling.

2.5.2. Plant monitoring, biomass production, chemical analysis, and pigment concentrations

The height of cattail was monitored every four days, and any instances of plant disease were recorded. Cattail height was calculated as the average height of leaves in the HCW-MFC. On the other hand, all the cattail biomass both aboveground and belowground was collected from the wetland units to determine and analyze dry biomass, and chemical composition of the plants at the end of the experiment. Dry biomasses of cattail were calculated by drying the harvested biomass in an oven at 65°C for 48h. After calculating, the plant parts were separated into leaves, stems and roots/rhizomes, and all the material were powdered and digested by HClO₄:HNO₃ acid at 1:3 proportion in a microwave digestion unit in order to determine B concentrations (Turker et al. 2013). Moreover, N content was determined by the Kjeldahl method.

Photosynthetic pigments (Chlorophyll a and b) concentrations of *T.latifolia* in the unit 1 and unit 2 were also measured during the experiment period. For the analysis, fresh tissues from mature leaves of *T.latifolia* were collected every four days from the units, and photosynthetic pigments were determined according to (Wellburn 1994).

2.5.3. Filtration media analysis and soil enzyme activities

The media samples from the units were collected homogenously at the end of the experiments. The samples were collected using a 4-cm diameter PVC corer. The concentration of B was determined by atomic absorption spectrometry method. The extractable B (plant available B) in sediment was measured by carminic acid method, N by Kjeldahl method and soil structure by Bouyoucos method (Turker et al. 2013).

Moreover, rhizosphere samples from the units were also collected every 4 days using hand-shaking method in order to determine the correlation existed between enzyme activities and bioelectric production in HCW-MFC (Zhang et al. 2010). In this respect, we measured the activities of 3 important enzymes in the rhizosphere of HCW-MFC. Those enzymes have crucial role associated with the cycling of carbon (dehydrogenase), nitrogen (urease), and phosphorus (phosphatase) (Zhang et al. 2010). The collected media samples 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 rhizosphere of the units were determined (Kong et al. 2009).

2.6. Calculation and statistical analysis

Boron removal performance (%) from wastewater through unit 1 and unit 2 was calculated as follows:

$$\text{B removal performance (\%)} = [(C_i V_i - C_e V_e) / C_i V_i] \times 100 \quad (1)$$

where C_i and C_e are the average B concentrations of inflow and outflow in mg L^{-1} ; V_i and V_e are the volumes of the outflow and the inflow collected and dosed into units.

The mass removal rate (MRT) of the HCW-MFC calculated using equation evaluated by (Türker et al. 2016):

$$\text{Mass removal rate (mg m}^{-2} \text{ d}^{-1}) = [(C_i \times V_i - C_e \times V_e) / \text{unit surface area} / \text{HRT}] \quad (2)$$

Total area of HCW-MFC was 0.405 m^2 and Hydraulic retention time (HRT) was 7 days describe in above.

$$\text{Current (I) was determined by according to Ohm's law using formula: } I = V/R \quad (3)$$

where V corresponds to cell voltage, and $R(\Omega)$ is the external resistance.

$$\text{Power (W) is determined by using formula: } P = I \times R \quad (4)$$

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 are (m^2) of anode (Oon et al. 2015).

Statistical between the concentrations of B and pH, EC, and temperature value in inflow and outflow water samples both unit 1 and unit 2 were determined a one-way ANOVA test. Correlations between soil enzyme activities and bioelectric production in the units were analyzed by Pearson's correlation coefficient. Prior to performing ANOVA, the data from the present experiment were checked for normality and homogeneity of variance (using the Shapiro-Wilks test) with significance set at 0.05. Statistical confidence was set at $p < 0.01$ and $p < 0.05$. All statistics were implemented using SPSS version 19.0 of the Statistical Software Package.

3. Results and Discussions

3.1. Treatment performance of HCW-MFC

The different B concentrations were applied to HCW-MFC combined with fluctuations in the inflow in order to create as possible as realistic wastewater composition and flow regime (Allende et al. 2014, Türker et al. 2016). Accordingly, overall B treatment efficiency of the HCW-MFC amounted to 63.4% with average inflow B concentration of 12.3 mg L⁻¹ during the experiment period (Table 1). Moreover, the results from the experiment demonstrated that B concentration in outflow was lower than B concentration in inflow, and the decrease in B concentration in the unit 2 was significant as well ($p<0.05$). This result indicates that HCW-MFC is capable of removing B from the wastewater, and thus this type of system could be used as a bio-filter tool to prevent the B pollution over the world. On the other hand, the removal efficiency for B in the present the HCW-MFC system was found to be relatively higher than efficiencies of other types of CW systems. For example, Ye et al. (2003) reported 32% B removal from electric utility wastewater in a surface flow CW. Kröpfelová et al. (2009), Türker et al. (2013), and Allende et al. (2014) found B removal of 22.5%, 18, and 32%, respectively in horizontal flow sub-surface CWs. For vertical flow CWs, Allende et al. (2012) reported 12.5% B removal at the end of the 7 weeks. Furthermore, the removal of B in horizontal subsurface CW amounted to 64% B removal rate reported by Türker et al. (2016), a performance very similar to what observed in the present experiment. In this respect, it can be concluded that B treatment performance differs according to the wetland types, thus the selection of wetland type is a key component to obtain higher B removal efficiencies with CW technologies.

In the present experiment, B has been reduced to the level of outflow concentrations after passing through the unit 1 and a significant decrease was determined after the passage through the unit 2 (Fig 2a). This phenomenon is seen very often while removing contaminants

through hybrid systems because more pollutant pass through the wetland unit during the first phase compared to the second phase, so more sorption sites were available to take up B in the rhizosphere or filtration media of the unit 1. Furthermore, applied B loading rates and corresponding mass removal rates of B, together with the coefficient of determination (R^2) values obtained by regression analysis for the HCW-MFC are also represent in Fig. 2b. It can be seen that the boron mass removal rates of the HCW-MFC (0.002-0.29 mg/m²/d) showed strong correlation with incoming loads, with R^2 = values of 0.9195. Various authors also found similar result associated with the relation between loading rates and mass removal rates in their studies (Turker et al. 2013, Allende et al. 2014, Ye et al. 2003). It is important to note that mass removal rate of the HCW-MFC is mostly related to sorption of B in filtration media, accumulation or precipitation in organic based media, and plant uptake (Türker et al. 2016).

Correspondingly, B concentration in outflow samples was stably below 2.4 mg L⁻¹ (drinking water safety limit) during the first 35 days, whereas outflow concentrations from unit 1 and unit 2 were found to be higher than 2.4 mg L⁻¹ when inflow exceeded 6.5 and higher B mg L⁻¹ (Fig 2). Therefore, it can be hypothesized that the HCW-MFC has a potential for remediating wastewater below drinking water safety limit and further experiment are needed to test this question.

HCW-MFC design and performance were not firstly aimed at COD, BOD, phosphorus removal, and therefore, no special measures were taken to enhance the removal of those parameters. However, we monitored nitrate and nitrite as nitrogen substrates in wastewater and physicochemical parameters (pH, oxidation reduction redox potential, EC, and temperature) during the experiment period. Monitored average nitrate and nitrite concentration and physicochemical parameters in the inflow, unit 1 outflow, and unit 2 outflows are given in Table 1. Overall treatment efficiency of nitrate and nitrite during the experiment period were found as 47.9% and 19.1%, respectively. The average nitrate and

nitrite amounted to 70.5 and 19.3 mg L⁻¹ and were reduced to 36.5 and 15.2 mg L⁻¹ respectively in the discharged final outflow (Table 1; Fig 3). The results from the present experiment concluded a positive role of the unit 1 on nitrate and nitrite removal because wastewater with high concentrations of these nitrogen substrate were loaded into unit 1 and thus nitrate and nitrite were removed from wastewater. On the other hand, one-way ANOVA indicated that the nitrate value of final outflow was significantly lower than the inflow ($p<0.05$), whereas differences for nitrite were not significant ($p>0.05$) (Fig 3). Nevertheless, it can be concluded that nitrite and nitrate together with B were efficiently reduced by the HCW-MFC.

It can be seen in Table 1 that average oxidation-reduction potentials were measured as 201, 150.6, and 158.2 mV for inflow, unit 1, and unit 2 outflows, respectively. This result indicated that a redox potential greater than 100 mV for unit 1 and unit 2 outflows and an aerobic environment development which facilitated the degradation of nitrate and nitrite by microorganisms to produce electrons in the entire wetland matrix (Oon et al. 2015). The redox values in the outflow samples were stable throughout the after 42th treatment days and remained close to +150 mV (Fig 3). These results indicated that oxidized iron in both wetland matrixes was relatively stable during the experiment period and the oxidation of ferrous ions may be maintained by the iron-depositing bacteria which are associated with the development of a redox gradient in the wetland matrix (Oon et al. 2015). Moreover, the precipitation of iron-hydroxides in the rhizosphere related to the activities of iron-depositing bacteria probably led to an iron plaque formation and this plaque promotes B binding affinity in the organic-based media, potentially being permanently immobilized in the wetland units as reported by Vymazal and Kröpfelova (2009) for other trace elements. On the other hand, it can be seen in Fig 3 that redox value of the unit 2 are mostly higher than the redox values in the unit 1 during the experiment period. The high redox potential in unit 2 was due to the fact

that oxygen was introduced from the air as the unit 1 outflow was dosed to unit 2. Hence, it can be hypothesized that increasing oxygen release into the wetland matrix may have resulted in more Fe precipitation as an iron plaque compared to unit 1, and this may have led to co-precipitation and high action of the iron-depositing bacteria in the unit 1 (Vymazal and Kröpfelová 2009). Theoretically, B removal dynamics could be related to the aerobic oxidation pathways in the wetland matrix, and increased oxygen in the HCW-MFC can have more positive benefits improving B removal during the experiment period (Türker et al. 2016). However, further researches are needed to understand the relationship between aerobic oxidation pathways or redox conditions of wetland matrix with B removal efficiency in wetland habitats.

As seen in Table 1, average pH values in unit 1 outflow and unit 2 outflow were determined to be higher than inflow, supporting that B as a boric acid form in wastewater were removed by HCW-MFC during the experiment period. Moreover, outflow pH values were very steady after the first 14 days of monitoring period, and then pH values for unit 1 and unit 2 had a gradually decreasing trend until 56th days. Afterwards, pH values showed a slight fluctuation near neutral pH until at the end of the experiment (Fig 3). As previously reported by various authors, availability of B for plants and media in the wetland habitats increases near neutral pH levels, and thus pH values obtained from the present experiment create a matrix which is available for B retention in filtration media and *T.latifolia* during the study period (Türker et al. 2016, Allende et al. 2012).

Average EC values of outflow from both the unit 1 and unit 2 were higher than those of the inflow during the experiment period (Table 2). Moreover, EC values in final outflow and unit 1 outflow showed high fluctuation ranged from 734 to 1994 $\mu\text{S cm}^{-1}$ and 796 to 1830 $\mu\text{S cm}^{-1}$, respectively (Fig 3). This result indicated that the unit 2 outflow contained more anion and caution ions; however those ions were reduced more in the unit 1 during the

experiment period. The data obtained from redox potential also support this phenomenon, and it can be seen that unit 2 outflow contained higher redox potential, suggesting that more electrons were found in the aforementioned environment compared to the unit 1 outflow.

Finally, temperatures of the outflows of both unit 1 and unit 2 showed slight fluctuation from 20 to 26 °C during the experiment period. Moreover, the temperature of the unit 1 outflow was slightly higher at the very beginning of the experiment while being very steady at the end of the study (Fig 3).

3.2. Bioelectric production with HCW-MFC

A series of comparative daily measurements of voltage in the unit 1 and the unit 2 were done to analyze the electricity generation from the HCH-MFC. Voltage readings across the 1000 Ω external resistor were taken during treatment and the results are shown in Fig 4a. As seen in the mentioned figure, the voltages of the two units were very stable during the first 21 days. Moreover, the voltages of unit 1 varied in the ranges between 0.4 and 1.4 V during the experiment period. However, the voltages obtained from unit 2 gradually increased after 21th treatment day, and they ranged between 0.9 and 1.6 V. Clearly, we obtained higher bioelectric production through the unit 2 compared to the unit 1 during the experiment period. The reasonable explanation of such behavior is to more B passing through unit 1 compared to unit 2 during the first phase and high level of B in the inflow was an inhibitor for the activity of microorganisms which are responsible for bioelectric production. Therefore, it can be suggested that the bioelectric production of the HCW-MFC was mostly promoted with B concentrations which were dosed into the wetland units during the experiment period. It is interesting to note that we found more nitrogen substrate such as nitrite and nitrate were reduced by unit 1 compared to the unit 2 during the experiment period. Therefore, our results also demonstrated that high B concentration in wastewater may have negative effects on the

decomposition reaction of nitrogen substrates such as nitrate and nitrite which are consumed by the electrogenic microorganisms to sustain their own growth and metabolism. Because excessive B is biologically toxic and it can lead to microbial inhibition which can promote microbial flora including electrogenic bacteria in the wetland units. Consequently, the efficiency of electric production would diminish due to the lower microbial population and the decrease in microbial activity at the higher B concentrations. However, further researches are needed to investigate the effect of B, other trace elements, as well as heavy metals on electrogenic bacteria in CWs coupled with microbial fuel cell.

The highest maximum power density for the wetland units were 40 mWatt/m² (with current density of 45 mA/m²) and 78 mWatt/m² (with 105 mA/m² current density) in the unit 1 (Fig 4b) and unit 2 (Fig 4c), respectively. On the other hand, the power density and the current density were decreased with increasing B concentration in wastewater. The average power density and current density were found to be 39.2 mWatt/m² and 7.98 mA/m² respectively for unit 1 average inflow with 12.3 mg L⁻¹ B concentration while the values were further increased to 75.7 mWatt/m² and 19.8 mA/m² respectively in case of 5.5 mg L⁻¹ of average B in wastewater. The decrease in power density and current density with an increase in concentration of B in average inflow is probably due to the effect of increased level of B toxicity on microorganism associated with bioelectric production. Therefore, it can be hypothesized that high B concentration negatively affects the microorganisms' activities which are catalyzed to the electron flow from anode to the cathode as a result of biodegraded nitrogen based substrate in wetland matrix. Similar results were also found by various researchers who showed toxic effects of contaminants when concentration were high (Corbella et al. 2015, Fang et al. 2015, Yadav et al. 2012).

3.3. Plant monitoring, development, and boron content

The selection of plant species is an important issue in the CWs and related to their lifespan (Vymazal and Kröpfelová 2009). In the monitored period, the survival rates of *T.latifolia* in units after the cultivation period were found to be higher than 75% according to Türker et al. (2013). The healthy growth trend of plants demonstrated that *T.latifolia* was acclimatizing and reproducing well in both unit 1 and unit 2 which were fed with wastewater containing Hoagland medium before B application. Plant height in both unit 1 and unit 2 were monitored during the operation of units and the results are shown in Table 2. The average plant height in the experiment was measured as 79.8 cm and 99.6 cm for unit 1 and unit 2, respectively. Moreover, *T.latifolia* height in unit 1 and unit 2 ranged from 48 to 100 cm and from 88 to 109 cm respectively during the experiment period. These results suggested that *T.latifolia* in the wetland units showed a good resistance to alterations in B dosage, and thus higher growth rate and proliferous germination level were achieved for *T.latifolia* in wetland units during the experimental period. Therefore, we recommend that *T.latifolia* is a well suited option against potential toxic effects of B.

The photosynthetic pigments in *T.latifolia* were determined to assess B toxicity during the experiment period. The levels of the chlorophyll *a* and *b* pigment concentrations for *T.latifolia* in the wetland units are shown in Table 2. The chlorophyll pigment concentrations of *T.latifolia* in unit 2 were higher than those in unit 1. It may be explained by several causes such as operational conditions and origin associated with excess B concentrations in the wastewater. Nevertheless, higher chlorophyll content in unit 2 may indicate an apparently higher resistance of *T.latifolia* to the application of B wastewater.

The results of the biomass production both in unit 1 and unit 2 are shown in Table 2. According to present the results at the end of the study, the whole plant biomass production was found as 268 and 305 (DW g m⁻²) for *T.latifolia* in unit 1 and unit 2, respectively.

Clearly, *T.latifolia* in unit 2 produced higher biomass compared to the plants in unit 1 during the experiment period. Its reasonable explanations might be that *T.latifolia* growing in unit 1 was exposed more B during the study as the inflow was firstly dosed into unit 1 according to operational conditions, and that's why we obtained lower biomass production in unit 1. Therefore, it is recommended that managers and environmental strategists should take into consideration the potential B toxicity effect on plant growth and biomass production for the HCW-MFC in their experiment plan. On the other hand, higher biomass production in unit 2 might also have catalyzed more bioelectric production compare to bioelectric production from unit 1 during the experiment period (Fang et al., 2013). Expectedly, more biomass production in unit 2 could have enhanced the cell voltage, and reduced the internal resistance of unit 2, so thus we obtained more bioelectric compared to unit 1 (Fig 4a).

Boron content for *T.latifolia* growing in unit 1 and unit 2 are also given Table 2. It can be seen in Table 2 that B content of *T.latifolia* growing in unit 1 was approximately 200 mg kg⁻¹ higher than the plants in the unit 2. It was an expected result because higher concentration of B predominantly flowed in unit 1, and so individuals of *T.latifolia* were exposed to more B compared to the plants growing in unit 2. Moreover, it can be also hypothesized that higher concentrations of B as a form of boric acid are transmitted to unit 1 and thus more B was available for *T.latifolia* uptake in this unit. It is because B is taken up by plant organisms from water mainly as a form of boric acid and stored in leaf, stem, root, and flower (Kröpfelová et al. 2009). Probably, the presence of high concentrations of B in unit 1 led to a significant effect on B uptake by the plants, suggesting that individuals of *T.latifolia* could play a more crucial role associated with B removal depending on its initial concentration (Türker et al. 2016). Various researchers also reported that some cattail species have direct role in B purification process in different type of CWs (Ye et al. 2003; Türker et al. 2014a; Türker et al. 2016).

The analysis of the nitrogen (N) content in *T.latifolia* revealed that the leaves had a higher nitrogen content than the stems and roots/rhizomes of individual plants growing in both unit 1 and unit 2 (Table 2). This result suggested that a nitrogen transport corridor occurred in *T.latifolia*, and that N is transported to upper parts of the plant organs such as leaves and stems after being taken up by roots/rhizomes. Therefore, harvesting of aboveground plant organs such as leaves from HCW-MFC might be an effective strategy to remove the nitrogen based pollutants from wastewater (Türker et al., 2013). On the other hand, it can be seen in Table 2 that nitrogen content in *T.latifolia* growing in unit 1 was found to be higher than *T.latifolia* growing in unit 2 in the experiment period. This result also showed evidence for more nitrogen substrates such as nitrite and nitrate was reduced by unit 1 during the first phase of the experiment.

3.4. Filtration media analysis of HCW-MFC

Total B concentration, extractable B (plant available B form as boric acid) amount, and other parameters (pH, EC, redox, nitrogen content, and sediment texture) at the end of the experiment are given in Table 3. The results showed that more than 700 mg kg⁻¹ and 500 mg kg⁻¹ B retained in the filtration media of unit 1 and unit 2, respectively. Therefore, it can be suggested that filtration medias in wetland units have the potential to remove B from wastewater and thus high level of B was retained in the organic based peat (as using filtration media) during the experiment period. Some reports from the literature also supported our findings that a sorption mechanism in organic based materials is the key element for removal of B in wetland matrix (Allende et al. 2014, Ye et al. 2003). Furthermore, it can be seen in Table 3 that extractable B concentration in the unit 1 was 3.39 mg kg⁻¹ higher than the unit 2, meaning that a higher number of the sorption sites have taken up B which was available for the plant in unit 1 compared to unit 2 during the experiment period. This situation may also

explain the higher B content in *T.latifolia* growing in unit 1 compared to individual plants in unit 2. On the other hand, other parameters such as pH, EC, redox, nitrogen content, and sediment texture can also be considered to have important roles on retained B content in the filtration media of the units. It is because these factors together with flow regime are known to control B adsorption in the wetland matrix (Türker et al. 2016, Allende et al. 2012).

3.5. Relationships between soil enzymes activities and bioelectric production

Dehydrogenase, urease, and phosphatase enzymes activities in wetland matrix can provide important information about the quantitative aspects of the quality of living dynamics related to microbial activity and microbial compositions in soil environment (Zhang et al. 2010). However, studies on the relationship between soil enzyme activities and bioelectric production in CWs is still lacking, and no direct research has been performed so far.

The activities of dehydrogenase, urease, and phosphatase in wetland units during the treatment days are shown in Fig 5. It can be seen that enzyme activities except phosphatase gradually increased with increasing bioelectric production both in unit 1 and unit 2 during the experiment period. Dehydrogenase activity in unit 1 and unit 2 increased from 12 to 54 $\mu\text{g TPF g}^{-1} \text{ h}^{-1}$ and 8 to 98 $\mu\text{g TPF g}^{-1} \text{ h}^{-1}$. Additionally, bioelectric production for the unit 1 and unit 2 increased from 0.55 to 1.43 V and 1.01 to 1.56 V, respectively. Moreover, we determined a similar trend for urease activity, and thus higher enzyme activity for unit 1 and unit 2 which were measured as 1188 and 2675 $\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ 48 h}^{-1}$ respectively when bioelectric production was high. On the other hand, the lowest activity of urease both in unit 1 and unit 2 was observed as 114 and 640 $\mu\text{g NH}_4^+ \text{ g}^{-1} \text{ 48 h}^{-1}$ respectively when bioelectric production was low. However, we did not note any discernible temporal trends between phosphatase enzyme activity and bioelectric production in unit 1 and unit 2, and the activity varied from 3.5 to 22.1 $\mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$ and 12.3 to 21.1 $\mu\text{g p-nitrophenol g}^{-1} \text{ h}^{-1}$,

respectively. Additionally, bioelectric production for unit 1 and unit 2 increased from 0.55 to 1.43 V and 1.01 to 1.56 V, respectively. These results indicated that bioelectric production in wetland matrix increased with increasing soil enzyme activities, especially of dehydrogenase and urease which are related to cycling of carbon and nitrogen. This may be because the presence of more microbial density catalyzes mineralization and transformation of organic matters and the hydrolysis of urea to carbon dioxide (Zhang et al. 2010). Notably, it can be seen from the present study that bioelectric capacity can be enhanced with increased soil enzyme activities. However, this phenomenon can also be related to B concentrations which were flowing through wetland units during the experiment period. In this respect, we found that soil enzyme activities in unit 2 were higher than the activities in unit 1, suggesting that high concentrations of B can decrease soil enzyme activities in wetland matrix. Therefore, it can also be hypothesized that when the high initial B concentration was dosed into unit 1, the production of the soil enzymes with microorganisms in the unit1 were inhibited more by B compared to unit 2, and so bioelectric production rises remarkably with wastewater passing from unit 1 to unit 2.

Furthermore, we determined that dehydrogenase and urease activities in unit 1 showed significant correlations as $r = +0.674$ ($p < 0.01$) and $r = +0.767$ ($p < 0.01$) respectively with bioelectric production, whereas there were no significant correlations among phosphatase ($r = 0.258$; $p > 0.05$) enzyme activity and bioelectric production for unit 1. A significant correlation was found between dehydrogenase activity and bioelectric production for unit 2 ($r = 0.686$; $p < 0.01$), while we did not find any significant correlations urease and phosphatase enzyme activities with bioelectric production for unit 2 in the experiment period. These results suggested that the monitoring and determining of soil dehydrogenase and urease in wetland matrix could serve as important biomarkers for bioelectric production during the contaminant purification process. Nevertheless, further studies are needed for a deeper understanding on

the relationship between soil enzyme activities and bioelectric production in the wetland systems treating both nitrogen and B from wastewater.

4. Conclusions

A HCW-MFC was designed, evaluated, and tested in term of feasibility for B removal and bioelectric production concurrently. 63.4% B removal average was achieved for wastewater containing average 12.3 mg L⁻¹ inflow B concentrations. This wetland system was also able to remove 47.5% and 19.1% nitrate and nitrite from wastewater, respectively. The maximum power density and current density was recorded as 78 mWatt/m² and 105 mA/m² for the last unit in the hybrid system, respectively. Dehydrogenase and urease enzyme activities were strongly correlated with bioelectric production, suggesting that these enzymes might play important roles in bioelectric production in HCW-MFC.

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Figure Captions

Fig. 1. The schematic demonstration of the hybrid constructed wetland couple with microbial fuel cell (HCW-MFC).

Fig. 2. Inflow and outflow properties both in unit 1 and unit 2 (a) and mass removal rate of HCW-MFC in the experiment period (b). Error bars indicate standard deviation.

Fig. 3. Nitrate and nitrite concentrations, as well as physicochemical parameter of inflow and outflow samples both in the unit 1 and unit 2 during the experiment period. Error bars indicate standard deviation.

Fig. 4. Voltage output of unit 1 and unit 2 (a), power density and current density monitoring of unit 1 (b) and unit 2 (c) in the experiment period.

Fig. 5. The activities of dehydrogenase, urease, and phosphatase enzymes both in unit 1 and unit 2 based on bioelectric production in the experiment period. Error bars indicate standard deviation.

Fig. 1. The schematic demonstration of the hybrid constructed wetland couple with microbial fuel cell (HCW-MFC).

Figure

Fig. 2. Inflow and outflow properties both in unit 1 and unit 2 (a) and mass removal rate of HCW-MFC in the experiment period (b). An asterisk (*) denotes significant difference ($p < 0.05$) of the mean value between the inflow and outflow in the treatment period. Error bars indicate standard deviation.

Fig. 3. Nitrate and nitrite concentrations, as well as physicochemical parameter of inflow and outflow samples both in the unit 1 and unit 2 during the experiment period. Error bars indicate standard deviation.

Fig. 4. Voltage output of unit 1 and unit 2 (a), power density and current density monitoring of unit 1 (b) and unit 2 (c) in the experiment period.

Fig. 5. The activities of dehydrogenase (a), urease (b), and phosphatase (c) enzymes both in unit 1 and unit 2 based on bioelectric production in the experiment period. Error bars indicate standard deviation.

Table 1. Overall treatment performance of hybrid constructed wetland with microbial fuel cell during the experiment period. Range in parentheses.

	Inflow	Unit 1 outflow	Unit 2 outflow	Overall treatment efficiency(%)
Boron (mg L ⁻¹)	12.3 (2-28)	5.5 (1.4-13)	3.7 (0.8-13)	63.4
Nitrate (mg L ⁻¹)	70.5 (44-88)	48.2 (30-64)	36.5 (26-50)	47.9
Nitrite (mg L ⁻¹)	19.3 (2.3-38.4)	16.9 (2.6-32)	15.2 (2.03-31)	19.1
pH (-log[H ⁺])	6.14 (5.98-6.33)	6.5 (4.66-8.01)	6.8 (5.18-8.05)	-
Redox (mV)	201 (157-271)	150.6 (103-198)	158.2 (88.5-308)	-
EC (µS cm ⁻¹)	993 (428-1937)	1249 (796-1830)	1395 (734-1994)	-
Temperature (°C)	22.5 (20.4-23)	22.8 (21-26.3)	22.5 (21.2-24)	-

Table 2. Plant monitoring, biomass production, boron (B) concentrations, and nitrogen (N) content in the experiment period. Range in parentheses.

	<i>Typha latifolia</i> (Cattail) growing in Unit 1				<i>Typha latifolia</i> (Cattail) growing in Unit 2			
	Leaves	Stems	Roots/Rhizomes	Whole plant	Leaves	Stems	Roots/Rhizomes	Whole plant
Plant Height (cm)	79.8 (48-100)	-	-		99.6 (88-109)	-	-	
Chlorophyll-a content (µg L ⁻¹)	24.1 (19.7-33.4)	-	-	-	28.06 (26.4-35.7)	-	-	-
Chlorophyll-b content (µg L ⁻¹)	13.4 (10.09-23.02)	-	-	-	16.1 (12.7-20.7)	-	-	-
Biomass Production (DW g/m ²)	175	15.5	77.4	268	132.5	29.1	100.1	305
Boron concentrations (mg/kg)	-	-	-	>700	-	-	-	>500
Nitrogen (N) content (%)	2.57 (2.51-2.68)	2.25 (2.19-2.33)	1.67 (1.51-1.74)	-	2.15 (2.05-2.22)	1.19 (1.01-1.38)	1.53 (1.48-6)	-

Table 3. Chemical and Physical analyzes of filtration media from unit 1 and unit 2 at the end of the experiment.
Range in parentheses.

	Unit 1	Unit 2
Total amount of B (mg kg ⁻¹)	>350	>300
Extractable B (mg kg ⁻¹)	10.83 (7.84-15.45)	7.44 (4.11-12.7)
pH (-log[H+])	8 (7.96-8.06)	7.99 (7.91-8.04)
Redox (mV)	81.2 (80.5-81.8)	82.7 (81.6-84.5)
EC	434 (408-443)	542 (515-542)
N (%)	0.527 (0.34-0.795)	0.2621 (0.237-0.272)
Sediment Texture	sand	sand