EFFECT OF PETROLUME REFINERY WASTEWATER ON PLANT GROWTH IN INTEGRATED MICROBIAL FUEL CELL-CONSTRUCTED WETLANDS SYSTEMS

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ABSTRACT
Three identically designed microbial fuel cell-constructed wetland (MFC-CW) systems were constructed and setup in this study for simultaneous biotreatment of petroleum refinery wastewater (PRW) and bioelectricity generation. MFC-CW1 and MFC-CW2 were planted with C*anna indica, and Phragmites australis*, respectively. MFC-CW3 was unplanted and considered as the control. These three systems were operated simultaneously in a batch mode for two cycles to evaluate the effect of PRW biotreatment on the growth and development of the selected plants and the potential of generated bioelectricity as well. The operation period for each cycle was 8 days. Results demonstrated that maximum removal efficiency of the organic content represented as chemical oxygen demand (COD) were 98.75%, 97.67%, and 97.83% observed in MFC-CW1, MFC-CW2, and MFC-CW3, respectively, whereby, the highest power generation were 19.86, 19.04, and 18.7 mW/m^2, respectively. On the other hand, both types of plants exhibited notable growth and new sprouts appearance. The potential convergence of the results in the three MFC-CWs, and the healthy growth of both types of plants clearly and potentially indicated that the dominant mechanism of organic pollutant removal was via biodegradation process by the anodic biofilm in the MFC rather than being removed by phytoremediation process.

Keywords: biodegradation, *Canna indica*, , electricity generation, irrigation, organic content

مجلة العلوم الزراعية العراقية -2020 :51 (4):1239-1248 **حسين واسماعيل**

تأثير المياه العادمة من مصافي النفط على نمو النباتات في النظم المتكاملة للأراضي الرطبة المشيدة مع خلايا الوقود الحيوي

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المستخلص
تم في هذه الدراسة تنفيذ ثلاثة منظومات متماثلة التصميم للأراضي الرطبة المشيدة – وخلية الوقود الحيوي نوع النبات المستخدم، بهدف معالجة المياه الناتجة من عمليات تكرير النفط وانتاج طاقة بيولوجية في آن واحد. تم زرع المنظومة الاولى بنبات القنب الهندي، والثانية بالقصب العادي، والثالثة بدون نبات ويعتبر كنظام مرجعي. تم تشغيل المنظومات الثلاث بنفس الوقت على النظام الدفقي وبدورتين, لغرض تقييم تأثير مياه تكرير النفط المعالجة بيولوجيا على تطوير ونمو النبات المزروع داخل انظمة المعالجة مع أمكانية أنتاج الطاقة في آن واحد .أستمرت فترة التشغيل لكل دورة ثمانية ايام. اوضحت نتائج الدراسة ان اعظم كفاءة ازالة للمحتوى العضوي والمتمثل بطلب الأوكسجين الكيميائي كانت 98.75% ,97.67 %, و95.83% للأنظمة MFC- CW2,MFC-CW1 ,و MFC-CW3 على التوالي. في حين أن اعظم كثافة طاقة ناتجة كانت 19.86 ,19.04, و 18.7 ملي واط/متر مربع للأنظمة CW2,MFC-CW1 -MFC, و MFC-CW3 على التوالي, مما يدل على تقارب أداء المنظومات من حيث الكفاءة وتوليد الطاقة. أما من حيث نمو النبات, فقد أشارت معدلات النمو النسبي (RGR) لكلا النوعين من النباتات المختارة الى أن نمو البراعم كان بشكل طبيعي طول فترة الدراسة مما يثبت أن آلية التحلل البيولوجي على قطب الأنود داخل خلية الوقود البيولوجية هي الآلية المسيطرة بعملية ازالة الملوثات العضوية بدلا من ازالتها بمساعدة النبات.

الكلمات المفتاحية: التحلل البايولوجي، نبات القنب الهندي، توليد الطاقة الكهربائية، السقي، المحتوى العضوي

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INTRUDUCTION

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 (20, 23). Refinery operations are quite complex and require large volumes of water, and the composition of refinery wastewaters can vary substantially among different sites (2). However, conventional wastewater treatment plants currently consumes a significant amount of electricity (23). Accordingly, in order to offset the costs of wastewater treatment and save energy, alternative methods are being developed to capture energy or produce useful chemicals from wastewaters (22). Microbial fuel cells (MFCs) can exploit a variety of soluble or dissolved complex organic waste/wastewater as substrate to fulfill renewable electricity generation along with simultaneous waste remediation (8, 26). A complex substrate composition helps in building a diverse and electrochemically active microbial community while a simple substrate is easier to degrade and improves the electricity generation of the system (5). On the other hand, plants growing in wetland environment have a large biomass, development roots, and robust stems. They can be planted in natural environments with a minimal disturbance of scenery without being competitive with agricultural lands which are needed for food production (15). In new systems of MFC integrated with constructed wetland (CW), plants can play an important role in the operation of MFCs. Their roots can also secrete oxygen allowing different oxygen levels to occur in different regions of the system helping survival of different microorganisms (12, 28) and provide the matrix for the generation of the electricity (30). Although the use of *Phragmitis* as the most expanded plant (29) and *Typha* species for wetland establishment is a tradition since 1950's and still going on. *Canna indica* is commonly used for wetland establishment in China and other countries (6, 25) as it has rapid growth rate, large biomass and beautiful flowers with great capability of nutrient removal (17). *Canna indica* is known as phytoremediation plant has great tolerance to the pollutants and has long root life span (3, 33). MFC-CW integrated system is an operationally friendly, economically sustainable approach for wastewater treatment (36). Previous studies indicated that the presence of the macrophyte increases microbial diversity and provides large surface areas for the development of biofilm (4). Thus, the reactions that occur in wetland environments generate aerobic and anaerobic zones that can be utilized for the implementation of MFCs. Yadav et al. (35) was the first who investigated the possibility of combining CW with MFC to treat a synthetic wastewater containing azo dye. The compatibility and combination of CWs and MFCs are based on the fact that they are both biological systems engaged in the degradation of organic matter. Additionally, an MFC requires a redox gradient, which can be naturally found in a CW, depending on flow direction and wetland depth (7). Most relevant studies have focused on improving anodic and cathodic efficiency or reducing the internal resistance of the MFC-CW to generate more energy or improve nutrient removal efficiency from wastewater (10, 11). This study aimed to investigate the followings:

1- The potential of simultaneous biotreatment of real petroleum refinery wastewater (PRW) biotreatment and bioelectricity generation in an integrated system of MFC-CW

2- The effect of using real hazardous PRW on the growth of two types emergent wetland macrophysics

3- The dominant mechanism for removal of organics from wastewater whether it is biodegradation by the anodic biofilm or phytoremediation process

MATERIALS AND METHODS

Substrate

Fresh petroleum refinery wastewater (PRW) was used to fuel the microbial fuel cell in this study. The samples were freshly and continuously collected from Al-Dora refinery in Baghdad. Table 1 shows the quality of this industrial wastewater in terms of Chemical oxygen demand (COD), pH, and Total suspended solid (TSS) Total petroleum hydrocarbon (TPH), phosphate ions (PO^{-4}), chloride ions (Cl^-), sulfate ions (SO_4^{-2}), copper (Cu), lead (Pb), zinc (Zn), and cadmium (Cd). The PRW is characterized by its high organic content as COD concentration is up to 1200 mg/l, and total petroleum hydrocarbons of 480 mg/l. The characteristics of PRW were determined according to the procedure outlined in the *standard methods* (1).

Biocatalyst

Activated sludge freshly collected from the existing wastewater treatment plant in Al-Dora refinery was used to inoculate the anodic section of the MFC embedded with the CW. Analysis of the collected sludge samples indicated that *Bacilli* species were the dominant type of bacterial cells in the sludge samples. The initial volatile suspended solids (VSS) concentration was 626.6 mg/g which represents the initial concentration of biomass in the sludge.

MFC-CW systems

In this study, three identically designed MFC-CW systems were constructed and set up out door (Plate. 1). These systems were identified as MFC-CW1, MFC-CW2, and MFC-CW3 and all of them were made of Perspex column of 0.5 cm thickness, 19 cm inner diameter and 40 cm height.

Table 1. Characteristics of the real petroleum refinery wastewater (PRW)

Constituent	Unit	Average concentration	Allowable* concentration
pH	-	7.75	6-9.5
COD	mg/l	1200	100
TSS	mg/l	75	30
TPH	mg/l	480	10
CL^-	mg/l	68	200
SO_4^{-2}	mg/l	55	200
PO_4^{-2}	mg/l	7.5	3.0
NO_3^-	mg/l	30	50
Cu	mg/l	Nil	0.2
Pb	mg/l	Nil	0.1
Zn	mg/l	Nil	0. 5
Cd	mg/l	Nil	0.01

*** According to the Iraqi regulation No. 25 in 1976 for rivers and public water reservation.**

Each MFC-CW system was filled with gravel only to the height of 37cm. The upper ends of the MFC-CW systems were maintained open to atmosphere. In each system, two identical plain graphite electrodes of 1cm thickness were used. The anode electrode was positioned at distance of 10 cm from the bottom in an anaerobic condition according to the suggestion reported by Yadav et al. (34) based on the fact that the stratified redox conditions in CWs can serve as cathode and anode compartments for developing a unique MFC-CW. In a flooded wetland system anaerobic and aerobic conditions exist throughout the bed depth. Near the surface aerobic conditions are likely to prevail; oxygen intrusion from the atmosphere in tandem with oxygen leakage from the rhizosphere of wetland plants should supply dissolved oxygen to sustain cathodic reactions, while increasing depth and water saturation will ensure anaerobic conditions in the wetland bed. The stratified redox conditions that exist may be exploited by incorporating an anode in the anaerobic section and a cathode in the aerobic section thereby creating an in-situ, non-destructive CW-MFC. In this study, the cathode was installed at the plant root zone at 30 cm from the base of the MFC-CW system. Both electrodes were layered between gravels. Each electrode had a projected surface area of 0.0565 m^2. The electrodes were placed horizontally in the system to maintain. the anode zone relatively anaerobic and the cathode zone more aerobic. The total volume of each MFC-CW was 12.56 L (0.01256 m^3) with the total void volume of the system (net liquid volume of 5 L (gravel porosity = 0.43%). A sampling port was located directly above the anodes in each MFC-CW system samples. MFC-CW1 was planted with common *C. indica* at a plant density of 4 plants/ reactor, which was placed on the upper surface of the cathode electrode. MFC-CW2 was planted with the common reed *P. australis*, which is a type of emergent wetland plants. Both types of plants were used in this study without pre- acclimation. MFC-CW3 was considered to be as the control system without planting. The cathode and anode electrodes in the MFC were connected to each other using copper wires to an external resistance 100Ω. Before constructing and operating the three MFC-CW systems, all anodic electrodes were inoculated with the biomass in an anaerobic environment, each electrode with 100 g of biomass, and then continuously monitored for 4 weeks with periodic feeding with the PRW in order to acclimate the biomass for this toxic type of wastewater.

Plate 1. Photo of the MFC-CW systems

Experimental procedure

After inoculation, the three MFC-CWs were operated in a batch mode at a hydraulic retention time (HRT) of 8 days. The PRW was added to each system from the top and was allowed to firmly trickle through the system. Two batch cycles were considered for each system. The purpose for considering two batches rather than a single batch was to observe the reproducible organic matter degradation in term of COD removal, and to ensure repeatable voltage outputs. During each cycle samples of influent and effluent were daily collected for analysis to observe the profile of COD reduction in the MFC-CW systems. Samples of effluent were withdrawn from the sampling port (S3) which was located 3cm below the cathode compartment before reaching the plant roots. Also, additional samples of the treated effluent were collected from the outlet of the cathodic compartment after passing through the plants roots.

Analytical methods

Samples were collected and analyzed on a daily basis for dissolved oxygen (DO), chemical oxygen demand (COD), total dissolved solids (TDS), electrical conductivity (EC) concentrations, and pH values as well. COD analysis was carried out using COD analyzer (Model: RD 125, Lovibond, Germany). DO concentrations were measured using dissolved oxygen meter (Model: DO-5510HA, Taiwan). pH, TDS, and EC value were measured using multi parameters analyzer (Model: Portable Milwaukee, MW 803, Romania). A volatile suspended solid (VSS) measurements were determined according to EPA-1684 Method. All measurements were performed in duplicate.

The COD removal efficiency can be calculated by using the formula below:

$$COD_E = \frac{C_{Inf} - C_{eff}}{C_{Inf}} \times 100\% \quad \text{...... (1)}$$

Where; C_{inf} is the COD concentration of the influent, and C_{eff} is the COD concentration of the effluent (mg/l).

Electrochemical measurements

The cell voltage were monitored in order to determine the power generation and the performance of MFC-CWs. The potentials between the edges of the fixed external resistance (100 Ω) were measured data logger as well as a portable handheld digital multimeter (model: MT-1233C, Proskit, Taiwan) for double checking. Once the voltage outputs of each reactor were stabilized, the electrochemical performance of the systems were analyzed in term of polarization and power density.

The electrical current (I) measured in amperes (A) was calculated by using Ohm's law:

$$I = \frac{V}{R} \quad \text{..... (2)}$$

Where; V is cell voltage (V), R is the external resistance (Ω).

Power was calculated using the following formula:

$$P = I \times V \quad \text{..... (3)}$$

Where; I is the current (A) and V is cell voltage.

Current density (ID, mA/m^2) and Power density (PD, mw/m^2) were normalized to anode surface area and determined by the Eqs. (4) and (5), respectively.

$$ID = V/(R \times A) \quad \text{..... (4)}$$

$$PD = ID \times V \quad \text{...... (5)}$$

Polarization and power density curves were obtained by varying the external resistance from 100000Ω to 3Ω every 10 min to ensure that the measured values were steady and repeatable. The potential of electrodes was measured using the anode or the cathode as the working electrode against a (SCE) saturated calomel Ag/AgCl reference electrode (Type: Ag-AgCl Beckman Laboratory, USA). and the values were recorded with the digital multimeter. The total internal resistance ($R_{int,\Omega}$) of MFC-CW was calculated by the peak power density method (linear region of polarization curve) with the aid of polarization and power density curves. When the maximum power density (P_{max}) was obtained, the internal resistance was equal to the external resistance (R_{ext},Ω) (21).

$$R_{int}= R_{ext}=P_{max} /I^2 \quad \text{.... (6)}$$

Where; I is the current corresponding to the maximum power density. The Coulombic efficiency (ɳ) is defined as the ratio of the actual charge generated to the maximum theoretical charge attainable. It is a critical parameter used to evaluate the performance of an MFC system. For the case of fed-batch mode, Coulombic efficiency was calculated as follows (18):

$$\eta_{Coulombic} = \frac{M\int_0^t I dt_{exp}}{FnV\Delta COD} \times 100 \times 1000 \quad \text{... (7)}$$

Where

$\eta_{Coulombic}$ = Coulombic efficiency, %

I = Current generated in the MFC system, Ma

t_{exp} = Interval of recording, seconds

t =Total period of the experiment, seconds

F =Faraday's constant, 96485 coulomb/mole

ΔCOD = Difference between the inlet and outlet chemical oxygen demand concentration, (mg/l)

n =Number of electrons exchanged per mole of substrate (eight mole of electrons were produced as acetate oxidation in anaerobic anode chamber).

V =Effective volume of the effluent involved in the work (mL)

M=Molecular weight of the substrate (g/mole), (for acetate M = 60 g/mole).

Plant growth measurement

Wetland plant growth and plant heights were daily monitored. The plant height was calculated as the average height of leaves in each system, The growth of emergent macrophyte was also monitored by visual observation. The relative growth rate (RGR) was used to reflect the growth of plants and was determined by Eq (8).

$$RGR = (\ln h_2-\ln h_1)/t \quad \text{...... (8)}$$

Where

t is the duration of the test, (days)

H_2 (cm) and H_1 (cm) represent the total height of plants above the interface at the beginning and the end of the cycle, respectively (36).

RESULTS AND DISCUSSION

COD and TPH removal

The profiles of COD removal in the three MFC-CW systems for the two batch cycles are given in Figure 1. The results demonstrated that maximum removal efficiencies of the organic content represented as COD were 98.75%, 97.67%, and 97.83% observed in MFC-CW1, MFC-CW2, and MFC-CW3, respectively indicating that the three MFC-CWs exhibited approximately similar potential for COD removal from the real PRW. This observation could be attributed to the fact that the removal of COD occurred due to the biodegradation process by the anodic biofilm rather than being removed due to phytoremediation process.

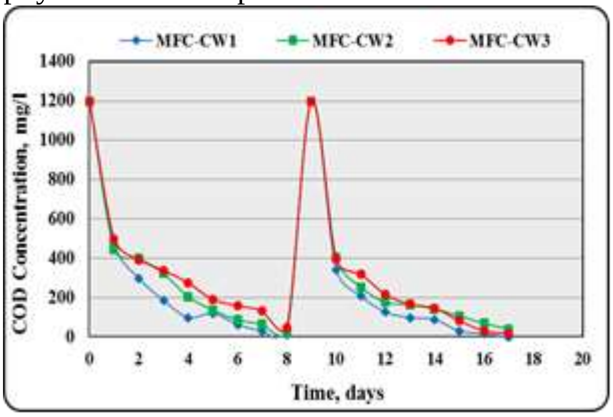

Figure 1. Profiles of COD removal in MFC-CWs

This suggestion was confirmed by the analysis of the treated PRW obtained from the port just above the anode at the level below the plant root. This fact explain the healthy growth of the plants since the treated PRW reaching their roots were free of the toxic hazardous organic pollutants which were completely biodegraded by the anodic biofilm. On the other hand, complete removal of the total petroleum hydrocarbons (TPH) up to 100% was observed in the effluent samples which were withdrawn directly above the anode electrode lower than the plant roots zone.

Power generation

The profiles of power generation in the MFC-CWs are shown in Figure 2.

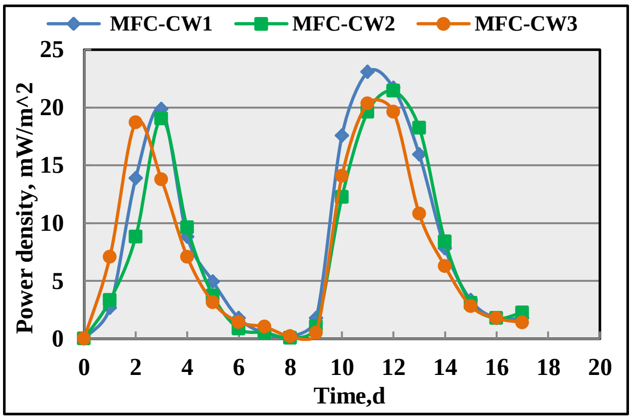

Figure 2. Profiles of power generation in MFC-CWs

As given in Figure 2, a sharp increase in the power generation was observed in the three systems during the first day of operation for each batch cycle, and then a steep drop was observed indicating the depletion of organic substrate. Also, it worth to mention that in wetlands, the plant increased the concentration of oxygen at the root zone creating greater redox potential gradient between anode and cathode thus enhancing the electron transfer rate through the external circuit (32). Moreover as the upper part of the wetland is exposed to atmosphere also contributes to some extent in maintaining aerobic conditions (32), and the higher availability of O_2 can amplify the cathodic potential. The results demonstrated that the maximum power generation in the first batch cycle were 19.86, 19.04, and 18.7 mW/m^2, for MFC-CW1, MFC-CW2, and MFC-CW3, respectively. Whereby for the second batch cycle, the results revealed that the maximum power densities were 23.1, 21.43, and 20.34 mW/m^2, respectively. It can be noticed that the maximum power densities in MFC-CW1, MFC-CW2 and MFC-CW3 systems were comparable and a very marginal differences were observed indicating that the existence and type of wetland plant had negligible effect on improving the power generation. This observation could be attributed to the fact that all the organic matters were oxidized by the anodic biofilm before reaching the plants roots and accordingly the released electrons will transfer via the anode to the cathode generating electrical current. The pH of influent PRWW samples was neutral or slightly alkaline (7.3-7.5), indicating that they were in a range suitable for bioelectrochemical treatment (13).

Polarization curves

Polarization curves were prepared and plotted for both the planted and unplanted systems represented by MFC-CW1 and MFC-CW3, respectively. The results revealed that maximum power density was 8.5 mW/m^2 at external resistance of 220Ω for MFC-CW1 as shown in Figure 3A, whereby, 8.16 mW/m^2 was the maximum power density observed at external resistance of 390 Ω for MFC-CW3 as given in Figure 3B. These results were in accordance with the fact that the maximum power achieved when internal resistance is equal to external resistance (Maximum point $R_{ext}=R_{int}$) (16). The internal resistance can be estimated as the slope of the linear section of polarization curves (21). These observations confirmed that the increase of power density in the planted systems compared to its value in the unplanted MFC-CW is due to the fact that the existence of plant roots directly on the cathode surface can wound around it with time, causing an increase in redox potential by releasing a sufficient amount of rhizospheric oxygen to run cathode reaction, and thus improved the power generation of the system. Oxygen reduction reaction is often considered at the cathode of MFCs, and described as:

$$O_2+4H^+ +4e^- = 2H_2O \qquad \text{….. (8)}$$

this process may be enhanced by the rhizosphere effect of plants (32). Overall, this analysis based on the polarization curves suggesting that the presence of plant at the cathode region could reduce internal resistance of the system and, therefore maximizes cell performance. These findings were consistent with the results outlined by Zhou et al. (36). The internal resistance which is also current-dependent, is defined as the sum of all internal losses (21) and the cathode is one of the main factors contributing to the internal resistance (9).

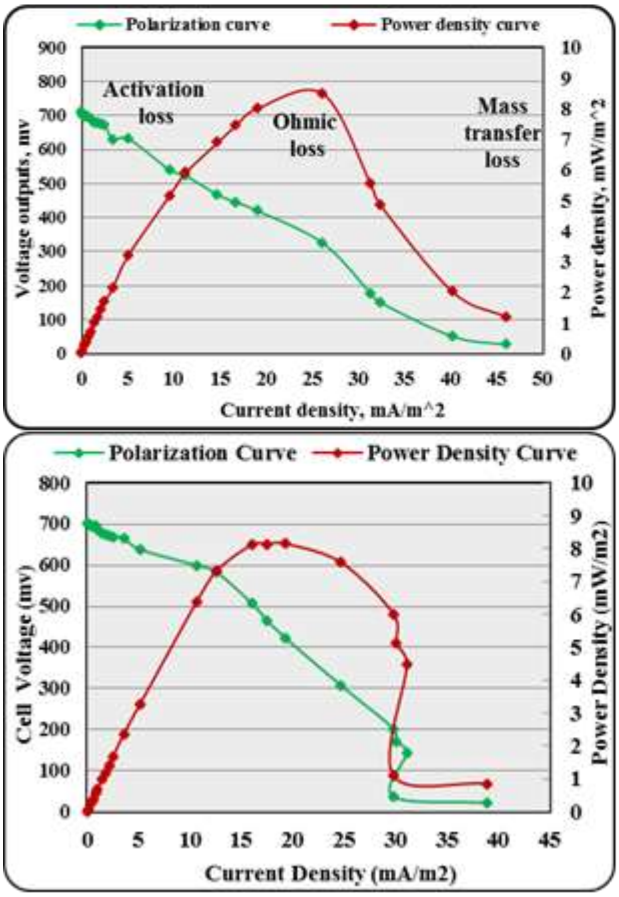

Figure 3. Polarization curves for; (A) MFC-CW1, (B) MFC-CW3

Coulombic efficiency

The Coulombic efficiencies for the three MFC-CW systems were calculated as a function of the COD removal and voltage output. Results of the Coulombic efficiencies revealed that the Coulombic efficiencies of MFC-CW1, MFC-CW2, and MFC-CW3 marginally increased in the second batch cycles as given in Table 2.

Table 2. Coulombic efficiencies in the MFC-CWs

Cycle No.	MFC-CW1	MFC-CW2	MFC-CW3
First	6.23	6.17	6.23
Second	6.64	6.62	6.32

However, all the Coulombic efficiency values were comparable. These results were in a good agreement the Coulombic efficiencies values ranged from 1% to 7% previously reported by He et al. (14). Villasenor et al. (32) reported columbic efficiency values between 0.27% and 0.45%.

Growth of plants

Both types of the plants in MFC-CW1 and MFC-CW2 exhibited healthy growth with an final average plant height of 35.1 cm and 22.67 cm for *C. indica* with three new shoots and common reed with four new shoots, respectively. Figure 4 A and B presents the growth potential, new shoots, and length increase of *C. indica* and common reed, respectively. However, lower growth rate was observed with the common reed seedlings compared to *C. indica* indicating that *C.indica* seedlings were well acclimated to the MFC-CW system. In general, *C.indica* shoots exhibited a relatively significant healthy gradual growth little bit more than the common reed. This observation is important because according to Fang et al. (11), denser biomass in a wetland matrix might also have catalyzed bioelectric production. Accordingly, more biomass production in the wetland module could enhance the cell voltage, and reduce the internal resistance of the system, resulting of more bioelectricity production (30). The fully consumption and degradation of substrate by microorganisms at the bottom of the MFC-CW, high stable COD removal efficiencies, and the potential reduction in the total petroleum hydrocarbons (TPH) contents have positively affected the plant growth.

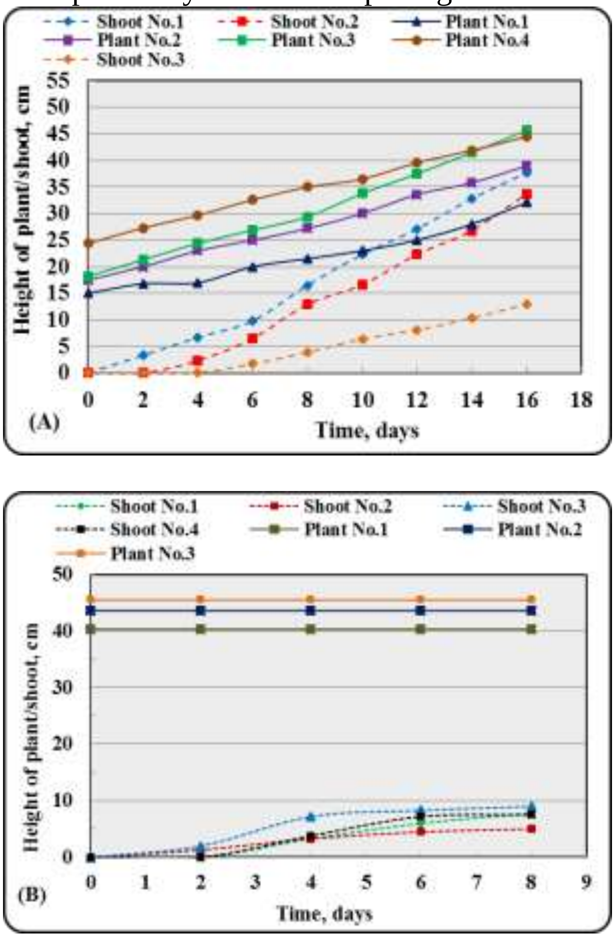

Figure 4. Profile of development of plants overtime for; (A) *canna indica*, (B) common reed

In addition, the relative growth rates (RGRs) of the two wetland plants are presented in Figure 5A and B. indicates that *C. indica* had notable increase in RGR during the first week, whereas the RGR of the common reed was unobservable. During the second week, the *C. indica* plant did survive and kept growing at relatively small positive rate, and their shoots steadily increase and remained positive all the period of study (16 days). Whereas the common reed plant did not survive and their shoots also withered and finally stop growing completely. Murr (24) suggested that a micro-current environment is beneficial to the growth of plants to some extent. Sufficiently high electric field have a definite effect on plant growth and growth responses.

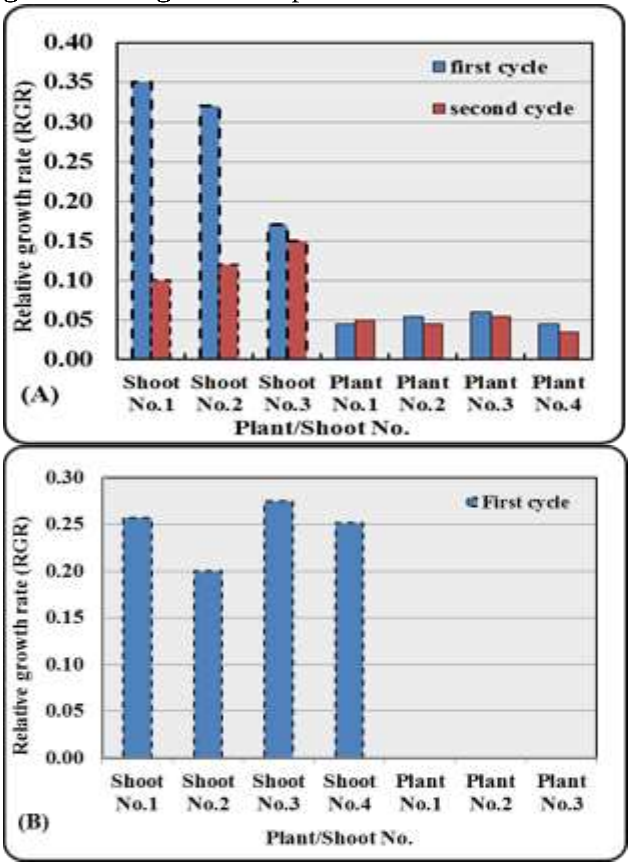

Figure 5. Profile of changes of the relative growth rate (RGR) into; (A) *C. indica*, (B) common reed

Results of this experimental study demonstrated that maximum removal efficiency of the organic content represented as COD were 98.75%, 97.67%, and 97.83% observed in MFC-CW1, MFC-CW2, and MFC-CW3, respectively, whereby, the highest power generation were 22.05, 21.1, and 20.71mW/m^2, respectively. In spite of the PRW toxicity, both types of plants *C. indica* and *P. australis* well survived, and maintain healthy and positive growth over the entire period of operation, indicating that the dominant mechanism of organic pollutant removal was via biodegradation process by the anodic biofilm in the MFC rather than being removed by phytoremediation process. However, compared with *P. australis* (common reed), *C. indica* was slightly more sustainable in growth during the treatment course of refinery petroleum refinery wastewater (PRW).

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