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Research Article

Technology of Microbial Electrolysis Cell with Statistical Optimization by Response Surface Methodology for Bio-Hydrogen Production and Phosphorus Recovery

A.N.Z. Alshehri[1]

Department of Biology, University College in Al-Jummum, Umm Al-Qura University, Makkah, 21955, Saudi Arabia.

Abstract

In this study, two chamber microbial electrolysis cells (MECs) were used to investigate effect of applied voltage and concentration of influent COD on bio-hydrogen (H_2) production and phosphorus (P) recovery. On the cathode chamber P as crystals were precipitated (the maximum was 94%), and verified as struvite, using X-ray diffraction and scanning electron microscopy analysis. Maximum of the H_2 production rate was $0.31 m^3/m^3/d$. H_2 production and P recovery have highly affected by applied voltage according to statistical optimization, while P recovery only had significantly affected by influent COD concentration. The range from 28 to 42%, was the total of energy recovery in the MEC. The current findings demonstrated capability of H_2 production and P recovery using MECs technology.

Keywords: Bio-hydrogen; Phosphorus; Struvite; Microbial electrolysis cell; Statistical optimization; Response surface methodology

Introduction

Demand for unsustainable resources worldwide has increased due to rising of population. Therefore, depletion of resource are attracting more concerns. Phosphorus is consider an essential unsustainable nutrient for all forms of life on the earth. There is wary worldwide that phosphorus rocks will be depleted within the next 50 – 100 years as some of estimates mentioned (Cooper *et al.*, 2011). Thus, new renewable sources of phosphorus should be discovered to face the high demand for phosphorus. Struvite (Magnesium ammonium phosphate) is consider one of most common phosphate fertilizers that can be recovered from different wastewaters. Struvite as fertilizer is an efficient be used for crop growth, and is an excellent replacement for phosphate rocks (Rahman *et al.*, 2014). Struvite deposition occurs in the equimolecular concentration of magnesium (Mg), ammonium (NH_4) and (P); which these elements combine with water to form struvite. pH is highly effective in precipitation of the struvite components, where struvite starts to precipitate at pH > 8 (Doyle and Parsons, 2002). Chemical addition and carbon dioxide stripping through aeration are the most common methods for P recovery as struvite, and they are effective processes, but the operations cost is too high. Up to 97% of struvite cost could be consumed by chemical addition to raise the solution's pH (Jaffer *et al.*, 2002; Morales *et al.*, 2013). Recently

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[1]***Corresponding author**
A.N.Z. Alshehri,
Department of Biology, University College in Al-Jummum, Umm Al-Qura University, Makkah, 21955, Kingdom of Saudi Arabia
Email: anshehri@uqu.edu.sa / Mobil: +966563708094

renewable clean energy source has attracted international attention; hydrogen is consider one of the most promising source (Datar *et al.*, 2007; Guo *et al.*, 2010; Kumar *et al.*, 2016). Technology of microbial electrolysis cells (MECs) are a new and promising approach for hydrogen (H_2) production from organic substances, including wastewater and other sustainable resources. Bacteria with electrochemical activity in MEC, oxidize organic substances and produce CO_2, electrons and protons. The electrons have been transferred by The bacteria to the anode then the protons are released into the solution. Thereafter the electrons travel via a wire to a cathode and combine with the free protons in the solution to form hydrogen gas (Logan *et al.*, 2008). pH of the catholyte would be increased by consumption of protons at the cathode chamber (Moussa *et al.*, 2006). Depending on this supposition, the cathode chamber in the MEC reactor could be used to deposit P as struvite. Technology of MECs have been used for production and generation different useful products such as bio-hydrogen production (Call and Logan, 2008), bio-methane production (Ding *et al.*, 2016), ammonium recovery (Kuntke *et al.*, 2014) and P recovery (Cusick and Logan, 2012; Cusick *et al.*, 2014). The recovery of P in MEC has not been studied in enough depth, just few researches have focused on the subject to date. Thereby, a comprehensive study should be carried out to estimate MEC performance for H_2 production and P recovery simultaneously. In this paper, a two-chamber MEC reactor was used to control the pH buffering between the anode and the cathode chambers. In addition, simultaneously hydrogen production and P recovery have been investigated to estimate MEC performance with applying response surface methodology to optimize the results. This work targeted understanding the role of applied voltage and COD concentration in P recovery as struvite and in H_2 production, using a two-chamber MEC reactor, and to optimize yield of the struvite precipitation and the hydrogen production under different operational conditions (applied voltage and COD concentration), using statistical optimization by a response surface methodology (RSM).

Materials and Methods

MEC Construction and Inoculation

MEC was constructed from two bottles-chambers in form H-type (Adams Scientific Glass, Berkeley: USA), as shown in Fig. 1. The compartments of the two bottles were identical with a volume of 200 mL for each chamber. Electrodes of the anode and cathode were made of carbon cloth measuring 2 × 4 cm, with a projected area of 20 cm^2 (Fuel Cells Etc, Texas: USA). The cathode included a Pt catalyst (0.25 mg/cm^2 20% Pt on carbon cloth electrode) to enhance cathode efficacy, while the anode was plain carbon cloth. All the electrodes were connected with a titanium wire (1.0 mm, purity > 99%, Alfa Aesar, Heysham, UK). A Nafion membrane (Nafion 117, Dupont CO., USA), with an area of 14 cm^2, was placed in the middle of the anode and the cathode compartments. The membrane was pretreated by 25% of H_2O_2 boiled, and deionized water, followed by 0.5 M H_2SO_4 and deionized water, each for 1 h. Then it was stored in deionized water prior to being used. A DC power supply PSD 30/3B (CALTEK, Hong Kong) was used to apply voltage for the MEC system. The voltage was added by connecting the positive pole of the power source to the anodes while the negative pole across a high resistor (13 Ω) to the cathodes. The anode chamber was inoculated with a 1:1 mixture of activated sludge (was obtained from Makkah Sewage Treatment Plant: KSA) and anolyte medium containing in (g/L): 3.28 Sodium acetate, 0.31 ammonium chloride, 0.13 potassium chloride, 2.69 sodium phosphate anhydrous monobasic, 4.33 disodium hydrogen phosphate, 10 mL of vitamins solution and 10 mL of a trace element solution. The cathode chamber was filled with 50 mM phosphorus buffer, and it was continuously aerated using an aquarium pump. Both electrodes were connected to 1000 Ω of external resistance at the initial stage of the operation, this was modified to 10 Ω after the inoculation process. A fresh medium combined with inoculum mixture was used to change the anolyte, when the voltage decreased.

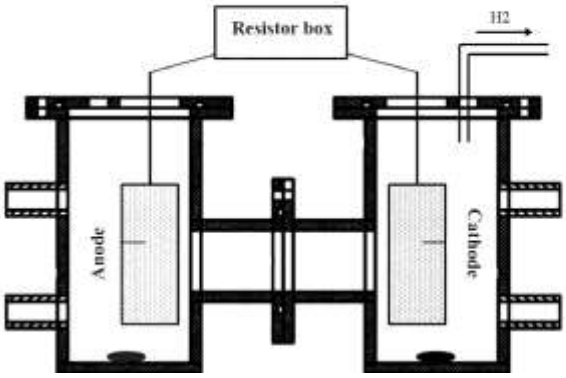

Fig. 1: Schematic view of the dual chamber MEC.

Mediums and Operation of MEC

Artificial wastewater in the anode chamber was contained (g/L): 0.5–2 (based on concentration of COD) Sodium acetate, 0.65 KH_2PO_4, 0.65 K_2HPO_4, 0.74 KCl, 0.58 NaCl, 0.375 NH_4Cl, 0.1 $MgSO_4 \cdot 7H_2O$, 0.1 $CaCl_2 \cdot 2H_2O$, 0.1 mL/L of a trace element mixture and vitamins. Artificial wastewater in the cathode chamber was contained (g/L) 0.5 sodium acetate 0.25 KH_2PO_4, 0.25 K_2HPO_4, 0.74 KCl, 0.58 NaCl, 0.38 NH_4Cl, 0.32 $MgCl_2$, 0.1 $MgSO_4 \cdot 7H_2O$, 0.1 $CaCl_2 \cdot 2H_2O$. Influent pH in the anode and cathode were adjusted to pH 7.0 . After each MEC cycle, the anode chamber was drained, then exposed to air for 30–40 min to inhibit methanogen growth (Call and Logan, 2008). Thereafter it was refilled with artificial wastewater solution, and both the anode and cathode chambers were sparged for 20 min with pure N_2 to make anaerobic conditions. The MECs were operated at applied voltages of 0.4, 0.5, 0.8, 1.1 and 1.2 V, for at least triplicate batch cycles at each voltage. As well, five COD concentrations were used in the anode feed solution as follows: 300, 500, 1000, 1500 and 1700 mg/L. Results of the current densities and operation time of a cycle were varied depending on the changes in anolyte compositions. The COD removal was calculated at the end of each cycle using DR3900 Spectrophotometer (HACH, UK). The displacement method was applied to estimate volume of the produced gas in the cathode chamber. Anolyte and catholyte pH and oxidation-reduction potential (ORP) were monitored constantly every 15 min during each cycle using 12-Channel measuring and monitoring data logger (EA Instruments, London, UK).

Monitoring and Calculation

The voltage across the external resistance was measured using a digital multimeter (Sanwa CD800a, Japan) was connected to a personal computer. Data was automatically recorded every 5 min via Picolog software (Pico Technology Limited). Current was calculated using Ohm's law by measuring the voltage across a resistor 10 Ω. Hydrogen production rate ($m^3/m^3/d$), coulombic efficiency (CE), the amount of energy added to the MEC by the power source (W_E), the amount of energy added by the substrate (W_S), energy efficiency (η_E), and overall system efficiency (η_{E+S}) were calculated as previously described (Logan *et al.*, 2008). In addition, the electrical energy input and the electrical energy recovered were calculated using Eq. (1) and (2). The electrical energy input per kg of COD removed (Wh_{in}/kg- COD)

$$Wh_{H2} = \int_2^t Windt/\Delta COD \cdot V \qquad (1)$$

Where, W_{in} is the electrical input, ΔCOD the change in solution COD, and V the reactor liquid volume.

The energy recovered as hydrogen per kg of COD (Wh_{H2}, kWh/ kg-COD)

$$Wh_{H2} = Y_{H2} \times HHV_{H2} \qquad (2)$$

Where, Y_{H2}(kg H_2/kg-COD) is the hydrogen yield, HHV_{H2} is the higher heating value of hydrogen (39.4 kWh/kg-H_2).

Analytical Methods

The results reported were average of triplicate for all experiments. Chemical oxygen demand (COD), ammonium (NH_4-N), orthophosphate (PO_4^{-3}) concentrations were measured after each batch. Total phosphorus (TP), magnesium (Mg), calcium (Ca), potassium (K), and sodium (Na) concentration were estimated using inductively coupled plasma optical emission spectrometry (ICP-OES). The total volume of the gas produced was measured using the water displacement method (Logan *et al.*, 2008). A gas chromatograph (compact GC, CE Instruments Ltd, UK) was used to analyze the produced gas in the cathode chamber during each batch, but anode gases were analyzed periodically. The compact GC was equipped with a thermal conductivity detector (TCD) and Flame ionization detector (FID). Argon gas was used as a carrier gas for the GC.

Phosphorus Precipitation in MEC

The theoretical P, Mg, and NH_4 concentrations in the cathode solution were approximately 3 mM at pH 7. For P precipitation as struvite a 1:1:1 M ratio of NH_4:Mg:P should be attained (Doyle and Parsons, 2002). Before and after each batch, the cathode chamber was washed with deionized water three times, thereafter cleaned and dried properly to eliminate any attached precipitates on the chamber walls. After each precipitation cycle, the used cathode was removed for maintenance and was changed with new electrode. Elimination of P precipitates from cathode surface was done, and it is essential step, as the precipitates decrease cathode performance, therefor dissolution treatment increases cathode performance to their initial level. The electrode was immersed 3 times in deionized water, pH 7, for 2 days each time. After deionized water dissolution, the electrode was immersed again 3 times in MES buffer ($C_6H_{13}NO_4S$ [MES]:10 mM, pH 5.5) each time for 30 h. Finally, the electrode was rinsed and dried before use. At the end of each batch, the catholyte was filtered using a 0.2 µm filter membrane (Fisher Scientific, UK). The precipitate was collected, weighed and analyzed by X-ray diffraction (XRD) and by scanning electron microscope coupled with energy dispersive X-ray spectroscope (SEM-EDXS). The recovered P was calculated using Eq. (3):

$$\text{Precipitation efficiency (\%)} = (P_{in} - P_{out}/P_{in}) \times 100 \qquad (3)$$

Where, P_{in} = P level in the catholyte influent, P_{out} = P level in the catholyte effluent. In addition, P deposition rate (g/$m^3_{cathode}$-d) was calculated using Eq. (4):

P precipitation rate

$$(g/m^3_{cathode}\text{-}d) = (TP_{in} - TP_{out}) \times V_{catholyte}/(V_{cathode} \times \Delta t) \qquad (4)$$

Where, TP_{in} = total phosphorus influent cathode concentration, TP_{out} = total phosphorus effluent cathode concentration, $V_{catholyte}$ = volume of catholyte solution (m^3), $V_{cathode}$ = volume of cathode chamber (m^3), and Δt = batch duration (d).

SEM and EDXS

The precipitates that accumulated on the cathode electrodes and in the cathode chamber were analyzed to examine the morphology of the crystal as well as its elemental composition. In addition, the used proton exchange membrane (PEM) was cut into pieces, carefully rinsed with deionized water and finally dried completely at ambient temperature. The microscopic structure and elemental components of the PEM surface were analyzed using a FEI-XL30 Environmental SEM equipped with an EDXS.

Crystals Composition and Purity

The purity of the collected crystals was evaluated by analyzing crystals composition. Struvite purity was estimated using SEM-EDXS and the dissolution method to identify the composition of the crystals. Almost 0.1g of crystals were dissolved in 50 mL of 0.5% nitric acid solution. In order to accelerate dissolution, the samples were stirred with a magnetic stirrer for 24 h, thereafter, samples were analyzed for magnesium, ammonia, orthophosphate, calcium, aluminum and iron using inductive coupled plasma (ICP) (Fattah *et al.*, 2008).

Statistical Optimization

Response surface methodology (RSM) is an effective statistical tool that helps in understanding and optimizing the system by determining the impact of different parameters on the response. STATISTICA program version 13 (USA) was used for the design, analysis, and optimization. The used variables were- applied voltage (X1) and influent COD concentration (X2). The responses were: cathode pH (Y1), Precipitation efficiency (Y2), and maximum volumetric hydrogen production (Y3). Analysis of variance (ANOVA) provided the statistical results and the diagnostic check tests to estimate the adequacy of the models. The quality of the fitted models was evaluated using the coefficient of determination R^2, and its statistical significance was tested by the Fisher F-test. Model terms were evaluated by the P-value (probability of error) with 95% confidence level. Three dimensional plots and their respective contour plots were obtained depending on the influences of the two factors (applied voltage and COD). In total, 19 experiments were carried out with 8 factorial points, 8 axial points, and 3 center point. Replicates of the center points were added to the design to examine the efficiency of the model and to get a good estimate of the experimental error. The nonlinear behavior of the responses (Y1 and Y2) were illustrated by the following quadratic model (Eq. 5):

$$y = \beta_o + \sum \beta_i X_i \qquad (5)$$

Where y is the response, β_o is the model intercept and β_i is the linear coefficient, and X_i is the level of the used parameters. Two replicates were employed at each factor combination. At the beginning of each factor combination, the system was run for at least 2 batches to let the system adapt to the new conditions.

Results and Discussion

Hydrogen production

Compact GC was used to analyze the gases produced in the cathode chamber, they were found to contain mainly hydrogen in all experiments, and methane was detected in the anode chamber. The hydrogen production rate in the two chamber MEC ranged from 0.09 to 0.31 $m^3/m^3/d$. The volume of the produced gas was variable and totally dependent on the applied voltage. Increasing the applied voltage caused an increase in the current density. Thus, an increase in the production of hydrogen gas was recorded (Fig. 2). An increase in applied voltage from 0.4 to 1.2 V caused a more than fourfold increase in hydrogen production. The system attained a peak volumetric hydrogen production rate of 0.22 $m^3/m^3/d$ at COD 1000 mg/L and applied voltage 1.2V. In addition, different COD concentrations were used to determine the impact of COD concentration on hydrogen production. The batch duration and the current densities varied when anolyte COD concentrations were changed. There was no correlation between COD concentration and hydrogen production rate, where increasing COD concentration from 300 to 1700 mg/L had no impact on hydrogen production. Namely that changing the anolyte COD concentration did not affect the H_2 production rate in the MEC. The peak hydrogen production rate (0.18 $m^3/m^3/d$) was achieved at COD 500 mg/L and applied voltage 0.8 V. And the H_2 production rate varied at different COD concentrations. The hydrogen production rate in this study was similar to previous studies using two chamber MEC (Yossan *et al.*, 2013; Ruiz *et al.*, 2015), where the maximum H_2 production rate was 0.2 and 0.5 $m^3/m^3/d$, respectively. In addition, H_2 production rate (114.46 mL/m2) was also observed in single chamber MEC (Pasupuleti *et al.*, 2015).

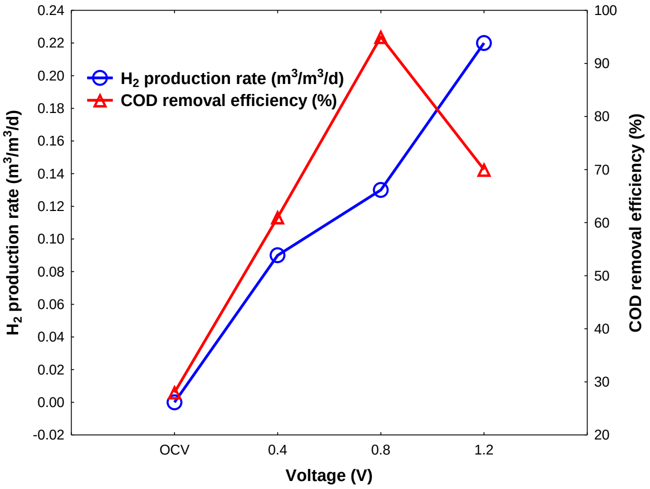

Fig. 2: The impact of applied voltage on H_2 production ($m^3/m^3/d$) and COD removal efficiency (%).

The total energy recovery rates in the MEC ranged from η_{E+S} = 28 to 42%. Total energy recovery was calculated under different applied voltages. The findings showed that there was no correlation between applied voltage and total energy recovery. The electrical consumption in the MEC was higher than the energy generation in all the experiments. An increase in applied voltage in the circuit, from 0.4 to 1.2 V, led to increase electrical energy input Wh_{in} from 0.5 to 1.9 kWh/kg-COD. The low H_2 production in the MEC was not adequate to recover the electrical consumption. However, the recovered power and struvite can be used to reduce the operational cost.

Phosphorus Recovery in MEC

When the pH of the cathode chamber reached 8, P started to precipitate as struvite. The reactor accomplished a peak precipitation efficiency of 94%. Recovery of P was in the cathode chamber, where the neutral solution was converted to alkaline by proton consumption. Previous studies reported similar results, like Cusick *et al.* (2014) and You *et al.* (2016), where high P removal was carried out by MEC (85%) and MFC (82%), respectively. Cathode pH was affected by applied voltage, where an increase in applied voltage from 0.4 to 0.8 V caused increased the average cathode pH from 8 to 9.1. However, increasing the applied voltage to 1.2 resulted a decrease in the average cathode pH, down to 8.5. Using high voltage may inhibit bacteria activity and impact the oxidation process in the anode compartment, with the result that low protons are released and cathode pH is influenced. To realize the role of the current on P recovery and cathode pH, the system was shifted to an open circuit system (OCV), where no resistance was used in the circuit, and MECs were operated for at least three cycles. Cathode pH remained at 7 without any increasing. These outcomes show the importance of defining the ideal applied voltage to obtain high pH in the cathode. Efficacy of precipitation in OCV was less than 1%, while when the circuit closed and 0.4 V was applied, the MEC attained 45 % precipitation efficiency as it shown at Fig. 3. Moreover, precipitation efficiency enhanced and reached 90 % when the applied voltage increased to 0.8 V. At 1.2 V, precipitation efficiency reached 92%. Precipitation of P was on the cathode electrode, suspended on the catholyte and on the chamber walls. The precipitation rate attained in the MECs ranged from 1.4 to 20 g /m^3/d. The highest precipitation rate was attained at 0.8 V. An increase in the applied voltage to 1.2 V decreased the precipitation rate. Increasing the applied voltage to 1.2 V prevents microorganism activity and increases the batch duration. The precipitation rate was influenced by batch duration, which decreased with applied voltage (Cusick and Logan, 2012). The concentrations of P and Mg in cathode influent were approximately 3 mm, and struvite precipitates when the molar ratio of NH_4 : Mg : P in the solution is 1:1:1 at an alkaline condition (pH > 8). Figs. 4 and 5 shows that in OCV there was no P eliminated from the cathode due to a neutral condition (pH = 7). This confirms that P was eliminated only by precipitation. However, more than 1 mm of Mg was transferred from the cathode chamber to the anode chamber via Nafion membrane exchange, due to the concentration gradient. When 0.4 V was applied to the circuit, catholyte pH increased to 8, and around 1.45 mm of P and almost 1.3 mm of Mg were recovered as struvite. When 1 mm of Mg was added, the transferring from the cathode to the anode was done. As well, increasing the applied voltage increased the pH. Thus, the precipitated P and Mg raised. This lowered the Mg transferred to the anode, due to most of the Mg was precipitated as struvite. Making the optimal pH in the cathode chamber is essential, to lower the diffusion of cations to the anode chamber. Furthermore, NH_4 concentration in the anode chamber minimized in all cycles due to NH_4 diffusion and microorganism consumption. As well, NH_4 elimination was noticeably increased when applied voltage was increased, ranging from 0.7 mm at 0.4 V to 2.25 mm at 1.2 V. Although, calculating NH_4 concentration in the cathode effluent was represented challenge. The concentration of NH_4 in the cathode effluent different and fluctuated in each batch, because ammonia volatilization caused by the high pH (Cusick *et al.*, 2014).

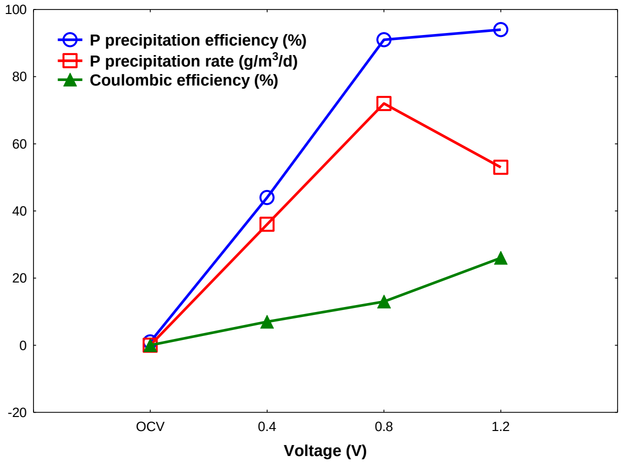

Fig. 3: The impact of applied voltage on P precipitation efficiency (%), P precipitation rate (g/m^3/d), and Coulombic efficiency (%).

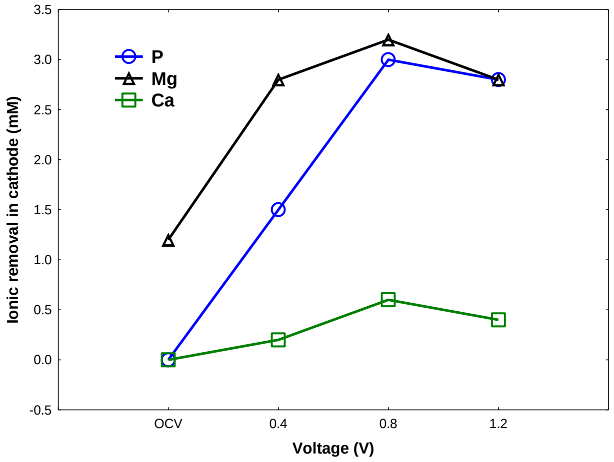

Fig. 4: Molar ionic removal in the cathode chamber.

Crystals Analysis

At end of all cycles, the precipitated crystals from the catholyte filtered were weighed and analyzed. In addition, the cathode electrode was treated, using the dissolution method, and changed with a new cathode electrode for the following batch. The XRD pattern showed that the precipitated crystals matched the standard pattern of struvite, as it is clear in Fig. 6A. The SEM images showed that the crystals had tubular morphology, which emphasizes that the precipitated crystals were struvite (Fig. 6B) (Hutnik *et al.*, 2011). Moreover, EDXS analysis showed that the main peaks of the crystals from all batches were O, Mg, and P, which are similar to the peaks of struvite standard (Fig. 6C) (Ronteltap *et al.*, 2010). The dissolution treatment for the cathode exhibited that the molar ratio of Mg:P in the solution was almost 1:1. This can also confirm that the precipitated crystals had a same molar ratio to the struvite standard. The SEM and XRD analysis exhibited that only struvite was precipitated in the cathode chamber. Struvite started to be supersaturated, only when the cathode pH reached 8.0, and struvite started to deposit.

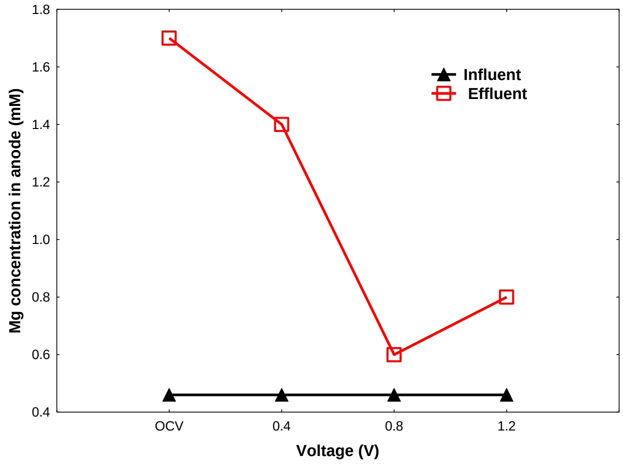

Fig. 5: Influent and effluent Mg concentration in the anode chamber.

COD Removal Efficiency

To estimate the ability of MECs to remediate wastewater and to figure out the correlation between applied voltage and COD removal. Thus, parameter of COD removal was monitored in experiments of MECs which were operated under different applied voltages (0.4, 0.5, 0.8, 1.1, and 1.2 V) at COD = 1000 mg/L. The efficiency of COD removal which has accomplished was ranged from 59 to 92%, as it is clear in Fig.2. When the applied voltage was modified from 0.4 to 0.8 V, the COD elimination efficiency increased ,where the system attained the peak removal efficiency. Subsequently, the removal efficiency dropped when the applied voltage rose above 0.8 V. Increasing of the removal efficiency by 30%, was achieved when the applied voltage was raised from 0.4 V to 0.8 V. As well, increasing the applied voltage above 0.8 V had a negative effect on COD removal, where high voltage could prevent bacterial activity. Thereby, low COD removal was recorded (Ding *et al.*, 2016). Identical results, with high COD removal efficiency, were previously reported in two chamber MEC (Ding *et al.*, 2016). It was shown that the applied voltage had a big influence on COD removal and that applying the optimal value can enhance COD removal and lower the operational cost. Moreover, MECs were operated under various COD concentrations (300, 500, 1000, 1500 and 1700) at applied voltage 0.8 V. The results were obtained of COD removal efficiency were ranged from 50 to 90%. At low influent COD concentration, the high COD removal was achieved, while long batch cycles was required, when the influent COD concentration was high, where the bacteria needs more time to degrade the organic substances. Thereby, the finding illustrated clearly that COD removal was impacted by current production and by the period of a batch cycle.

Coulombic Efficiency (CE)

Fig. 3 shows level of the attained coulombic efficiency, which was ranged from 8 to 21% . It was clear that the CE was influenced by the applied voltage and by COD influent concentration, where increasing the applied voltage from 0.4 to 1.2 V at COD = 1000 mg/L, increased the CE from 8 to 21 %. Furthermore, rising COD concentration from 300 mg/L to 1700 mg/L, at an applied voltage of 0.8 V, caused of a drop in coulombic efficiency from 21 to 13.3 %. Thus, this means that a small part of the substrate was consumed for current production and the remnant was used for methane forming (Sleutels *et al.*, 2011). The availability of excess substrate in the anode compartment caused of the methanogens to utilize it, instead of using it in current generation. Thus, most of COD was utilized by methanogens in long batch duration and that caused of decrease the CE. As well, the current was deteriorated and the CE was further decreased because struvite precipitation on the surface of cathode electrode (Almatouq and Babatunde, 2016).

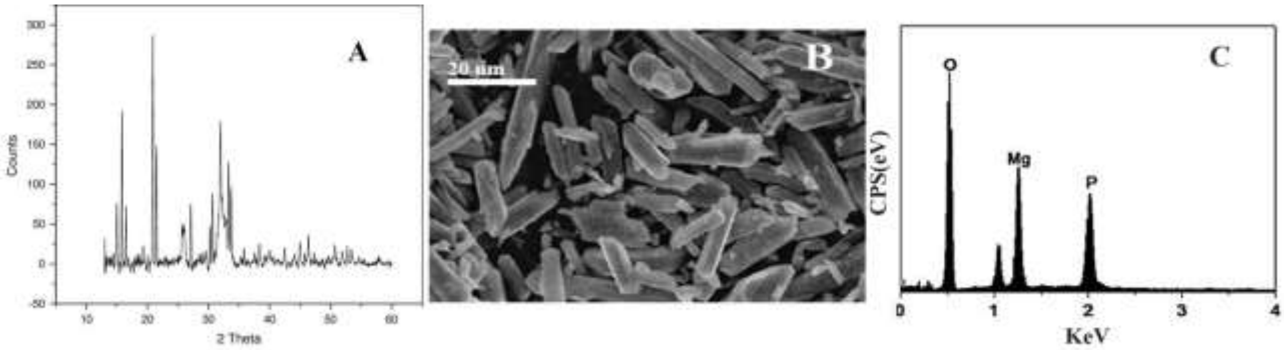

Fig. 6: (A) The XRD pattern showed that the precipitated crystals matched the standard pattern of struvite. (B) the SEM images showed that the crystals had tubular morphology, which confirms that the precipitated crystals were. (C) EDS analysis showed that the main peaks of the crystals (from all cycles) were O, Mg, and P, which are similar to the peaks of struvite standard.

Table 1: Experimental design and the responses of the duplicates of RSM runs.

The variables			The experimental results		
Run	X1: Applied voltage (V)	X2: COD (mg/L)	Y1: cathode pH	Y2: precipitation efficiency (%)	Y3: max. H2 production ($m^3/m^3/d$)
1	0.5	500	7.43	50	0.06025
2	0.5	1500	8.25	70	0.08514
3	1.1	1500	8.47	91	0.1318
4	1.1	500	7.95	62	0.28057
5	0.5	500	7.4	44	0.061
6	1.1	1500	8.67	95	0.13987
7	0.8	1000	8.25	90	0.10052
8	1.1	500	8.067	60	0.2674
9	0.8	1000	8.24	88	0.13101
10	0.5	1500	8.189	72	0.09755
11	0.4	1000	7.7	57	0.076
12	0.8	300	7.44	68	0.15939
13	1.2	1000	8.49	87	0.20943
14	0.8	1700	8.4	91	0.06564
15	1.2	1000	8.4	85	0.22482
16	0.8	1000	8.1	90	0.11677
17	0.8	1700	8.35	90	0.09103
18	0.4	1000	7.6	59	0.08678
19	0.8	300	7.5	66	0.1223

Statistical Optimization

Statistical optimization was employed to determine the effect of each factor, in addition the interaction influence on MEC efficiency, and to define the optimum operating conditions for the two chamber MEC. In this work, applied voltages were varied from 0.4 to 1.2 V. COD levels were ranged from 300 to 1700 mg/L, dependent on the concentration of COD in wastewater (Hu *et al.*, 2017).

Response surface methodology (RSM) was employed to define the effect of applied voltage (X1) and COD concentration (X2) on MEC efficiency, and to identify the optimum operating conditions, and to fit a quadratic model to the data. Four axials with $\alpha = \pm 1.4$ and three center points were conducted to have a rotatable design. The levels of variables for RSM are given in Table 1. The efficiency of the MEC was studied with respect to cathode pH, precipitation efficacy and maximum volumetric hydrogen production rate. Cathode pH is the most important parameter for P recovery, where P solubility is based on solution pH. Precipitation efficacy was applied to estimate P recovery as struvite in the cathode compartment. Eventually, peak volumetric hydrogen production rate was employed to estimate hydrogen production in MEC. The experimental design and the outcomes are summarized in Table 1. Before completing the models of all responses, trials of hypotheses were carried out to emphasize that none of these conditions were violated. The standard deviations between the actual and the predicted response values followed a normal distribution. The studentized residuals, against predicted values, showed that there was no evidence for the violation of constant or independence hypotheses during the response space. Check for outliers if any are available. In these statistical processes, Cook's distance was applied to exam if there were any influential values. The Box-Cox plot for power transformation was examined, to see if the data required any transformation. After all these checks, the models were finalized and the RSM was drawn up.

Cathode pH

Cathode pH is consider the most important factor for P recovery. Cathode pH was investigated to define the effect of applied voltage and COD level on MEC efficiency. The Analysis of Variance (ANOVA) is summarized in Table 2, where the findings showed that a quadratic model with an F value of 126.76 and a P-value of <0.0001 was significant. There was only a 0.01% opportunity that this level of fit could occur because error. The lack of fit was not significant, with a P-value of 0.3481. The coefficient of determination (R^2) of 0.96 and adjusted (R^2) of 0.95 implied that the model was fit to express almost 96% of the variability in the response. The response surface of the cathode pH is shown in Fig. 7. The following model was considered satisfactory in illustrating cathode pH (Eq. 6):

$$\text{Cathode pH} = 8.10 + 0.19X_1 + 0.23X_2 - 0.161X_1X_2 - 0.071X_1^2 - 0.31X_2^2 \quad (6)$$

Table 2 shows that the impacts of all factors were significant on cathode pH. In addition the interaction was significant too, which means the influence of applied voltage on cathode pH is based on the level of COD concentration. As well, Eq. (6) showed that COD concentration had the biggest impact on the response. The RSM graph in Fig. 7 shows that the impact of applied voltage was linear on cathode pH and that cathode pH increased with an increase in applied voltage. However, the influence of COD concentration was quadratic on the response, and increasing of COD concentration led to increasing cathode pH. Proton utilization in the cathode chamber for hydrogen formation in the MEC resulted in a pH increase. Thus, increasing of COD concentration from 300 to 1700 mg/L, at 1.1 V caused of an increase in average cathode pH from 7.0 to 8.0. Due to P solubility is based on solution pH, the optimal pH for struvite crystallization is 8.0 (Cusick *et al.*, 2014). Eq. (6) was fit to predict a cathode pH of 8.0 at applied voltage of 1.1 V and COD concentration of 1700 mg/L. Three experiments were performed to check model sufficiency. A cathode pH average of 8.1 was accomplished, emphasizing the reliability of the model.

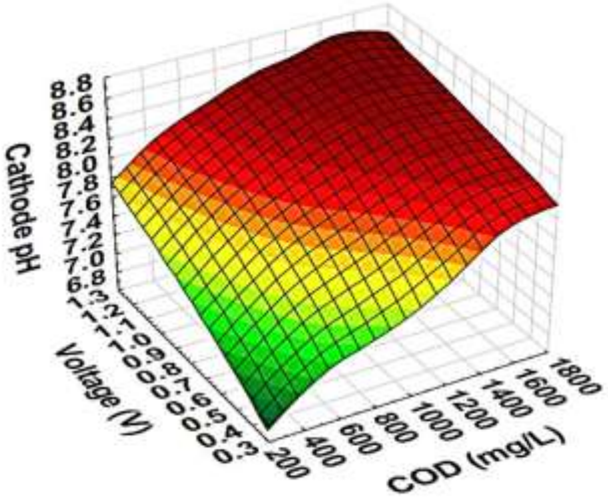

Fig. 7: Response surface of cathode pH as a function of applied voltage and COD concentration.

Precipitation efficiency

Precipitation efficiency was measured to estimate the P recovery efficacy in the MEC. Eq. (3) was applied to calculate the precipitation efficiency. The ANOVA analysis presented in Table 2 illustrates the results which showed that quadratic model with F-value of 130.15 and P-value of <0.0001 was significant. There was only 0.01% opportunity that this level of fit could take place because error. The lower of fit was not significant, with a P-value of 0.099. The coefficient of determination (R^2) of 0.99 and adjusted (R^2) of 0.97 implied that the model was fit to express almost 99% of the variability in the response. The response surface of the precipitation efficiency is shown in Fig. 8. The following model was considered satisfactory in illustrating precipitation efficiency (Eq. 7):

$$\text{Precipitation efficiency} = 91.0 + 8.85X_1 + 9.3_1X_2 + 3X_1X_2 - 15.15X_1^2 - 6.17X_2^2 + 7.14X_1^2X_2 \quad (7)$$

Table 2 shows that all factos were significant on precipitation efficiency, and that applied voltage had the biggest impact on precipitation efficiency. The RSM in Fig. 8 shows that the applied voltage and COD concentration had a quadratic influence on precipitation efficiency. A low applied voltage (0.5 V) increased the COD concentration from 300 to 1700 mg/L, and as well as, increased the precipitation efficiency from 50 to 72%. Similar result was recorded at high applied voltage (1.1 V), where the precipitation efficiency increased from 65 to 94%, when COD concentration increased from 300 to 1700 mg/L. Increasing of COD concentration led to increasing of the electrons and protons that transferred to the cathode electrode and compartment. Thereby, increasing the catholyte pH because proton utilization. P precipitation reached the super saturation point at high pH >8. Thus, more than 90% of P can be precipitated when pH reaches 8.3 (Adnan *et al.*, 2003). Moreover, an increase in the applied voltage resulted an increase in the current and, an increase in precipitation efficiency. At a low COD concentration (300 mg/L), an increase in applied voltage from 0.5 to 0.8 V increased the precipitation efficiency from 47 to 79%. However, precipitation performance started to drop when applied voltage was increased above 0.8 V. The cathode pH was raised due to increasing of the applied voltage. Modifying of the applied voltage from 0.5 V to 1.1 V, resulted increasing the pH from 7.5 to 8.1, thereby, caused of an increase in ammonia volatilization and diffusion to the anode chamber (Zhou and Wu, 2012; Rahman *et al.*, 2014). Eq. (7) was fit to predict a peak precipitation efficiency of 96% at an applied voltage of 1.0 V, and a COD concentration of 1700 mg/L. Three experiments were carried out to exam model sufficiently. A precipitation efficiency average of 94% was accomplished, and emphasizing the reliability of the model.

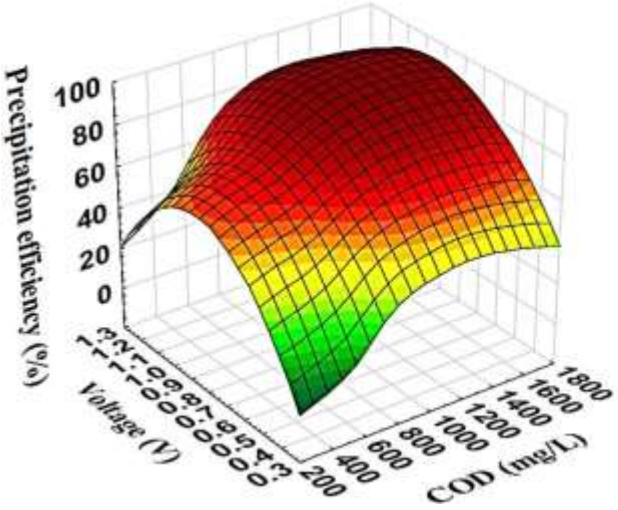

Fig. 8: Response surface of precipitation efficiency as a function of applied voltage and COD concentration.

Table 2: ANOVA for the quadratic model of cathode pH, precipitation efficiency and H_2 production rate.

Source	Sum of squares	DF	Mean square	F-value	P-value
Cathode pH					
Block	0.052	2	0.642		
Model	2.96	4	0.90	126.76	<0.0001
X1	1.87	1	2.07	204.82	<0.0001
X2	1.95	1	5.75	784.28	<0.0001
X1X2	0.130	1	0.930	6.48	0.0256
Residual	0.855	8	8.054		
Lack of Fit	05013	3	4.7659	0.97	0. 3481
Pure Error	0.141	8	023		
Total	3.067	16			
Precipitation efficiency					
Block	176.14	1	179.14		
Model	4744.64	5	747.34	130.15	<0.0001
X1	7528.76	1	4528.76	281.94	<0.0001
X2	582.25	1	502.25	83.52	<0.0001
X1X2	32.80	1	2.50	5.02	0.0400
Residual	74.96	13	5.41		
Lack of Fit	66.46	4	3.23	2.09	0.0990
Pure Error	30.50	12	4.8		
H2 production rate					
Block	2.060	1	5.60		
Model	1.080	7	1.013	112.08	<0.0001
X1	0.018	1	0.318	180.27	<0.0001
X2	5.588	1	9.588	54.18	<0.0001
X1X2	0.914	1	0.614	97.60	<0.0001
Residual	2.921	12	7.838	0.42	
Lack of Fit	3.732	2	2.660		0. 8756
Pure Error	013	6	014		
Total	0.662	17			

Hydrogen Production Rate

In this work, the hydrogen production rate was examined to estimate the ability of MEC to recover P and produce H_2 simultaneously. The Analysis of Variance (ANOVA) results showed that a quadratic model with an F-value of 112.08 and a P-value of <0.0001 was significant (Table 2). There was just a 0.01% chance that this level of fit could occur due to error. The lack of fit was not significant, with a P-value of 0.8756. The coefficient of determination (R^2) of 0.96 and adjusted (R^2) of 0.94 implied that the model was able to express approximately 96 % of the variability in the response. The response surface of the H_2 production rate is shown in Fig. 9. The following model was considered satisfactory in illustrating hydrogen production rate (Eq. 8):

$$H_2 \text{ production rate} = 0.23 + 0.051X_1 - 0.034X_2 - 0.038X_1X_2 + 0.08X_1^2 + 3.144\times10^6X_2^2 + 0.014X_1X_2^2 \qquad (8)$$

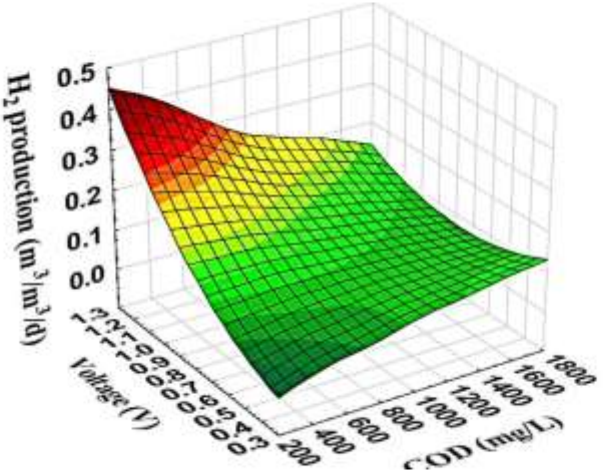

Fig. 9: Response surface of H_2 production rate as a function of applied voltage and COD concentration.

Table 2 shows that the effects of all terms were significant on H_2 production rate, except X_2, which was statistically insignificant. More importantly, the interaction term was significant. Eq. (8) shows that the effect of applied voltage had double the effect of COD concentration on the response. The RSM graph in Fig. 9 shows that the effects of applied voltage and COD concentration were linear and quadratic on the H_2 production rate, respectively. H_2 production increased linearly along with the applied voltage. An increase in applied voltage from 0.5 to 1.1 V led to increasing H_2 production rate from 0.06 to 0.264 $m^3/m^3/d$. although, at high COD concentration (1700 mg/L) the effect of increasing applied voltage on H_2 production rate was minimal. In general, two chamber MECs was operated with a phosphate buffer solution (PBS) to keep pH balance in the cathode chamber, because high cathode pH causes a lot losses in the system. Operating MEC with a high cathode pH will deteriorate the MEC efficiency (Nam and Logan, 2012). The production of H_2 in low COD concentration (300 mg/L) was much better than the high COD concentration. Hydrogen production was negatively impacted by the lack of a high concentration of PBS in the cathode. The change of anode and cathode pH at high COD concentration was bigger than the low COD concentration. A high pH difference between anode and cathode chambers led to high potential losses, negatively affecting MEC efficiency. Thereby, PBS was used in most of the two chamber MECs to keep the pH balance during the operation of the reactor (Luo *et al.*, 2014).

Conclusion

Technology of microbial electrolysis cell reactor with two compartments was an efficient approach to recovery Phosphorus via the precipitation as struvite in the cathode chamber. Where the maximum precipitation efficiency of 94%, was at applied voltage 1.1 V, and COD 1500 mg/L. In addition, by applied 1.1 V of voltage and 300 mg/L of COD, the MEC accomplished a peak H_2 production rate of 0.31 $m^3/m^3/d$. The produced H_2 in MEC can be used as an energy source to lower struvite operational cost.

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