

## Response Surface Methodology for Optimization of Microbial Electrolysis Cell Performance Using Carbon Nanotube-Based Cathode

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DOI: <https://doi.org/10.30880/peat.2021.02.02.006>

Received 13 January 2021; Accepted 01 March 2021; Available online 02 December 2021

**Abstract:** Hydrogen gas is a promising clean energy carrier and alternative renewable energy. Microbial electrolysis cell (MEC) is a new biological process that converts low-grade organics from wastewater to hydrogen gas. The MEC performance can largely be affected by the cathode material and applied voltage. Thus, this study was conducted to obtain the optimum operational conditions of polyaniline/multi-walled carbon nanotube (PANI/MWCNT) cathode and applied voltage for the hydrogen production efficiency in MEC by using the application of response surface methodology (RSM). ANOVA was used to evaluate the mathematical and statistical analysis of the regression model via historical data design. The independent parameters used were applied voltage and different weight percent of PANI/MWCNT cathode, while the responses were hydrogen production rate (HPR), coulombic efficiency ( $C_E$ ), cathodic hydrogen recovery ( $r_{cat}$ ) and energy recovery (ER) of MEC. The results showed the optimum condition was 75 wt. % PANI/MWCNT cathode at an applied voltage of 1.0V with responses of 1.06 m<sup>3</sup>/m<sup>3</sup>/day HPR, 48.0 %  $C_E$ , 57.0 %  $r_{cat}$  and 90.0 % ER, whereas the predicted values were 1.08 m<sup>3</sup>/m<sup>3</sup>/day HPR, 47.8 %  $C_E$ , 57.9 %  $r_{cat}$  and 91.0 % ER. Overall, RSM has proved to be a reliable optimization technique that can be used to design experiments and determine the performance of MEC.

**Keywords:** Microbial Electrolysis Cell, Response Surface Methodology, Hydrogen Production Rate, Polyaniline/Multi-walled Carbon Nanotube

### 1. Introduction

Wastewater generation is increasing noticeably worldwide as urbanization and population keeps growing rapidly, particularly in developing countries [1]. Instead of letting wastewater to be contaminated continuously, waste management should enhance its role by making them reusable and sustainable. Hence, microbial electrolysis cell (MEC) is introduced as it is a new biological process that

converts low-grade organics from wastewater to hydrogen gas. Hydrogen is the most abundant gas with promising clean energy carriers alternative renewable energy. MEC consists of anode and cathode, the exoelectrogenic bacteria from the wastewater will create a layer of biofilms on anode surfaces [2]. The electron and proton will then generate at the anode, the proton will transfer along the circuit, while electrons will merge at the cathode during oxidation is operated. Instead of generating voltage, an external voltage is applied to the MEC and operates the circuit in a state of anaerobic.

Carbon nanotube (CNT) is one of the potential cathode materials in the utilization of MEC. Due to the nanostructure materials, CNT has the potential of high electrical conductivity, high accessible surface area, high stability and the ability to withstand high temperature [3]. It is also a superb adsorbent for gas molecules due to the huge surface-to-volume ratio, hollow geometry and unique electrical properties [4].

Response Surface Methodology (RSM) is a statistical technique that aids experimental design for the development of a statistical model, operating variable assessment and obtain optimum operating conditions of the parameters that result in maximizing desirable responses [5]. The parameter that influences the process is known as independent variables while the response of the operation is known as dependent variables. RSM is effective in most production processes, as some theoretical models that relate in parameters to a response are very complex or not available. Thus, to have a more sufficient result, RSM can be practical as it implies less laboratory test, shorter time consuming and lesser costing.

### 1.1 Problem statement

The implementation of MECs for wastewater treatment is costly due to the cathode catalysts which catalyze the hydrogen evolution reaction [6]. Therefore, the problem facing is to choose the most suitable yet economical cathode to produce a greater efficiency for the MEC. RSM is a useful tool that helped the study to determine an optimum condition for the performance of MEC. By applying historical data design, RSM has attained relevant data that affects the efficiency of MEC in terms of hydrogen production rate, coulombic efficiency, cathodic hydrogen recovery and energy recovery. The parameters that being investigated were applied voltage and the weight percent of polyaniline/multi-walled carbon nanotube (PANI/MWCNT) cathode. RSM is used to study for the regression modeling of historical data and evaluate the value of optimum conditions of input voltage and the electrical conductivity of PANI/MWCNT cathode.

### 1.2 Objective

This study was conducted to address the following objectives:

- i. To establish the regression model equation of applied voltage and weight percent of PANI/MWCNT cathode towards hydrogen production rate, coulombic efficiency, cathodic hydrogen recovery and energy recovery.
- ii. To investigate ANOVA on the applied voltage and weight percent of PANI/MWCNT cathode in MEC for validation of the model.
- iii. To determine the effect of applied voltage and weight percent of PANI/MWCNT cathode towards MEC performances by three-dimensional response surface plot.
- iv. To obtain the optimum operational conditions of PANI/MWCNT cathode and applied voltage in MEC system for the best hydrogen production efficiency.

### 1.3 Scope of study

The scope of this study covers on the performance of carbon nanotube-based cathode in MEC towards the performance of MEC. The experiment of the past study has used PANI/MWCNT material as a low-cost cathode to assess the proficiency of MEC. The applied voltage was also one of the impacts that affect the performances of MEC. In this study, RSM was used from Design-Expect software (Stat-Ease, Inc., Minneapolis, MN, USA) to help in understanding and optimizing the system by identifying

the effect of various parameters on the responses. The study was determined by using historical data from the previous researcher [7]. The operation parameters used were applied voltage in MEC and weight percent of PANI/MWCNT cathode. Whereas the performance of MEC is in terms of hydrogen production rate, coulombic efficiency, cathodic hydrogen recovery and energy recovery of MEC. The experimental data were evaluated to a regression model, consequently examined by the analysis of variance (ANOVA). Subsequently, the obtained results were analyzed based on the design strategy which culminates in a mathematical model. This mathematical model needed to be fitted to the experimental results to validate the whole procedure. The effect of the two variables was examined and illustrated by three-dimensional surface plots. The graph of predicted versus actual plots have also been evaluated to show the adequacy of the model. RSM has also identified the overall optimal operating conditions for all the responses from the effect of variables.

## 2. Methodology

The application of RSM is used to study the effect of MEC cathode made of PANI/MWCNT material and the applied voltage towards the performance of MEC. This study used Design-Expert Version 12.0 software for mathematical modeling and statistical analysis. Historical data design was used in RSM. The role of RSM is to investigate the relationship between parameters (applied voltage and wt. % PANI/MWCNT cathode) toward the responses (HPR,  $C_E$ ,  $r_{cat}$  and ER). The applied voltages were ranged 0.3 V to 1.0 V with using 0.1 g of MWCNT and 0.1 g of PANI/MWCNT for each 25.0 % wt. and 75.0 % wt. The three steps in RSM were firstly statistically designed experiments, secondly was estimating the coefficients in a mathematical model and third was predicting the response and tested the adequacy of the models in the experiment. The parameters were fitted by the regression step to acquire a mathematical model for experimental data. Equation 2.1 shows the quadratic regression model for the responses. ANOVA was presented statistically to identify the affecting factors.

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^n \sum_{j=i+1}^n \beta_{ij} X_i X_j + \varepsilon \quad Eq. 1$$

Where Y is the predicted response,  $\beta_0$  is the constant coefficient,  $\beta_i$  is the linear coefficients,  $\beta_{ii}$  is the quadratic coefficients,  $\beta_{ij}$  is the interaction coefficients, n is the total number of variables studied and optimized in the experiments,  $X_i$  and  $X_j$  are the coded independent variable parameters for the process and  $\varepsilon$  is the random error.

In ANOVA analysis, R<sup>2</sup> is the statistical measure that determines the amount of variance of response data that evaluated by an independent variable. The model acceptance was also determined from the adjusted R<sup>2</sup> and predicted R<sup>2</sup>. Adj R<sup>2</sup> value is the modified version of R<sup>2</sup>, which adjusted the number of terms that are insignificant in a regression model, while Pred R<sup>2</sup> is to determine how well the regression model to make predictions. Verification and the adequacy of the regression model were completed numerically and graphically. 3D surface plot graphs were shown to visualize the effect of the input parameters on the studied responses. Process optimization was obtained to compare the experimental values and predicted values of the responses during the optimum operational conditions of variables. Figure 1 shows the flow chart of this study.

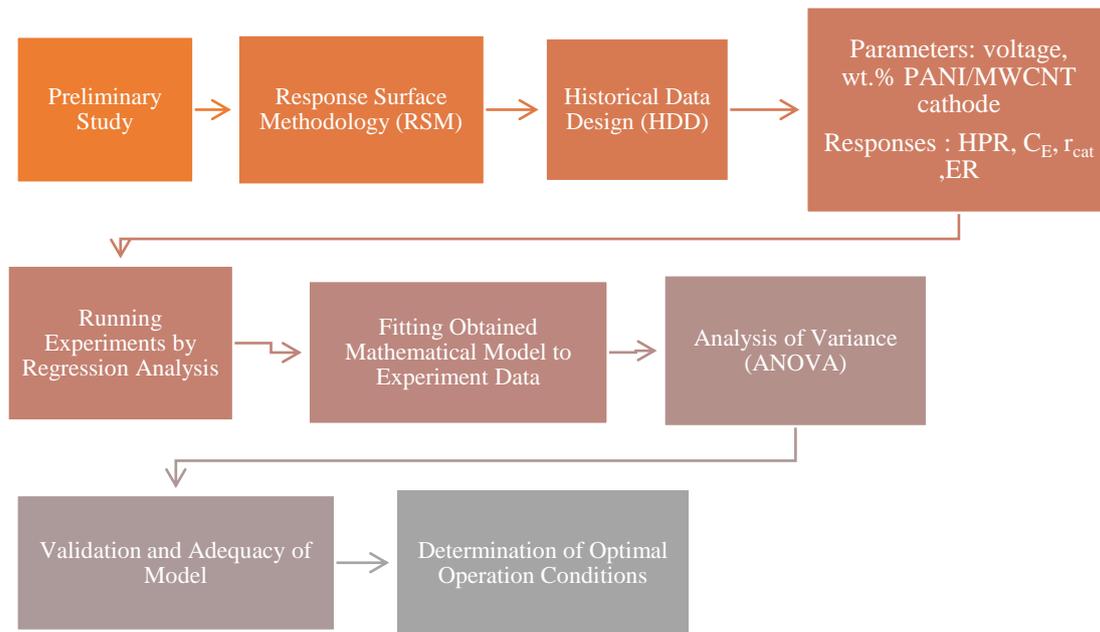


Figure 1: Flow chart of the study

### 3. Results and Discussion

Historical data design was utilized to estimate the performances in terms of hydrogen production rate, coulombic efficiency, cathodic hydrogen recovery and energy recovery. The input parameters of applied voltage and weight percent of PANI/MWCNT cathode was analyzed through RSM. Table 1 shows the parameters and responses used in RSM. The regression analysis was performed on the responses to determine the coefficients of the model terms. Quadratic equation was selected as the regression model. The equations were used to make predictions on the responses. For giving the level of each parameter, the relative effect of the factors was identified by comparing the factor coefficients. Equation 2 to 5 are the quadratic regression model for all studied responses in terms of coded factors.

For Hydrogen Production Rate (m<sup>3</sup>/m<sup>3</sup>/day):

$$Y = 0.2924 + 0.4012A + 0.1255B + 0.0954AB + 0.1051A^2 + 0.0621B^2 \quad Eq. 2$$

For Coloumbic Efficiency (%):

$$Y = 35.98 + 8.27A + 6.91B + 2.40AB - 3.45A^2 - 2.341B^2 \quad Eq. 3$$

For Cathodic Hydrogen Recovery (%):

$$Y = 31.50 + 20.71A + 2.06B + 0.3482AB + 3.79A^2 - 0.5625B^2 \quad Eq. 4$$

For Energy Recovery (%):

$$Y = 75.86 + 9.43A + 5.19B - 0.7679AB + 2.94A^2 - 1.31B^2 \quad Eq. 5$$

Where  $Y$  is the predicted response,  $A$  indicates the parameter of applied voltage while  $B$  indicates the parameter of weight percent of PANI/MWCNT cathode. In addition, high levels of the factors are coded as +1 while low levels are coded as -1.

Table 1: The data obtained from previous study [7]

No.	Variables		Responses			
	V	wt (%)	HPR (m <sup>3</sup> /m <sup>3</sup> /day)	c <sub>E</sub> (%)	r <sub>cat</sub> (%)	ER (%)
1	0.3	0	0.02	16.0	13	64
2	0.4	0	0.04	21.0	17	65
3	0.5	0	0.12	23.0	21	66
4	0.6	0	0.16	25.0	27	68
5	0.7	0	0.30	27.0	31	69
6	0.8	0	0.40	28.0	37	72

7	0.9	0	0.52	31.0	43	76
8	1.0	0	0.64	31.0	55	85
9	0.3	25	0.04	23.5	13	64
10	0.4	25	0.06	28.0	18	68
11	0.5	25	0.12	32.0	22	71
12	0.6	25	0.18	32.5	28	74
13	0.7	25	0.30	34.0	33	75
14	0.8	25	0.46	34.0	41	79
15	0.9	25	0.56	35.5	48	83
16	1.0	25	0.70	36.0	56	88
17	0.3	75	0.10	24.0	15	74
18	0.4	75	0.16	32.5	20	75
19	0.5	75	0.26	36.0	25	76
20	0.6	75	0.38	38.0	31	80
21	0.7	75	0.58	42.0	37	82
22	0.8	75	0.76	45.0	43	85
23	0.9	75	0.90	47.0	49	86
24	1.0	75	1.06	48.0	57	90

### 3.1 ANOVA

ANOVA test for the regression model was performed to fit a good model. The variables were evaluated by the significance of model terms, which depends on the probability value (p-value) at 95.0 % confidence interval [8]. The p-value determines the closeness of the results achieved to the actual experimental data across the model variables [9]. P-value should be less than 0.05 to be significant on the responses. P-values were associated with F-values. The F-value should be large enough to determine the model is statistical significance. Consequently, for all the modal in the responses, each p-value was less than 0.0001, while for each F-value, they were more than 100 which were large enough to support the statement. ANOVA test indicated all of the models were significant. Table 2 shows the ANOVA for all responses.

**Table 2: ANOVA for all responses**

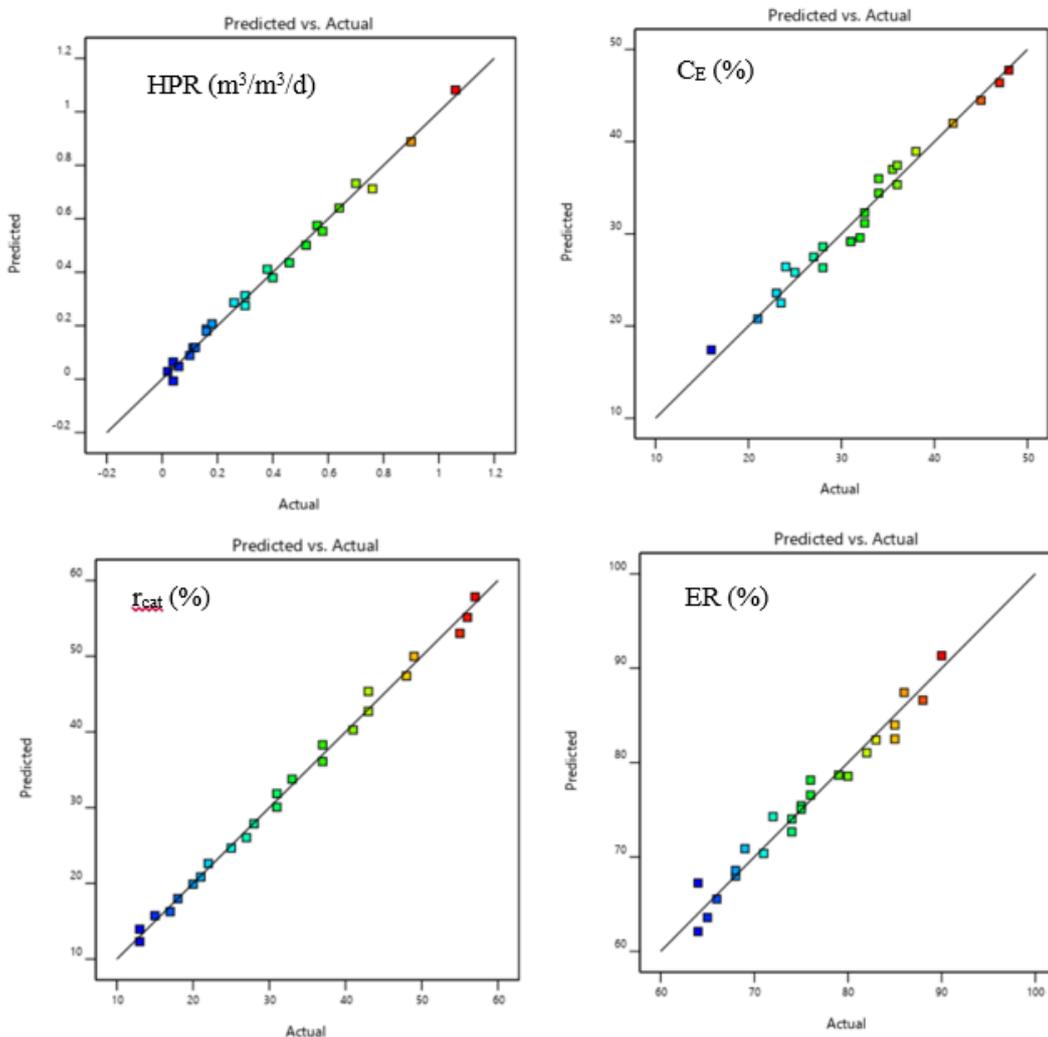
Modal	F-value	p-value	
Hydrogen production rate, HPR	517.05	< 0.0001	Significant
Coulombic efficiency, $C_E$	141.32	< 0.0001	Significant
Cathodic hydrogen recovery, $r_{cat}$	756.69	< 0.0001	Significant
Energy Recovery, ER	102.33	< 0.0001	Significant

The validation of the model is determined theoretically to study responses by the input parameters. Table 1 shows the ANOVA results of fit statistics for each of the responses.  $R^2$  value should be more than 0.9. A model with an  $R^2$  value that is near or equal to one indicates the model is ideal for good prediction, hence shows a significant effect on the response [9]. It was found all responses have a high value of  $R^2$ , which ranged from 0.9660 to 0.9953. This means that the models are a good fit for the experimental values as well as the predicted values. Moreover, Adjusted  $R^2$  (Adj  $R^2$ ) and predicted  $R^2$  (Pred  $R^2$ ) were also used to assist the regression analysis to obtain more significant results. Adj  $R^2$  value has adjusted the number of terms that are not significant in each regression model. In Table 3.1, all responses have slightly smaller Adj  $R^2$  values than  $R^2$  values. This is because by decreasing Adj  $R^2$  will turn out to increase the adequacy of a regression model. Whereas Pred  $R^2$  is to determine the predictions to have a better model fitting, thus the Pred  $R^2$  value should be more than 0.9 as well. Each Pred  $R^2$  value of all the responses has achieved the standard. After evaluating the model, the ANOVA results were used to cross the design space.

**Table 3: ANOVA results of fit statistics for all responses**

Response	Std. Dev.	Mean	R <sup>2</sup>	Adj R <sup>2</sup>	Pred R <sup>2</sup>
Hydrogen Production Rate	0.0276	0.3672	0.9931	0.9912	0.9875
Coulombic efficiency	1.46	32.08	0.9752	0.9683	0.9461
Cathodic Hydrogen Recovery	1.09	32.50	0.9953	0.9939	0.9901
Energy Recovery	1.65	75.63	0.9660	0.9566	0.9279

The adequacy of the regression model was determined by the predicted versus actual plot graphs. This is presented in Figure 3.1. As observed, the majority of the experimental value points for each response was close to the straight line as the predicted values. This indicates that each of the R<sup>2</sup> and Adj-R<sup>2</sup> values proven to be acceptable towards the relationship between the predicted and actual data. This is due to the fact that all coefficients are high values and similarly close to each other. Besides, their p-values are also less than 0.05, verified that they are statistically significant. Hence, the values were found to be adequate for all the regression models in this study.

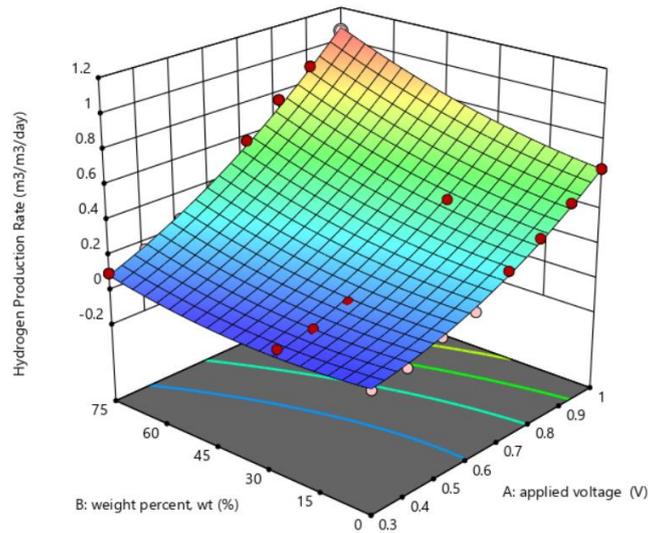


**Figure 2: Predicted vs. Actual values plot graph of all responses**

### 3.4 Effect of parameters on dependent variables

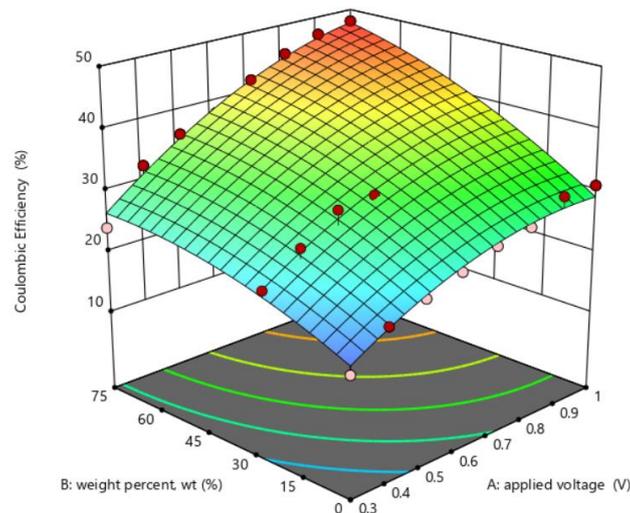
The three-dimensional (3D) response surface plot in RSM analyzes the effects of different parameters on the dependent variable visually. 3D surface response plot for HPR is shown in Figure 3.

As observed, the maximum HPR of  $1.06 \text{ m}^3 \text{ H}_2/\text{m}^3/\text{d}$  is obtained at the applied voltage of 1.0 V with a 75.0 % wt. of PANI/MWCNT cathode in MEC, while the minimum rate was  $0.02 \text{ m}^3 \text{ H}_2/\text{m}^3/\text{d}$  at 0.3 V with a fully MWCNT cathode. It shows that MEC exerted higher performance in hydrogen production when consuming a higher applied voltage than it did at lower. It is due to the rise of applied voltage can significantly suppress methanogen and increases the rate of reaction in MEC [10]. The amount of methanogens should be controlled as hydrogen losses will occur and hence results in a decreasing HPR. Moreover, different weight percent of PANI/MWCNT cathode in MEC also become a great impact on HPR. It was found that 75 wt % PANI/MWCNT cathode obtained the highest performance in MEC compared to the lower weight percent cathodes. This is due to the higher conductivity in 75 wt % PANI/MWCNT material which performs a better adsorption of the electrons on the cathode surface of the cathode [7]. As a result, combined effects for both the variables have shown that performance of hydrogen production rate increases when weight percent of PANI/MWCNT cathode and applied voltage increased.



**Figure 3: 3D surface response plot for hydrogen production rate**

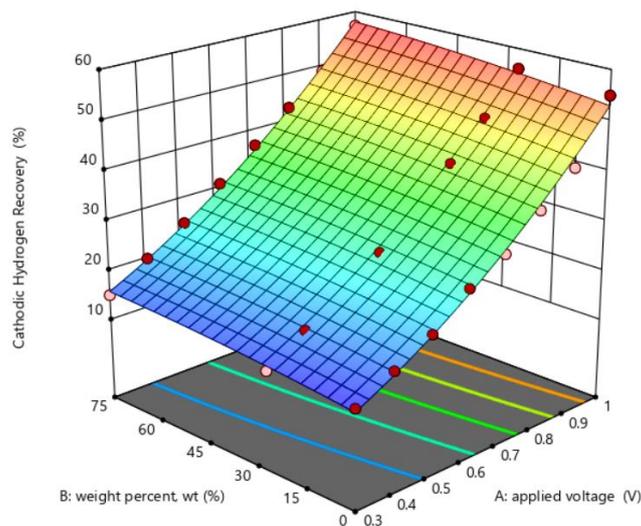
As for coulombic efficiency,  $C_E$ , is noticeably affected by the applied voltage and the weight percent of PANI/MWCNT cathode. Increasing applied voltage at 75.0 % wt. of PANI/MWCNT cathode has shown an increase in  $C_E$ . Due to the increasing sign, it is proved that the growth of exoelectrogenic microorganisms in biofilms has affected the electron transfer rates which increased the electron transfer and improved  $C_E$ . The diffusion of substrates decreased the dead cells and debris as the microorganisms will consume them during the process and thus enhanced electrocatalysis [11]. Furthermore, increasing of weight percent of PANI/MWCNT cathode at an applied voltage of 1.0 V has increase  $C_E$  from 36.0 % to 48.0 %. This indicates that electrocatalysis can also be affected by the electrode reactions that are catalyzed by the exoelectrogenic microorganisms. It is shown that 75 wt. % of PANI/MWCNT cathode was the highest reaction rate among the cathodes used in MEC. Both variables can improve the percentage of  $C_E$ . The maximum  $C_E$  was 48.0 % when the applied voltage was 1.0 V, combining with 75.0 % wt. of PANI/MWCNT cathode in MEC. Some losses of efficiency may due to minor substrates in MEC which having the methanogens to consume it, thus reduced the use in current production. Figure 4 displayed the 3D response for coulombic efficiency.



**Figure 4: 3D surface response plot for coulombic efficiency**

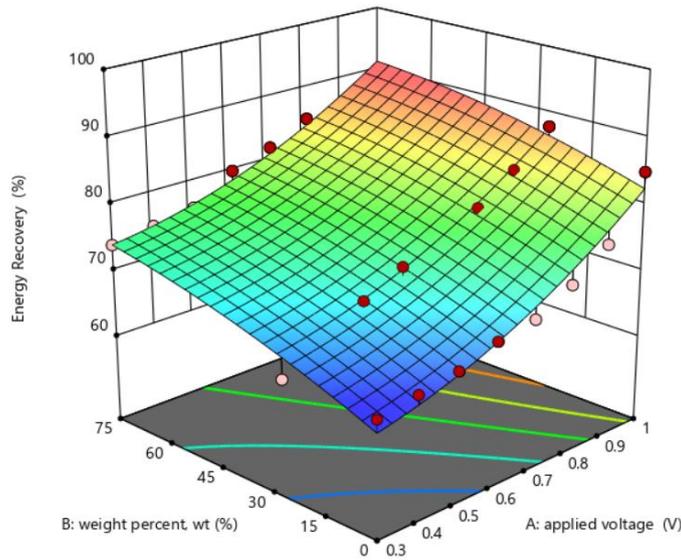
Cathodic hydrogen recovery,  $r_{cat}$  is also relative to electrical input in the MEC circuit. Cathodic hydrogen recovery is calculated in terms of electron recovery as hydrogen gas. The combined effect of applied voltage and weight percent of PANI/MWCNT led to increasing the  $r_{cat}$ . The maximum of  $r_{cat}$  was found 57.0 % at the applied voltage of 1.0 V combined with 75.0 % wt. of PANI/MWCNT cathode in MEC. Hydrogen recovery increases due to the increase in the hydrogen production rate. In contrast, when the applied voltage was 0.3 V,  $r_{cat}$  was less than 16% when combining with all the weight percent of PANI/MWCNT cathodes, the minimum percentage was 13.0 % at 0.3 V with fully MWCNT cathode and 25 wt. % of PANI/MWCNT cathode.

For the effect of PANI/MWCNT material, high conductivity and catalytic activity, can also enhance the efficiency of  $r_{cat}$ . Figure 5 illustrated the 3D surface response plot for  $r_{cat}$ . The percentage of  $r_{cat}$  was low as it may due to the diffusion of hydrogen from cathode to anode in MEC. Hydrogen at the anode could be consumed by the microorganism, especially methanogenic microorganisms. It is because the MEC is designed as a single-chamber, so there is no membrane to separate anode chamber and cathode chamber in the MEC circuit. The major problem of single-chamber MEC is the crossover of hydrogen to the anode and the methane formation caused by methanogenic microorganisms consuming the hydrogen production [12].



**Figure 5: 3D surface response plot for cathodic hydrogen recovery**

Energy recovery (ER) efficiency represents the ratio of the energy content of the hydrogen produced to the electrical energy input required. As observed in Figure 6, ER efficiency was significantly affected by the cathode in MEC. At an applied voltage of 1.0 V and 75.0 % wt. of PANI/MWCNT cathode, showed 90.0 % of ER, which contributed as the highest ER in the experiment. Increasing the applied voltage can slow down the process of hydrogen turning to methane, leading to harvest a higher hydrogen production, hence increases the rate of energy recoveries [12]. Besides applied voltage, the high electrochemical activity of the PANI/MWCNT electrode due to the combined catalytic effect of the conductive polymer and the high catalytic surface area of PANI/MWCNT also contributed to the ER of MEC. The MEC with MWCNT cathode exhibited the lowest energy recovery, which presented the minimum energy recovery of 64.0 % at the applied voltage of 0.3 V. This shows that the technology of MEC may be a capable and promising system for simultaneous hydrogen production and wastewater treatment [7].



**Figure 6: 3D surface response plot for energy recovery**

3.5 Process optimization

Optimization of RSM is to determine the optimum condition of parameters affecting each of the responses. The RSM model is capable to predict the maximum performance of MEC. It was found that the optimum operating condition of the parameters towards all the performance of MEC was at 1.0 V of applied voltage and using a 75 wt. % PANI/MWCNT cathode. The experimental values and predicted values were observed to ascertain the reliability of this technique. Table 4 shows the optimization conditions and model validation of all responses. The optimum experimental values, were 1.06 m<sup>3</sup>/m<sup>3</sup>/day of HPR, 48.0 % of C<sub>E</sub>, 57.0 % of r<sub>cat</sub> and 90% of ER, whereas optimum predicted values were 1.08 m<sup>3</sup>/m<sup>3</sup>/day of HPR, 47.8 % of C<sub>E</sub>, 57.9 % of r<sub>cat</sub> and 91.0 % of ER. Therefore, this shows that the experimental design using RSM can be applied to optimize the parameters affecting MEC performance.

**Table 4: Optimization conditions and model validation of responses**

Optimum conditions	Applied voltage (V)	Weight percent (wt. %)	Experimental	Predicted
HPR (m <sup>3</sup> /m <sup>3</sup> /day)	1.0	75	1.06	1.08
C <sub>E</sub> (%)	1.0	75	48.0	47.8
r <sub>cat</sub> (%)	1.0	75	57.0	57.9
ER (%)	1.0	75	90.0	91.3

#### 4. Conclusion

RSM is a broadly used mathematical and statistical method for modeling and analyzing a progression in which the specific response is influenced by several variables. In this study, RSM has successfully shown that the effect of applied voltage in MEC and the different weight percent of polyaniline/multi-walled carbon nanotube cathode for the MEC system can influence hydrogen production rate, coulombic efficiency, cathodic hydrogen recovery and energy recovery of MEC. The best optimum condition of the parameters was obtained at an applied voltage of 1.0 V and weight percent of 75.0 % of PANI/MWCNT cathode. The experimental values of hydrogen production rate, coulombic efficiency, cathodic hydrogen recovery and energy recovery were 1.06 m<sup>3</sup>/m<sup>3</sup>/day, 48.0 %, 57.0 % and 90.0 %, respectively, while the predicted values were 1.08 m<sup>3</sup>/m<sup>3</sup>/day, 47.8 %, 57.9 % and 91.3 %, respectively. This study proved that RSM is a reliable optimization technique that can be used to design the experiments and determine the performance of MEC.

##### 4.1 Recommendation for future study

Throughout this study, there are several suggestions for improvement as follows:

1. Other types of design for statistical analysis and process optimization such as full factorial design (FFD), Box-Behnken design (BBD), central composite design (CCD) or Doehlert design (DD) can be used to evaluate the optimization process of MEC.
2. Conduct the experiment based on optimization of RSM by using the optimum conditions of the predicted value for validating the results.
3. Changes of other independent variables such as pH, temperature or substrate concentration in MEC can affect the performance of MEC in hydrogen production by using RSM.
4. The more experimental data can increase the adequacy of the regression model and the precision of the response for better understanding on the effect of independent variables.

#### Acknowledgement

The authors would like to thank the Department of Civil Engineering Technology, Faculty of Engineering Technology and Universiti Tun Hussein Onn Malaysia for its support throughout this research activity

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