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# Optimization of anolyte solution in Microbial Fuel Cell using statistical experimental design

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## ABSTRACT
This study aims to optimize factors that affect power generation in microbial fuel cell (MFC) using a statistical experimental design. A MFC reactor with two compartments has been constructed from cheap materials and used for electricity generation. The performance of MFCs can be influenced by several factors, hence; the Plackett–Burman design was employed to determine the most significant factors that affect power generation. The factors that affected the electricity generation significantly were magnesium sulfate, yeast extract, and sodium acetate. A near optimum medium formulation was obtained using this method with increased power density by 71.57 %. In this respect, the three levels Box–Behnken design was applied. A polynomial model was created to correlate the relationship between the three significant variables and power density. Under the optimized condition, the power density was 390 $mW/m^2$. These results indicate that the optimized conditions accelerated the power generation and the maximum power point (MPP) was about 91.2 % higher than that recorded with the basal condition. Microbial fuel cells (MFCs) are new types of bioreactors that use bacteria to generate electricity from organic and nonorganic compounds and are considered one of the prospective alternative technologies.

## INTRODUCTION

Microbial fuel cells (MFCs) are new types of bioreactors, which use exoelectrogenic biofilms for electrochemical energy production (**Ulusoy and Dimoglo, 2018; Xu *et al*., 2019**). In recent years, a large number of studies have been conducted to explore microbial fuel cells in many aspects, such as electron transfer mechanisms, enhancing power outputs, reactor developments and applications (**Parkash, 2016; Cao *et al*., 2019**).

Many studies practiced the classical optimization methods for bioelectricity generation, which involve the alteration of one factor at a time, while keeping all other factors at a predetermined level (**Flimban *et al*., 2018**). In this approach, a series of experiments are carried out using a large number of variables which need to be tested to determine the optimum level.

This process is very time-consuming, labour-intensive, expensive and the interaction between the variables is ignored **(Haaland, 1989; Deng and Tang, 1999; Montgomery, 2012; Scibilia, 2014**). On the other hand, for improving the power generation, statistical experimental design approaches were employed. Statistical experimental designs are powerful tools for searching the key factors rapidly from a multivariable system and minimizing the error in determining the effect of parameters. Therefore, results are achieved in an economical manner (**Anderson and McLean, 2018**).

Optimization of bioelectricity generation using a statistically planned experiment is a sequential process. First, a large number of continuous factors are screened to find the most affecting factors in bioelectricity generation that could be optimized by a response surface modeling (**Mabrouk *et al*., 2013, 2014; Ghanem *et al*., 2015, 2016; Vilas Boas *et al*., 2019)**. Response surface methodology, which is supported by software, is an empirical modelization technique derived to evaluate the relationship between a set of controlled experimental factors and observed results. This optimization process involves three major steps: estimating the coefficients in a mathematical model, predicting the response and checking the adequacy of the model (**Ferreira *et al*., 2007; Annadurai *et al*., 2008; Mahmoodi-Babolan *et al*., 2019**).

The present work aims to investigate the parameters affecting the performance of dual chamber MFC through statistically designed experiments. First, Plackett–Burman design was applied to screen fot the most significant medium components affecting voltage yield. Second, Box–Behnken design was applied to determine the optimum level of each of the significant parameters.

## MATERIALS AND METHODS

### MFC setup

Double chamber MFC, medium composition, inoculum, and operation conditions were constructed as previously described by El-Badan ***et al***. (2019).

### Measurement of Output:

The output of the MFC was expressed by means of voltages (E) after the MFC had reached steady state. For this purpose, multimeter (SanwaCD800a – Japan) was used and calibrated each time before use. The voltage and current generated were converted to power according to Ohm’s law, P=IV, where, P=power (mW), I=current (mA), and V=voltage (mV). Power density ($mW/m^3$) and current density ($mA/m^3$) were calculated by dividing power and current by the treated wastewater volume ($m^3$). The polarization curve was obtained at different external resistances (100–740 Ω). The internal resistance was derived from the polarization curve as the slope (**Alshehri *et al*., 2016)**. Triplicate of all experiments was done and the average was taken. The standard deviation of the studied parameters ranged between 0.4 and 3.8 %.

### Plackett-Burman experimental design

Application of the statistical design was carried out in a "two-phase" optimization approach. The first step was to evaluate relative importance of the various constituents in the culture media and selecting levels of variables that have the significant influence on power generation, the second was verification of the experiments to validate results under specific optimized experimental conditions (**Huang *et al*., 2019**). The Plackett-Burman experimental design can provide indication and tendency regarding necessity of each factor in relatively few experiments. Seven independent variables were screened in eight combinations organized according to the Plackett–Burman design matrix (**Table 1**). Each variable was examined at two levels, low (-) and high (+). Main effect of each variable can be calculated using the following standard equation:

$$\text{Main effect} = [\sum R(H) - \sum R(L)]/N$$

Where R(H) and R(L) are observations of trials where independent variables were present in high and low concentrations, respectively, and N is the number of trials divided by 2.
Another important part of the Plackett-Burman screening design was the choice of dummies (**Mohajeri Amiria *et al*., 2019**). A dummy is a component whose level does not change in the design. Factors known to have no effect can be chosen as dummies, or any factor not chosen as a variable can be included as a dummy. In the experiments performed, temperature served as a dummy variable. Excel (Microsoft office, 2010) was used for the experimental design and all statistical analysis. The variables with confidence levels more than 95% were considered to influence power generation.

### Box-Behnken design

In the second phase of medium formulation for optimum power generation, the Box-Behnken experimental design (**Selamat *et al*., 2018; Mohajeri Amiria *et al*., 2019**), which is a central composite design (**Box and Behnken, 1960**), was applied. In this model, the most significant independent variables, namely; yeast extract ($X_1$), sodium acetate ($X_2$), and $MgSO_4.7H_2O$ ($X_3$) are included and each factor can be examined at three different levels, low (-), high (+) and central or basal (0). Fifteen combinations were fitted to the following second order polynomial model:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2$$

Where, Y is the dependent variable (MPP); $X_1$, $X_2$ and $X_3$ are the independent variables; $b_0$ is the regression coefficient at centre point; $b_1$, $b_2$ and $b_3$ are linear coefficients; $b_{12}$, $b_{13}$ and $b_{23}$ are second-order interaction coefficients; and $b_{11}$, $b_{22}$, and $b_{33}$ are quadratic coefficients. The values of the coefficients were calculated using STATISTICA (data analysis software system), version 7, and the optimum concentrations were predicted using Microsoft Excel 2010. The quality of the fit of the polynomial model equation was expressed by the coefficient of determination, $R^2$ (**Uwadiae and Ihaza, 2018**). Three-dimensional graphical representations were also constructed using STATISTICA software to reflect the effects as well as the interactions of independent variables on the power generation.

## RESULTS

### Optimization of medium composition

The anolyte composition has a great effect on the performance of the microbial fuel cell; it is where bio-electrochemical reactions take place. The composition of the anolyte not only affect the type of microorganisms available for electricity generation, but also their electrogenic activity and interactions with each other as well as with the electrode. The performance of the cell is also greatly affected by the ionic strength of the anolyte solution **(Logan, 2008; Thygesen *et al*., 2009; Slate *et al*., 2019).** Therefore, the aim of this experiment was to optimize medium composition for maximum electricity production and to evaluate the factors significantly affecting power generation, using Plackett–Burman experimental design. The Plackett–Burman design for 8 trials with two levels of concentrations for seven different variables was carried out according to the experimental matrix as shown in Table 1. The corresponding results for the seven examined variables were summarized in Table 2.

A large variation in the results of the Plackett–Burman design experiments was observed, the maximum values of voltage, 24.27mV, electric current, 0.728mA, current density, 0.124mA/cm$^2$, electric power, 17.67mW, power density, 300mW/m$^2$, and volumetric power density, 9.5mW/m$^3$ was achieved in trial number 1, while the minimum values of voltage, 14.01mV, electric current, 0.42mA, current density, 0.071mA/cm$^2$, electric power, 5.89mW, power density, 100mW/m$^2$, and volumetric power density, 3.167mW/m$^3$ was achieved in trial number 4 (Table 2).

The main effects of the examined variables on the MPP generation were calculated and presented in Table 1. A main effect value with a positive sign indicates that high concentration of this variable is near to optimum and the negative sign indicates that the low concentration of this variable is near to optimum, while a mean close to zero means that a factor has little or no effect. As shown in Table 1, it was found that all of the tested variables, except phosphate buffer solution (PB), had a positive effect on power generation within the test ranges. The highest improvement percentages of 47.1, 31.4, and 17.6% were observed in trials numbers 1, 2, and 8; respectively (Table 2). Fig. 1 shows the ranking of factor estimates in a Pareto chart.

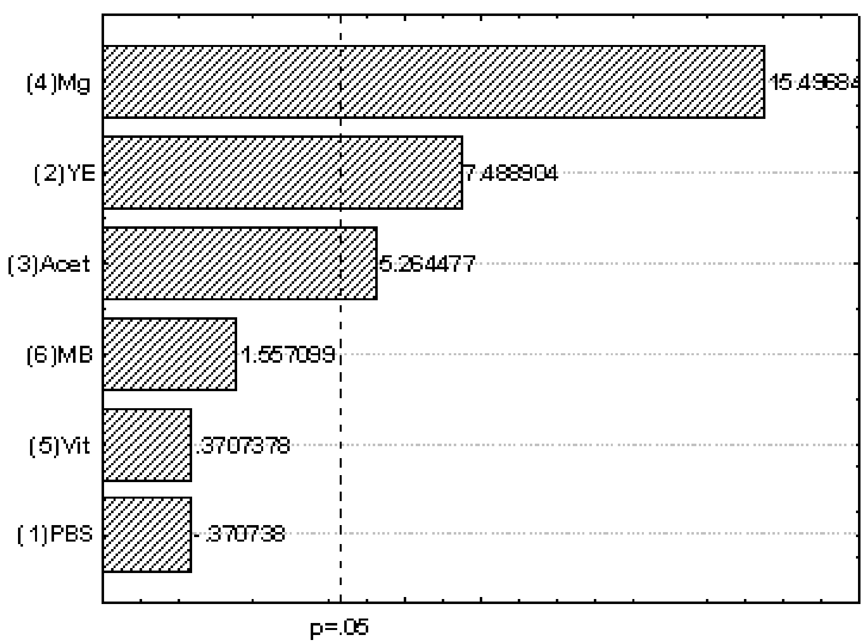



**Fig. 1: Pareto chart for Packett-Burman analysis, rationalizing the effect of each variable on power generation. The vertical line indicates confidence level of 95% for the effects.**

**Table 1: Plackett–Burman design matrix and Statistical analysis of results showing estimated effect, corresponding, *p*-value, regression coefficient, and confidence level for each variable on power generation**

| Run order/ Measuring unit | Experimental values | | | | | | |
|---|---|---|---|---|---|---|---|
| | PB mM | YE g/l | SA g/l | Mg g/l | VI Ml | MB μM | TP* °C |
| **1** | 25(-) | 5(+) | 8(+) | 0.61(+) | 0.5(-) | 60(-) | 30(+) |
| **2** | 25(-) | 5(+) | 2(-) | 0.61(+) | 1(+) | 240(+) | 30(+) |
| **3** | 100(+) | 1(-) | 2(-) | 0.61(+) | 0.5(-) | 240(+) | 30(-) |
| **4** | 25(-) | 1(-) | 2(-) | 0.21(-) | 0.5(-) | 60(-) | 30(+) |
| **5** | 25(-) | 1(-) | 8(+) | 0.21(-) | 1(+) | 240(+) | 30(+) |
| **6** | 100(+) | 5(+) | 8(+) | 0.21(-) | 0.5(-) | 240(+) | 30(-) |
| **7** | 100(+) | 5(+) | 2(-) | 0.21(-) | 1(+) | 60(-) | 30(-) |
| **8** | 100(+) | 1(-) | 8(+) | 0.61(+) | 1(+) | 60(-) | 30(-) |
| **9** | 50(0) | 3(0) | 5(0) | 0.41(0) | 0.75(0) | 150(0) | 30(0) |
| **Main Effect** | -2.5 | 50.5 | 35.5 | 104.5 | 2.5 | 10.5 | - |
| ***p*-value** | 0.75 | 0.02 | 0.03 | 0 | 0.75 | 0.26 | - |
| **Coefficient regression** | -1.2 | 25.3 | 17.8 | 52.3 | 1.3 | 5.3 | - |
| **Confidence %** | 25.36 | 98.26 | 96.58 | 99.59 | 25.36 | 74.03 | - |

* Dummy variable, Phosphate-Buffered Saline solution (PB), Yeast extract (YE), Sodium acetate (SA), $MgSO_4.7H_2O$ (Mg), Vitamin solution (VI), Methylene blue (MB), Temperature (TP)

**Table 2. Results of Plackett-Burman design**

| Trials | Electric current $I_{(33\,\Omega)}$ (mA) | Voltage $E_{(33\,\Omega)}$ (mV) | Current density $I_d$ ($mA/cm^2$) | Electric power P (mW) | Power density MPP ($mW/m^2$) | Volumetric Power density $P_{v\,(An)}$ ($mW/m^3$) | Percentage of improvement in power density |
|---|---|---|---|---|---|---|---|
| **1** | 0.728 | 24.27 | 0.124 | 17.67 | 300 | 9.500 | 47.06 |
| **2** | 0.688 | 22.94 | 0.117 | 15.79 | 268 | 8.487 | 31.37 |
| **3** | 0.626 | 20.88 | 0.106 | 13.08 | 222 | 7.030 | 8.82 |
| **4** | 0.420 | 14.01 | 0.071 | 5.89 | 100 | 3.167 | -50.98 |
| **5** | 0.528 | 17.61 | 0.090 | 9.31 | 158 | 5.003 | -22.55 |
| **6** | 0.586 | 19.52 | 0.099 | 11.43 | 194 | 6.143 | -4.90 |
| **7** | 0.532 | 17.72 | 0.090 | 9.42 | 160 | 5.067 | -21.57 |
| **8** | 0.651 | 21.71 | 0.111 | 14.14 | 240 | 7.600 | 17.65 |
| **9** | 0.600 | 20.01 | 0.102 | 12.02 | 204 | 6.460 | 0.00 |

The Pareto chart has been described as a useful tool for identifying the most important effects. It displays the magnitude of each variable and is a convenient way to view the results of a Plackett– Burman design (**Haaland, 1989; Strobel and Sullivan, 1999)**. In this chart, the length of each bar on a standardized Pareto chart is proportional to the absolute value of its associated regression coefficient or estimated effect.

The *t*- test for any individual effect allows an evaluation of the probability of finding the observed effect purely by chance. The statistical confidence shown in Table 1, which shows the analysis done for the Plackett-Burman design, was calculated as:

$$\text{Statistical confidence} = (1\text{-}p)*100$$

So, a value of $P = 0.05$ corresponded to a statistical confidence of 95%. Hence, any component showing a statistical confidence higher than 95% was considered significant. Based on the statistical analysis of confidence level of 7 variables (Table 1), yeast extract, sodium acetate, and $MgSO_4$ had confidence levels above 95% and hence were considered the significant parameters influence power generation. None of these components had a negative effect on power generation.

The goodness of fit of the model was checked by determination coefficient ($R^2$). In this case, the $R^2$ value was calculated to be 0.9939, indicated that 99.39% of the total variability in the response could be explained by this model and only 0.61% of the total variation were not explained. A regression model with $R^2$ close to 1.0 is considered as having a very high correlation **(Yong *et al*., 2011).** Therefore, the present $R^2$ value reflected a very good fit between the observed and predicted responses and implied that the model is reliable for predicting power generation.

After applying the ANOVA statistical test, it was found that the first order models for power generation was satisfactory, the polynomial model equation was proposed to calculate the optimum levels of these variables for power generation can be written as:

$$Y = 205.1\text{-}1.2*PB+25.3*YE+17.8*SA+52.3*Mg+1.3*VI+5.3*MB,$$

Where Y represents power generation in $mW/m^2$

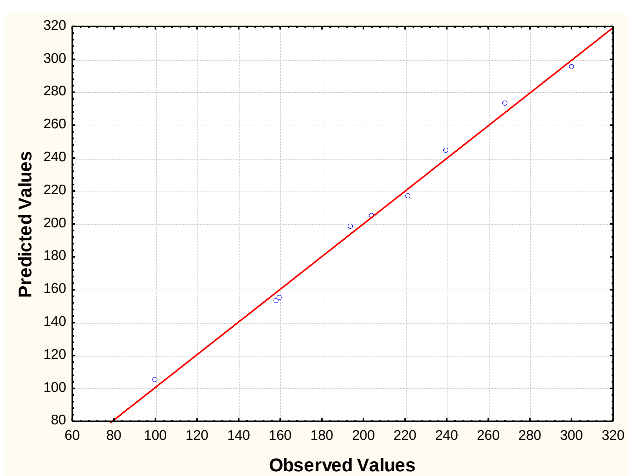



**Fig.2: Correlation between predicted and observed values in Plackett-Burman experiment.**

## Desirability and prediction profile for power generation

The maximum experimental value for power generation was 300 $mW/m^2$, while the predicted response, based on RSM, was estimated to be 295.1 $mW/m^2$ (Fig. 2). The close correlation between the experimental and predicted data indicates the appropriateness of the model.

## Validation of the model

Based on the data obtained from Plackett-Burman experimental results, the following composition is predicted to be near optimum: PB, 25 mM; YE, 5 g/l; SA, 8 g/l; Mg, 0.6 g/l; VI, 1 ml; and MB, 240 µM. In order to determine the accuracy of the applied Plackett-Burman screening test, a verification experiment was carried out. The applied near optimum condition resulted in power generation of approximately 350 $mW/m^2$. This result represented a 71.57% folds increase in power generation when compared with the average of the results obtained under the basal condition.

## Optimization of medium composition by Box-Behnken design

The variables so identified by Plackett-Burman design were further optimized by RSM using Box-Behnken design experimental plan (**Bin Halmi *et al*., 2016**). The three key variables were examined at three different levels (-, 0, +) (Table 3). Table 3 shows various combinations of the three selected variables according to Box-Behnken design. The concentrations of the remaining components in all assemblies were the same as those in the pre-optimized medium of Plackett-Burman design. The polarization data obtained from Box-Behnken experimental design were summarized in Table 4.

The maximal power generated of 380 $mW/m^2$ was in trial number 5, with an improvement percentage of 8.6 (Fig. 4) compared with the control (obtained from pre-optimized Plackett-Burman design), followed by the trials number 14, and 15; which gave an improvement percentage of 3.4, and 0.8, respectively. The maximum experimental value for power generation was 380 $mW/m^2$, while the predicted response based on the RSM was estimated to be 382.13 $mW/m^2$ (Table 4). The close correlation between experimental and predicted data indicates the appropriateness of the model.

The analysis of variance (ANOVA) for the response quadratic model is presented in (Table 5). The model was significant at the 99.94% confidence level and the quality of the model can also be checked using various criteria. Desirability and prediction profile for the MPP are shown in Fig. 3 that emphasize the tendency and ranges of the main factors that affect the MPP.

**Table 3: Box-Behnken design for the most significant three variables that affected power generation (yeast extract ($X_1$), sodium acetate ($X_2$), and MgSO4.7$H_2$O ($X_3$)).**

| Trial | Variable | | |
|---|---|---|---|
| | $X_1$ | $X_2$ | $X_3$ |
| **1** | 5(0) | 6(-) | 0.71(+) |
| **2** | 5(0) | 6(-) | 0.51(-) |
| **3** | 5(0) | 8(0) | 0.61(0) |
| **4** | 4(-) | 10(+) | 0.61(0) |
| **5** | 5(0) | 10(+) | 0.51(-) |
| **6** | 6(+) | 8(0) | 0.51(-) |
| **7** | 6(+) | 6(-) | 0.61(0) |
| **8** | 5(0) | 8(0) | 0.61(0) |
| **9** | 6(+) | 10(+) | 0.61(0) |
| **10** | 4(-) | 6(-) | 0.61(0) |
| **11** | 5(0) | 8(0) | 0.61(0) |
| **12** | 4(-) | 8(0) | 0.71(+) |
| **13** | 4(-) | 8(0) | 0.51(-) |
| **14** | 6(+) | 8(0) | 0.71(+) |
| **15** | 5(0) | 10(+) | 0.71(+) |

The calculated regression equation for the optimization of media constituents assessed power generation (Y) as a function of these variables. By applying quadratic regression analysis on the experimental data, the following equation was found to explain power generation:

$$\mathbf{Y = -2266 + 486\,X_1 + 304\,X_2 - 24\,X_3 - 5.88\,X_1X_2 - 68.0\,X_1X_3 - 171\,X_2X_3 - 37.6\,X_1^2 - 9.42\,X_2^2 + 1576\,X_3^2}$$

Where, Y is the dependent variable (MPP) in $mW/m^2$; $X_1$, $X_2$ and $X_3$ are the concentrations of the independent variables as shown in (Table 4).

**Table 4. Results of Box-Behnken design experiment.**

| Run | Electric current $I_{(33\,\Omega)}$ (mA) | Voltage $E_{(33\,\Omega)}$ (mV) | Current density $I_d$ (mA/cm$^2$) | Electric power P (mW) | Power density MPP (mW/m$^2$) | Volumetric Power density $P_{v\,(An)}$ (mW/m$^3$) | Percentage of improvement in power density |
|---|---|---|---|---|---|---|---|
| **1** | 0.780 | 26.00 | 0.132 | 20.29 | 344.4 | 10.906 | -1.60 |
| **2** | 0.644 | 21.48 | 0.109 | 13.84 | 235 | 7.442 | -32.86 |
| **3** | 0.786 | 26.21 | 0.134 | 20.62 | 350 | 11.083 | 0.00 |
| **4** | 0.734 | 24.47 | 0.125 | 17.96 | 305 | 9.658 | -12.86 |
| **5** | 0.819 | 27.31 | 0.139 | 22.38 | 380 | 12.033 | 8.57 |
| **6** | 0.774 | 25.80 | 0.131 | 19.97 | 339 | 10.735 | -3.14 |
| **7** | 0.688 | 22.94 | 0.117 | 15.79 | 268 | 8.487 | -23.43 |
| **8** | 0.786 | 26.21 | 0.134 | 20.62 | 350 | 11.083 | 0.00 |
| **9** | 0.758 | 25.26 | 0.129 | 19.14 | 325 | 10.292 | -7.14 |
| **10** | 0.596 | 19.86 | 0.101 | 11.84 | 201 | 6.365 | -42.57 |
| **11** | 0.786 | 26.21 | 0.134 | 20.62 | 350 | 11.083 | 0.00 |
| **12** | 0.765 | 25.49 | 0.130 | 19.50 | 331 | 10.482 | -5.43 |
| **13** | 0.705 | 23.49 | 0.120 | 16.55 | 281 | 8.898 | -19.71 |
| **14** | 0.800 | 26.65 | 0.136 | 21.31 | 361.8 | 11.457 | 3.37 |
| **15** | 0.790 | 26.32 | 0.134 | 20.79 | 352.9 | 11.175 | 0.83 |

**Table 5: Analysis of variance for the fitted quadratic polynomial model.**

| | Coefficients | Standard Error | *t* Stat | *P*-value |
|---|---|---|---|---|
| **Intercept** | -2265.98 | 65.87665 | -34.3973 | 3.91E-07 |
| $X_1$ | 486.08 | 12.31472 | 39.47146 | 1.97E-07 |
| $X_2$ | 303.8625 | 5.558238 | 54.66885 | 3.87E-08 |
| $X_3$ | -24.15 | 137.747 | -0.17532 | 0.867706 |
| $X_1X_2$ | -5.875 | 0.483703 | -12.1459 | 6.69E-05 |
| $X_1X_3$ | -68 | 9.674063 | -7.0291 | 0.000899 |
| $X_2X_3$ | -170.625 | 4.837032 | -35.2747 | 3.45E-07 |
| $X_1^2$ | -37.5625 | 1.006908 | -37.3048 | 2.61E-07 |
| $X_2^2$ | -9.42187 | 0.251727 | -37.4289 | 2.56E-07 |
| $X_3^2$ | 1576.25 | 100.6908 | 15.65435 | 1.93E-05 |

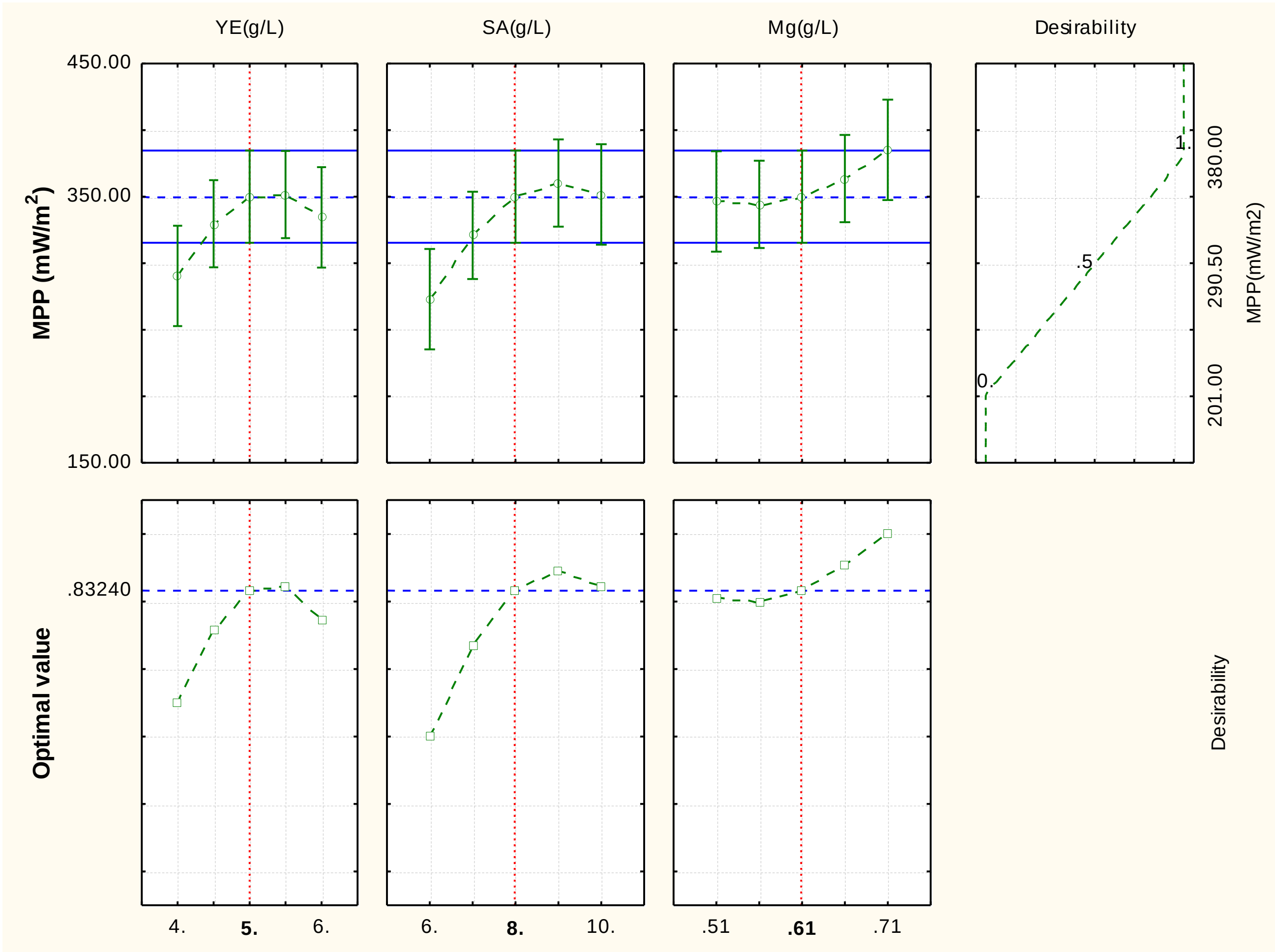


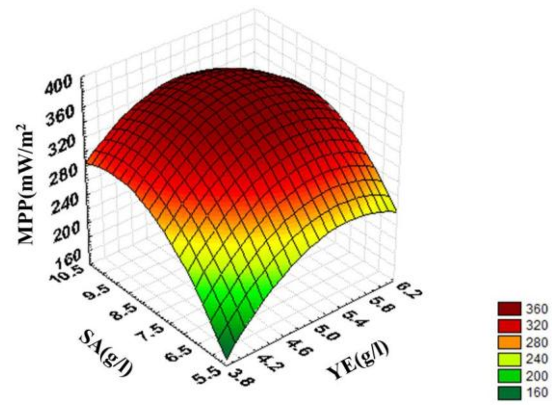

**Fig.3: Desirability and prediction profile for the MPP in Box-Behnken design.**

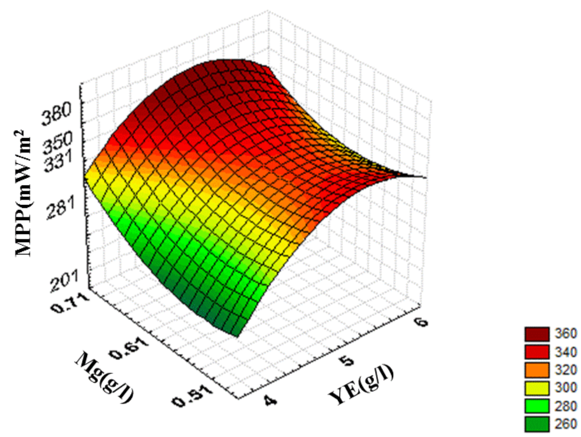
Normally, a regression model having an $R^2$ value higher than 0.9 is considered as having a very high correlation and a model with an $R^2$ value between 0.7 and 0.9 is considered as having a high correlation (**Guilford and Fruchter, 1973; Haaland, 1989)**. That means, in the present case, the coefficient of determination, i.e., $R^2$, was 0.9997. This means that 99.97% reflected a very good fit between the observed and predicted responses and it was reasonable to use the regression model to analyze the trends in the responses.

Using a confidence interval of 95%, the analysis suggested that, the factors that affected the response significantly were; yeast extract, sodium acetate ($P < 0.05$), square of yeast extract, sodium acetate, MgSO4.7$H_2O$ ($P < 0.05$), and the interaction between yeast extract and sodium acetate, yeast extract and $MgSO_4.7H_2O$, sodium acetate and $MgSO_4.7H_2O$ ($P < 0.05$). According to the polynomial equation, all these factors except the concentration of yeast extract and sodium acetate had a negative effect on power generation.

The three dimensional response surface plots are graphical representations of the regression equation. Higher responses were observed for high concentration of yeast extract and sodium acetate (Fig.4A), high concentration of yeast extract and $MgSO_4.7H_2O$ (Fig.4B); and high concentration of sodium acetate and low concentration of $MgSO_4.7H_2O$ (Fig.4C).

**(A)**




**(B)**

**(C)**

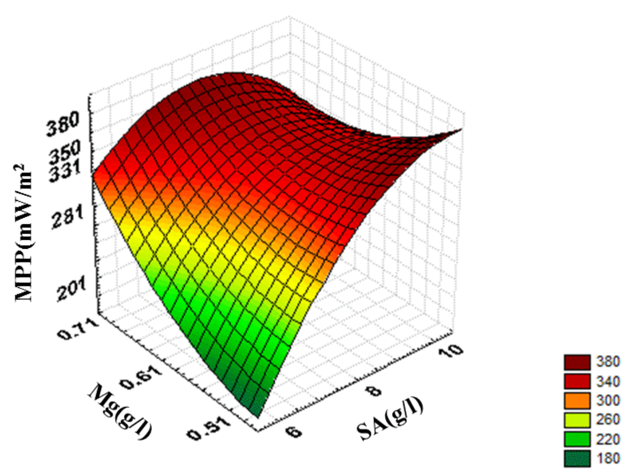



**Fig.4: Response surface of the interaction of (A) yeast extract and sodium acetate, (B) yeast extract and $MgSO_4.7H_2O$ and (C) sodium acetate and $MgSO_4.7H_2O$ on power generation.**

These trends in response surfaces suggested the use of a medium containing elevated concentrations of yeast extract, sodium acetate and low concentration of $MgSO_4.7H_2O$. It is important to understand why these three components were identified as critical factors for power generation, i.e., yeast extract, sodium acetate, and $MgSO_4.7H_2O$. The effect of yeast extract is likely because it is the source of nitrogen, which is very important for the cell, as it is an integral component of amino acids, proteins and nucleic acids**. Tiwari *et al*. (2018),** who tested the effect of four different nitrogen sources on power output of MFC, reported that the presence of any of the tested nitrogen sources, increased the electricity generation by almost the double, when compared with a cell running with no nitrogen source added.

The effect of sodium acetate is likely because carbon compounds are the sources of carbon skeleton and energy for bacterial cell growth and hence increased the power generation (**Ong and Yamagiwa, 2018**). **Webb (1951)** reported that in complex media, conditions of magnesium deficiencies or excess were found to inhibit the process of cell division, where as in simple chemically-defined media, these conditions predominantly limited growth (i.e. synthesis of cell substances). The optimum concentration of the three independent variables as obtained from the maximum point of the model were calculated using Microsoft Excel 2010 solver, and were found to be; yeast extract, 5.2 g/l; sodium acetate, 8.1 g/l and $MgSO_4.7H_2O$, 0.71 g/l. Response "Y" of MPP predicted by applying these levels is 386.74 $mW/m^2$.

**Verification of the optimized results**

According to Box-Behnken and Plackett- Burman experimental results, an optimum response for power generation is predicted with the following medium composition: PBS, 25mM; YE, 5.2 g/l; SA, 8.1 g/l; Mg, 0.71 g/l; VI, 1 ml; and MB, 240 µM. A confirmatory experiment for maximal power generation was performed under the predicted optimal condition, where MPP was estimated after 10 days of performing the MFC. A basal culture medium was used as a control. Under the optimized condition, the MPP was 390 $mW/m^2$. These results indicate that the optimized conditions accelerated the power generation and the MPP was about 91.2 % higher than that recorded with the basal condition (204 $mW/m^2$). Matching the predicted MPP (386.74 $mW/m^2$) and the observed one (390 $mW/m^2$) under optimal condition also proved the accuracy and validity of the model.

## CONCLUSION

Recent researches are focused on the use of the MFC technology for wastewater treatment while simultaneously generating electricity. If the performance of this technology was successfully improved to be applicable on a larger scale, it would not only save us time and energy, but also would help in the utilization of what would otherwise be considered as waste. Researchers are trying to make this technology applicable on a larger scale and are trying to enhance the power produced by the cell. If successfully applied on a large scale, microbial fuel cells would be of great promise especially in developing countries where water treatment, when available, is very limited due to economic and political issues. Other applications of microbial fuel cell technology include the use in powering implanted medical devices (e.g. the pace maker), and powering environmental sensors. MFCs can also be modified to produce valuable chemicals (e.g. hydrogen and caustic soda).

**Author of conflict:** There is no author of conflict

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