IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. III (Jul – Aug 2017), PP 43-59
www.iosrjournals.org

Using Box–Wilson statistical design to optimize biodegradation of phenanthrene and generation of sustainable power in microbial fuel cell simultaneously

*Alshehri A.N.Z.
Department of Biology, University college in Al-Jummum, Umm Al-Qura University, Makkah, 21955, Saudi Arabia.
Corresponding Author: Alshehri A.N.Z.

Abstract: *Microbial Fuel Cells (MFCs) are increasingly being used as a source of sustainable energy, as this technology can generate bioelectricity from the chemical energy present in organic pollutants. This potential was demonstrated in this study using the toxic and highly resistant polycyclic aromatic hydrocarbon by degrading as substrate in MFC. Optimization of the phenanthrene biodegradation and the resultant power generation in MFC was made by applying the Box-Wilson Statistical Design (BWSD) with the following six as parameters: (1) phenanthrene degradation, (2) voltage, (3) power density, (4) current density, (5) coulombic efficiency, and (6) COD removal. Optimization was achieved by conducting 23 experiments involving five independent operating factors: phenanthrene glucose concentration, temperature, pH value, NaCl concentration, and volume of inoculum. The levels of these factors, which were predicted to be 30-500mg/L, 35ºC, 6, 2% and 35mL respectively, resulted in increased phenanthrene degradation from 87%, to 100%, with improvement 115%. The voltage improved 226.3% from 292mV to 663mV, the power density improved 512% by increasing to 732.62mW/m^2 from 143.1mW/m^2, the current density by 231.3% from 0.43mAm^2 to 1.11mAm^2, the coulombic efficiency by 223.1% from 13% to 29%, and COD removal by 118.9% from 79% to 94%. The BWSD proved able to be applied efficiently for optimizing the phenanthrene biodegradation and generation of electricity from MFCs with regression coefficients for all factors exceeding 90%.*

Keyword: *Box–Wilson statistical design/ Biodegradation/ Microbial fuel cell, Phenanthrene/ Sustainable power.*

Date of Submission: 08-07-2017 *Date of acceptance: 22-07-2017*

I. Introduction

Petroleum based hydrocarbons pose a serious threat to the environment, thereby to health due to they are toxic, carcinogenic and mutagenic (Juana et al., 1998). Some approaches have been taken to clean the contaminants, such as chemicals oxidation, photo oxidation and use of dispersants (Morris & Jin, 2008), but these processes tend to be expensive, slow, environmentally unfriendly, and therefore ineffective. Biodegradation presents an environmentally friendly and cost effective way of removing pollution. However, aerobic degradation requires oxygen to be injected, which can be difficult and unsustainable (Zhang et al., 2010). Anaerobic degradation requires terminal electron acceptors (TEAs) to be present, such as metallic oxides, nitrate or sulphate, and is still a slow process. Furthermore, deploying TEAs presents problems, such as making them diffuse away from their point of application easily due to their high solubility in water as a result of hydrodynamic forces (Rozendal et al., 2008). Also, TEA depletion from anaerobic degradation imposes limitations on its rate, and it is unsustainable to provide a continuous supply due to their high maintenance and energy costs.

Microbial Fuel Cells (MFCs) are electrochemical devices with the capability to generate electricity from chemical energy by means of biocatalysts from exoelectrogenic bacteria (Liu and Logan, 2004; Patil et al., 2009; Alshehri et al., 2011; Alshehri et al., 2016). These cells have the unique property of being capable of extracellular electron transfer to solid materials, such as an anode electrode which can allow for continuous electron transfer to a cathode. Such electrodes are in inexhaustible supply, insoluble, and they can be exposed to the air to be consumed by oxygen as indirect TEAs, which tend to improve hydrocarbon degradation relative to degradation through anaerobic respiration. Although MFCs have been applied in treating wastewater along with concomitant generation of electricity (Hawkes et al., 2010), MFC technology also has the potential to be applied for treating more recalcitrant compounds, including petroleum hydrocarbons (Alshehri, 2015a; 2015b; 2015c). Previously, the occurrence of anaerobic biodegradation of petroleum hydrocarbons has been recorded to result from carboxylation following aromatic ring cleavage prior to entering a benzoate metabolic pathway (Meckenstock et al., 2005). Biotransformation products are formed during the degradation of organic pollutants such as phenanthrene, many of which are acidic metabolites that result from incomplete biodegradation due to hydrocarbon degrading microorganisms, such as naphthoic acid, salicylic acid, catechol, and 1-hydroxy-2-naphthoic acid (Foght, 2008; Tsai et al., 2009; Cooper et al., 2010).

In order to test for MFC robustness in treating recalcitrant pollutants, an investigation would be necessary of systemic response under different operating conditions with respect to concentration of the contaminant, temperature, pH value, salinity, and volume of inoculum. This may help to assess the feasibility of the system, as any change in these conditions could affect the characteristics of the microbial population in the anodic chamber of an MFC adversely (Guerrero et al., 2010). For instance, a high temperature condition may increase the rate of bio-kinetics, such as activation energy levels and their mass transfer coefficient, as well as that of substrate utilization and the system's thermodynamics generally. Conversely, a low temperature condition may inhibit methanogenic bacterial growth although it may be favorable for electrogenesis. Although sodium chloride may be added to increase salinity, this can decrease the internal resistance of MFCs, as the conductivity of the anodic solution would increase (Lefebvre et al., 2012). In addition, contradictory effects may be induced by increasing anolyte conductivity, which would affect the microbial population physiology adversely (Tehrani et al., 2009).

Notably, statistical methods have scarcely been used in previous studies for optimizing processes by controlling for other parameters than the one being investigated (Rao et al., 2000; Adour et al., 2008). It would be necessary to conduct multiple experiments for this purpose, which would be time consuming and it would not take into account effects of interaction between the different parameters thus leading to an insufficient level of efficiency for optimization. Circumventing such limitations would necessitate the implementation of a response surface methodology, which combines several statistical techniques in order to develop, improve and optimize processes for evaluating the relative significance of causative factors irrespective of the complexity of the interactions (Khayet et al., 2011). This methodology would involve adoption of a statistical design in which all factors can be varied collectively over the entire set of experiments by devising symmetric models and a polynomial equation to fit the data obtained, and thereby describe the behavioral characteristics of the independent factors (Bezerra et al., 2008).

The BWSD provides a highly analytical second-order symmetric model that can be applied in various scientific disciplines (Myers and Montgomery, 2002; Vargas et al., 2010; Auta and Hameed, 2011; Gan and Latiff, 2011; Prasad et al., 2011; Alshehri et al., 2013). In particular, it provides an empirical technique for modeling to evaluating relationships based on controlled conditions and taking into account the results obtained (Box and Wilson, 1951). As far as can be ascertained, the literature does not presently contain studies relating to investigating statistical optimization of phenanthrene biodegradation and electricity generation in MFC, involving Box–Wilson statistical design (BWSD). Therefore, the present study proposed adapting the BWSD for optimizing phenanthrene degradation in MFC. The relationship between responses: phenanthrene degradation (%), voltage (mV), power density (mW/m^2), current density (mA/m^2), coulombic efficiency (%) and COD removal (%), and five independent factors: phenanthrene – glucose concentration (mg/L), temperature (^oC), pH, NaCl concentration (%) and inoculum volume (mL/100mL), were investigated in this work.

II. Materials and methods

2.1 Chemicals

Phenanthrene (97.0% purity) was purchased from Acros (UK). All other chemicals used were obtained from Sigma Aldrich (UK). Chemical solvents including FicodoxPlusTM mixed chemical oxygen demand (COD) reagent were obtained from Fisher Scientific (Loughborough, UK). All chemicals were of analytical grade and used without further purification.

2.2. Microbial inoculum and culture medium

Two pure strains along with Anaerobic sludge were used as anodic biocatalysts (inoculum). Anaerobic sludge (AS) was obtained from Makkah Sewage Treatment Plant (KSA). The two pure strains as *Shewanellaoneidensis*(SO) and *Pseudomonas aeruginosa* (PA)were purchased from the German collection of microorganisms and cell cultures, Braunschweig, Germany. Anaerobic sludge, *S.oneidensis*and *P.aeruginosa*were grown anaerobically separately in mineral salts medium (MSM) supplemented with 100 mg/L of D-glucose and subsequently incubated at 30°C for 48h. The Mineral salts medium (MSM) was composed of (g/L of deionized water) 3 NH_4Cl, 0.5 KH_2PO_4, 0.5 $K_2HPO_4{\cdot}3H_2O$, 0.008 $MgSO_4{\cdot}7H_2O$, 0.002 $CuSO_4{\cdot}5H_2O$, 0.002$MnSO_4{\cdot}H_2O$, 0.002 $FeSO_4{\cdot}7H_2O$and 0.002 $CaCl_2{\cdot}2H_2O$. The pH was adjusted to 7.0 with either HCl or NaOH solutions. Culture medium was sterilized in an autoclave at 121^oC for 15 min. Mixing of the different inocula (AS, SO and PA) was done in a 1:1:1 ratio. Volume of the initial inoculum was 15 ml.

2.3. MFC set up and operation

Single – chamber air cathode MFCs were constructed as described previously (Liu and Logan, 2004) with some modification. Briefly, the anode and cathode were placed in parallel on the opposite side of the chamber (total volume is 200 mL, working volume is 100 mL) with distance of 5cm. Non – wet proofed carbon cloth (type A,E – TEK, Somerset, NJ, USA, 4cm^2) which was used as anode. Wet – proofed (30%) carbon cloth (type B, E – TEK, Somerest, NJ, USA, 10cm^2) was used as cathode pressed to proton exchange membrane (Nafion 117, Dupont CO., USA) on the water – facing side. The anode chamber was filled with anolyte medium (MSM) (pH 7.0). The MFCs were sterilized by autoclaving at 121^{o}C for 15 min, followed by addition of anolyte to the anode chamber which was done aseptically. All experiments conducted in this study were operated in fed-batch mode for 10 days. The MFCs were inoculated initially (before statistical optimization) with 15 mL of the mixed inoculum (AS, SO and PA). Anaerobic conditions were maintained in the anode chambers by purging them with 100% N_2 for 15 min before MFC operation began. The pH was adjusted by adding NaOH or HCl. The temperature of experiments were controlled using an incubator (LAB – LINE ® AMBI – USA). The net volume of the anolyte was 100 mL for each experiment. Immediately after adding the fuel and inoculum, MFCs were hooked up to a data acquisition system to start monitoring the voltage generation (150Ω). The controls in all sets of experiments were not seeded with inoculum.

2.4. Monitoring and calculation

Voltage was measured after the MFC has reached the steady state by a digital multimeter (Sanwa CD800a, Japan) was connected to a personal computer. Data was automatically recorded every second via Picolog software (Pico Technology Limited). The corresponding current was based on equation $I=E/R_{ext}$, where : I is current (mA) , E is voltage (mV) and R_{ext} is external resistance. The power (P) was obtained by $P=IE$. The current density and the power density have been normalized based on the projected surface area of the anode via equations $I_{An}= I/A_{An}$, where I_{An} is current density and A_{An} is the surface area of anode, $P_{An}=E^2/A_{An}R_{ext}$, where P_{An} is power density. The polarization curve was obtained at different external resistance (50 - 1000Ω). Internal resistance was derived from the polarization curve as the slope. Coulombic efficiency (CE) was derived from the equations $C_p=It$, $C_{max}=FfS_{COD}V_{An}$,and $CE=Cp/C_{max}$, where C_p is the coulombs of energy produced , t is the time of stable voltage output, C_{max} is the theoretical maximum coulombs , F is Faraday's constant (96.485 C/mol of electrons), $f$is a factor of 1mol electrons/8g COD, S_{COD} is substrate concentration g COD/L, and V_{An} is a net volume of anolyte (mL). The COD of the samples was estimated using the closed reflux titrimetric method as described in the Environment Agency (UK) Standard method 5220D (APHA Standard Methods for the Examination of Water and Wastewater, 20th ed.). Appropriately diluted 1mL samples were used for each determination. The COD of samples was expressed as percentage COD removal and COD removal rate. The percentageCODremoval wascalculated as follows:

PercentageCODremoval(%) = CODi– CODf/ CODi× 100 (1)

CODremovalrate(*mg/L /h*) = CODi–CODf / *t*(2)

Where:CODi and CODf are initial COD and final COD values respectively and *t* is time of theexperiment. All the experiments were replicated twice.

2.5. Phenanthrene analysis

Phenanthrene was analysed by HPLC (Dionex GS50, USA) using a Photo-diodeArray (PDA) detector (DIONEX,PDA-100) at 254 nm. The injected volume was 20 μL. The analytical column was a reversed phase column, SupelcosilTM LC-PAH column (150 mm × 4.6 mm). The mobile phase (80% acetonitrile and 20% deionized water) flow rate was 0.5 mL/min. The column oven temperature was set at a constant temperature of 25°C. The minimum detectable concentration for phenanthrene was 5 μg/L. Phenanthrene extraction procedures used were adopted from Kermanshahi pour et al. (2005) but with slight modification: 1 mL of aliquots were withdrawn at intervals from the MFC and transferred to 2 mL eppendorf tubes. Subsequently, 1 mL of methanol was added to make up to 2mL and the eppendorf tubes (which were placed in a 200 mL glass beaker) were incubated in an incubator shaker for 1 h at 25°C and 150 rpm. Thetubes were then centrifuged at 10000*g*for 10 min and 500 μL of the supernatant was carefully transferred into 1.5 mL HPLC glass vials prior to analysis by HPLC. In order to quantify the total amount of phenanthrene degraded, the amount of phenanthrene adsorbed on the anode was determined by soaking the anode electrodes in 20 mL methanol at the end of each experiment for 1 h at 200 rpm. Aliquots were transferred into 2mL eppendorf, immediately followed by centrifugation at 10000*g* for 10 min. All liquid samples were immediately analysed within few hours after sampling in order to minimize adsorption onto the wall of the sample vials. Degradation efficiencies and rates were determined based on the remaining phenanthrene in solution and that adsorbed on the anode at the end of MFC operation.

2.6. Box – Wilson design

Box–Wilson statistical design (BWSD) was used as an experimentaldesign. It provides further information with less number of experiments (Box and Wilson, 1951). In order to obtain required data, series of 23 experiments, including twelve factorial points, tenaxial points and one central points was done based on five independent factors,phenanthrene– glucose concentration (mg/L), temperature (^oC), pH, NaCl concentration (%) and inoculum volume (mL/100mL). STATISTICA computer software was used for the estimation of the coefficients of Equations (3) by regression analysis of the experimental data:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X^2_1 + b_{22}X^2_2 + b_{33}X^2_3 \quad (3)$$

Where:Y is the predicted yield, b_o the constant, b_1–b_3 the linear coefficients, b_{12}, b_{13}, and b_{23} the cross product coefficients, and b_{11}, b_{22}, and b_{33} are the quadratic coefficients.

BWSD with a full factorial was developed using STATISTICA software (Version 5.5, Stat-Soft Inc., USA). Each factor is varied over five levels: the high level (1), the low level (-1), the center points (coded level 0) and two outer points the very high (1.86) and the very low (-1.86), as shown on Table 1, (Tan et al., 2008; Ghani et al., 2010; Ghani et al., 2011). The data, which has been obtained by design BWSD, are then used in the optimization. In this study, the responses that have been measured were phenanthrenedegradation (%), voltage (mV), power density (mW/m^2), current density (mA/m^2), coulombic efficiency (%) and COD removal (%). The optimum condition for the fiveindependent factors, phenanthrene– glucose concentration (A), temperature (B), pH (C),NaCl concentration (D) and inoculum volume (E) were obtained using data from the statistical analysis. STATISTICA software searches for a combination of variables that simultaneously satisfy the requirements placed on each of the response and factors (Ghani et al., 2010).

III. Results And Discussion

3.1. Basal experiment

Performance of phenanthrenedegradation in a MFC operated in mode of fed batch was assessed, in terms of phenanthrenedegradation (%), voltage (mV), power density (W/m^2), current density (mA/m^2), coulombic efficiency (%) and COD removal (%). The MFC has been operated initially on a basal experiment (before the statistical optimization) at conditions, initial phenanthrene-glucose concentration 30-500mg/L (Phenanthrene taken from a 1000-fold concentrate in 100% methanol), temperature 25^oC, pH 7.0, NaCl 1% and inoculum volume 15mL/100mL . The methanol used in dissolving the phenanthrene (0.1% v/v of the working volume) is considered to be nontoxic since the concentration used is far below the minimum inhibitory concentration for microorganisms (Caldwell, 1989; Wadhwani et al., 2007). After 24 h following the inoculation of the anode in the MFC, voltage, power density, current density,phenanthrenedegradation and COD removal, were increased slightly to 12 mV, 0.24mW/m^2, 0.02 mA/m^2, 4.0 % and 0.9%, respectively. Subsequently the voltage, power density and current density recorded maximum values of 293mV, 143.1$mW/m^2$and 0.48 mA/m^2, respectively , after 192 h of the operation (Fig. 1). At the experiment end (240 h),the phenanthrene degradation, COD removal and coulombic efficiency, were87, 79 and 13%, respectively.

Good degradation efficiency of phenanthreneusing a MFC system in this study was obtained by anaerobic inoculum microbial consortia which have been employed in fedbatch operation. High degradation rates and removal efficiency observed in this study and previous study(Adelaja, 2014) could be imputed to the presence of aromatic degrading enzymes in the microbial consortia, as well as the availability of an insoluble electron acceptor in the anodic chamber. The presence of these enzymes could have encouraged raised cell metabolic rate that might have resulted into higher substrate consumption. The availability of the anode (as an electron acceptor) for microbial respiration continuously, andcoupled with substrate degradation give credence to its choice as a sustainable and affordable bioremediation technology. Oxidation of substrates by anaerobic microorganisms through different metabolic pathways (based on the nature of the substrate) has been well reported in the literature (Meckenstock et al., 2005; Foght, 2008). Generally, anaerobic degradationofaromatichydrocarbonsproceedsintwo steps;

Carboxylationofthearomaticring followed byringcleavage. The degradationpathwayprogressesuntilitentersintoacentral intermediate (i.e.thebenzoate)metabolicpathwaybeforefurther oxidation intoCO_2 finally (Coates et al., 2002). The electrochemicalperformances(interms of poweroutputandcoulombicefficiency)recordedinthisstudy are patter than a lot of previous worksthathave investigated thetreatmentofpetroleumhydrocarbonsinMFCs (Chandrasekhar and Mohan, 2012; Morris and Jin, 2012). In astudyontheanaerobicbiodegradationofdieselinMFCs, Morris etal. (Morris et al., 2009) reported maximumvoltageoutputof50–65mV but withimproveddegradationefficiencyof82%comparedtoan anaerobically incubatedcontrolcell(31%).Similarly,Wuetal. (2013) documented maximumpowerdensityof0.028to2.1mW/m^2 (using potassium ferricyanideascatholyte)ascatholyteconcentration was increasedfrom0to200mMrespectively; in contrast ,whole degradationofbenzene(21.74mg/L)wasaccomplished.Future work will be focused on improving electrochemical performances of MFC system with petroleum hydrocarbon as sole substrate. This will make this technology more suitable for use as preferred bioremediation approach in treatment of contamination with concomitant electricity generation.

3.2. Statistical analysis and optimization

The relationship between responses (phenanthrenedegradation, voltage , power density, current density, coulombic efficiency and COD removal) and five independent factors (phenanthrene– glucose concentration, temperature, pH, NaCl concentration and inoculum volume) were investigated in this study. The independent factors of designhave been suggested by the software (Table 2), and the experimental results have obtained at each point are shown in Table 2. The experimental sequence was randomized in order to minimize the effects of the uncontrolled factors (Halim et al., 2009). For the model fitted, the software generated model coefficients, R^2-values, F-values, and significant probabilities, and from these values the significance of each experimental factor can be justified. In this study, the responses and factors were fitted to each other by multiple regressions. Regression analysis is the general method to fit the empirical model with the collected response factor data (DC, 2001). The coefficients of the full regression model equation and their statistical significance were determined and evaluated using STATISTICA 5.5 software from State-Soft Inc. The final model in terms of coded value is given in Equations (4) - (9) for phenanthrenedegradation, voltage , power density, current density, coulombic efficiency and COD removal.

$$Y_{Phenanthrene\text{-}Degr.(\%)} = 37.21 – 44.57A – 8.32B – 0.73C + 63.92D +11.03E+11.19A^2 + 3.24B^2 + 5.03C^2+ 0.27D^2 + 1.16E^2 +3.92AB + 0.11AC +10.06BC + 30.34DE \quad (4)$$

$$Y_{Voltage\ (mV)} = 97.35 – 63.57A – 28.76B – 9.02C + 01.54D +29.54E+ 62.01A^2 + 06.51B^2 + 53.82C^2+ 88.14D^2 + 25.16E^2 + 0.72AB + 0.31AC +45.27BC + 90.43DE \quad (5)$$

$$Y_{power\ density\ (mW/m^2)} = 63.10+ 7.35A + 9.72B - 1.67C - 0.32D -1.13E - 7.08A^2 - 0.19B^2 + 5.52C^2 +5.07D^2 + 3.68E^2+1.93AB\ 7.59AC - 1.84BC + 10.01DE \quad (6)$$

$$Y_{current\ density\ (mA/m^2)} = 72.81+04.71A +63.91B – 20.43C + 69.02D +23.23E+ 02.13A^2 + 03.54B^2 + 05.93C^2+ 80.57D^2 + 71.13E^2 + 30.02AB + 39.11AC + 15.36BC + 0.11DE \quad (7)$$

$$Y_{coulombic\ efficiency\ (\%)} = 42.13 +72.34A - 23.09B +7.31C +6.02D +0.41E + 0.02A^2+ 0.74B^2 - 0.26C^2 -2.61D^2 + 9.00E^2 +6.38AB - 1.17AC + 0.27BC+45.25DE \quad (8)$$

$$Y_{COD\ removal(\%)} = 17.24 – 35.50A + 27.12B – 9.70C + 22.14D +51.53E+ 81.10A^2 + 22.14B^2 + 15.16C^2+ 1.07D^2 + 56.16E^2 + 86.23AB + 92.21AC +37.16BC + 41.74DE \quad (9)$$

Where: Y is the response, and A, B, C, D and E are the coded terms for the fivefactors that has been selected, i.e.phenanthrene– glucose concentration (A), temperature (B), pH (C), NaCl concentration (D) and inoculum volume (E). The positive sign in front of each term represents synergistic effect, while antagonistic effect represented by the negative sign.

The statistical analysis of the models (based on BWSD) according to the obtained data from Table 2 and, it is shown in Fig. 2A, B, C, D, E and F, it revealed that the optimized new levels of the independent factors, phenanthrene– glucose concentration, temperature, pH, NaCl concentration and inoculum volume, were, 30-500 mg/L, 35^{o}C, 6.0, 2.0% and 35mL/100mL , respectively, where the BWSD, predicted that the values of responses would be recorded the highest levels as phenanthrenedegradation 100 %, voltage 671mV, power density 750.40 mW/m^2, current density 1.11 mAm^2, coulombic efficiency 31 % and COD removal 97 %.

Fig. 3 A, B, C, D, E and F, is showing the relationships between the response surface plot for phenanthrenedegradation (%), voltage (mV), power density (mW/m^2), current density (mA/m^2), coulombic efficiency (%) and COD removal (%)as a function of the independent factors: phenanthrene– glucose concentration (A), temperature (B), pH (C), NaCl concentration (D) and inoculum volume (E). By moving along the X and Y axes, it could be demonstrated that keeping of the phenanthrene– glucose concentration at initial level (30-500 mg/L), have a positive effect on the responses (phenanthrenedegradation, voltage, power density, current density, coulombic efficiency and COD removal). While increasing of the levels of temperature, pH, NaCl concentration and inoculum volume, have a conspicuous positive effect on results of the responses. As a result, the stationary ridge shape was observed on the surface plot of the five operating conditions.All the variablesplayed major effects to all the responses investigated in this study.

Analysis of variance (ANOVA) was used to assess the goodness of fit. The significant quadratic models and the corresponding significant model term for the most main three responses (phenanthrenedegradation, power density and coulombic efficiency) are tabulated in Tables 3-5.

From Table 3, the model F-value of 126.26, implies that the model is significant. Interestingly with respect to the phenanthrenedegradation. From Table 4, the model F-value of 142.76, implies that the model is significant with respect to the power density.F-value of 136.21 that has been shown in Table 5, implies that the model is significant for the response of coulombic efficiency.

In order to determine the fit of the model, the regression equation and the determination coefficient (R^2) were estimated. For the response of phenanthrenedegradation, the value of determination coefficient (R^2 = 0.9472) indicates that the sample variation of 94.72% for phenanthrenedegradation is imputed to the independent factors, and only 5.28% of the total variation could not be illustrated by the model. The value of adjusted determination coefficient (Adj R^2 = 0.9183) is also very high to advocate for a high significance of the model. Meanwhile for the response of power density, the value of determination coefficient (R^2 = 0.9701) indicates that the sample variation of 97.01% for power density is imputedto the independent factors, and only 2.99% of the total variation could not be illustratedby the model. The value of adjusted determination coefficient (Adj R^2 = 0.9206) is also very high to advocate for a high significance of the model. On the other hand, the response of coulombic efficiency, the value of determination coefficient (R2 = 0.9587), indicates that the sample variation of 95.87% for coulombic efficiency is imputedto the independent factors, and only 4.13% of the total variation could not be illustratedby the model. The value of adjusted determination coefficient (Adj R^2 = 0.9345) is also very high to advocate for a high significance of the model.

The correlation between experimental values and predicted values of phenanthrenedegradation, voltage, power density, current density,coulombic efficiency and COD removal, are shown in Fig. 4 A, B, C, D, E and F, respectively. A higher value of the correlation coefficient for all responses justifies an excellent correlation between the independent factors (Ghani et al., 2010).

3.3. Confirmatory experiment

In order to verify the optimization results, an experiment was performed under the predicted optimal conditions (phenanthrene– glucose concentration30-500 mg/L, temperature 35°C, pH 6.0, NaCl concentration 2.0% and inoculum volume 35mL/100mL). The responses were phenanthrenedegradation, voltage, power density, current density, coulombic efficiency and COD removal. The basal operating conditions(before applying the optimization process of the BWSD)were used as a control experiment (phenanthrene-glucose concentration 30-500mg/L, temperature 25°C, pH 7.0, NaCl 1% and inoculum volume 15mL/100mL). The optimized operating conditions, which have been obtained by BWSD, recorded maximum levels of the responses asphenanthrenedegradation 100%, voltage 663mV, power density 732.62mW/m^2, current density 1.11mA/m^2, coulombic efficiency 29% and COD removal 94%.

Results of the confirmatory experiment showed obviously, massive optimization in values of the responses versus the basal experiment. Generation of the voltage was optimized, from 293mV (in the basal experiment) to 663mV (in the confirmatory experiment)as 226.3% optimization. In addition the power density and current density from 143.1 mW/m^2 and 0.48 mA/m^2 to 732.62mW/m^2 and 1.11mA/m^2, as 512% and 231.3% optimization, respectively. As well, the BWSD through the confirmatory experiment accomplished improvement in the phenanthrenedegradation, coulombic efficiency and COD removal, from 87, 13 and 79% (in the basal experiment) to 100, 29 and 94%, respectively.These results reflect the accuracy and applicability of the BWSD model as an extremely powerful method for optimization processes.

Increasing of the temperature until 35°C was positively affected in the responses, that may be because of the high temperature reduced the internal resistance via increasing of the movement of molecules, as well as raising of oxygen reaction and protons on the cathode, that have positive effect on protons exchange via the membrane (Khanal, 2008).

pH is critical for all microbial based processes, In MFCs, pH not only affects bacterial metabolism and growth, but also affects the proton transfer, the cathode reaction, and thus the MFC performance (Khanal, 2008). Most MFCs are operated at range 6.0 – 7.0 pH to maintain optimal growth conditions for microbial communities involved in electricity generation(Logan, 2008). Gil et al. (2003) reported maximum power generation at pH 6.0 in a two-chamber MFCs using a mixed culture enriched from activated sludge.

The positive effect of the new level of NaCl, it could be interpreted,ionic strength of the anolytemolecules was increased, which caused, raising in protons exchange throughmembrane of MFC, and reducing in the possibility of oxygen permeability from the cathode to the anode, all this led to the reduction of internal resistance and increasing of potential difference between the electrodes.

By increasing the inoculum volume to 35mL/100mL , the responses have recorded highest values. This result may be because of increasing population of microbial consortium in the anolyte.

The BWSD in the current work achieved optimization, in the phenanthrenedegradation, voltage, power density, current density, coulombic efficiency and COD removal, as 115, 226.3, 512, 231.3, 223.1 and 118.9%, respectively, of the basal experiment.These results are promising and similar to the number of previous studies (Niessen et al. 2004;Kim et al. 2007; Huang et al. 2008; Zhu and Ni 2009; Chae et al. 2009; Zhang et al. 2009 ; Antonopoulouet al. 2010 ; Luo et al. 2010).

The statistical optimization has been successfully applied in many recent biotechnological applications (Al-Ahmadi et al., 2008; Lopes et al., 2008; Akhir et al., 2009; Ghanem et al., 2009; Alalayah et al. 2010;

Ghanem et al., 2011a; 2011b; Alshehri et al., 2013; Alshehri, 2015c; 2015d; Alshehri et al., 2016). However to the best of our knowledge no single report was obtained on using Box–Wilson statistical design to optimizebiodegradation of phenanthrene and generation of sustainable power in microbial fuel cell simultaneously.

IV. Conclusions

The present study succeeded in using MFC technology to generate clean and renewable bioelectricity from a recalcitrant pollutant compound as phenanthrene. MFC technology can be a green and sustainable solution for the global energy crisis, and it can help decrease carbon dioxide emissions, and environmental pollution to help slow climate change. In this work, for the first time, the biodegradation of phenanthrene, and performance of MFC reactor through the role of microorganisms,were improved by optimization of operating conditions(five independent factors: phenanthrene– glucose concentration, temperature, pH, NaCl concentration and inoculum volume) using Box–Wilson statistical design (BWSD). The responses as phenanthrenedegradation, voltage, power density, current density,coulombic efficiency and COD removal, were increased significantly, and this method was found to be reliable and accurate. However, more research is required to optimize MFC performance, especially in terms of biodegradation of pollutants and power generation, to make MFC technology more attractive and applicable.

Table 1: Levels of the investigated factors in the Box–Wilson statistical design.

Factors	Levels					
	Symbol	-1.86	-1	0	1	1.86
Phenanthrene-glucose (mg/L)	(A)	10+50	20+250	30+500	40+1000	50+1500
Temperature (°C)	(B)	10	15	25	35	45
pH	(C)	5.0	6.0	7.0	8.0	9.0
NaCl (%)	(D)	0.1	0.5	1.0	2.0	3.0
Inoculum volume (mL/100mL)	(E)	5.0	10	15	25	35

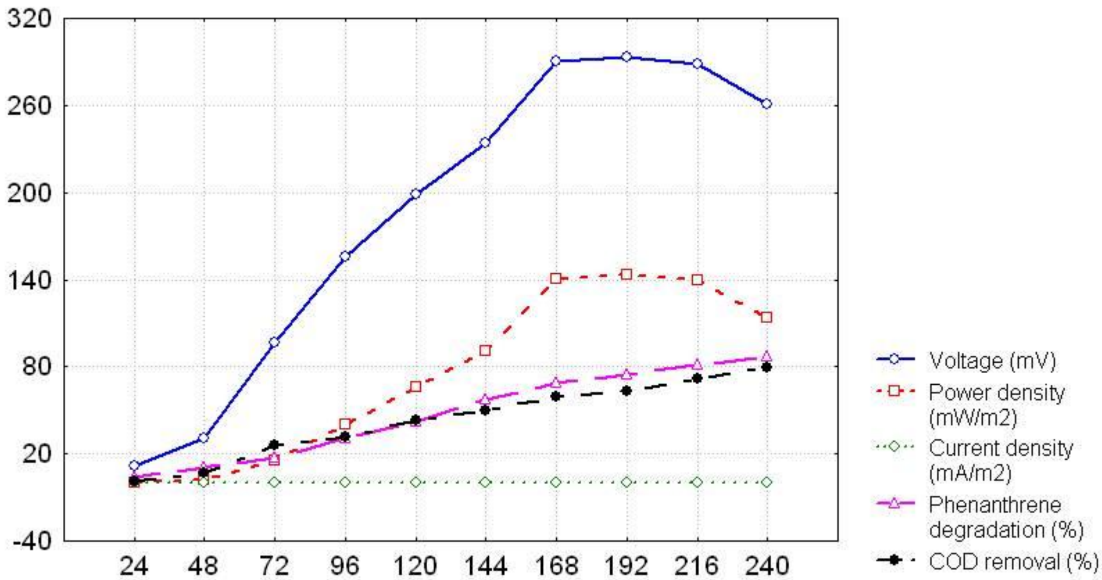
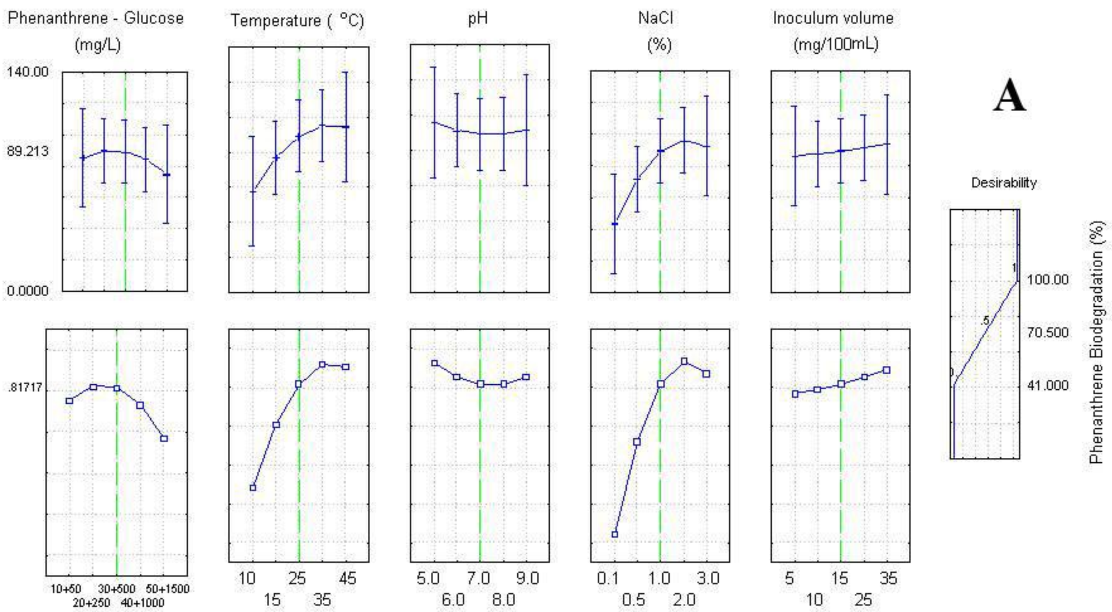
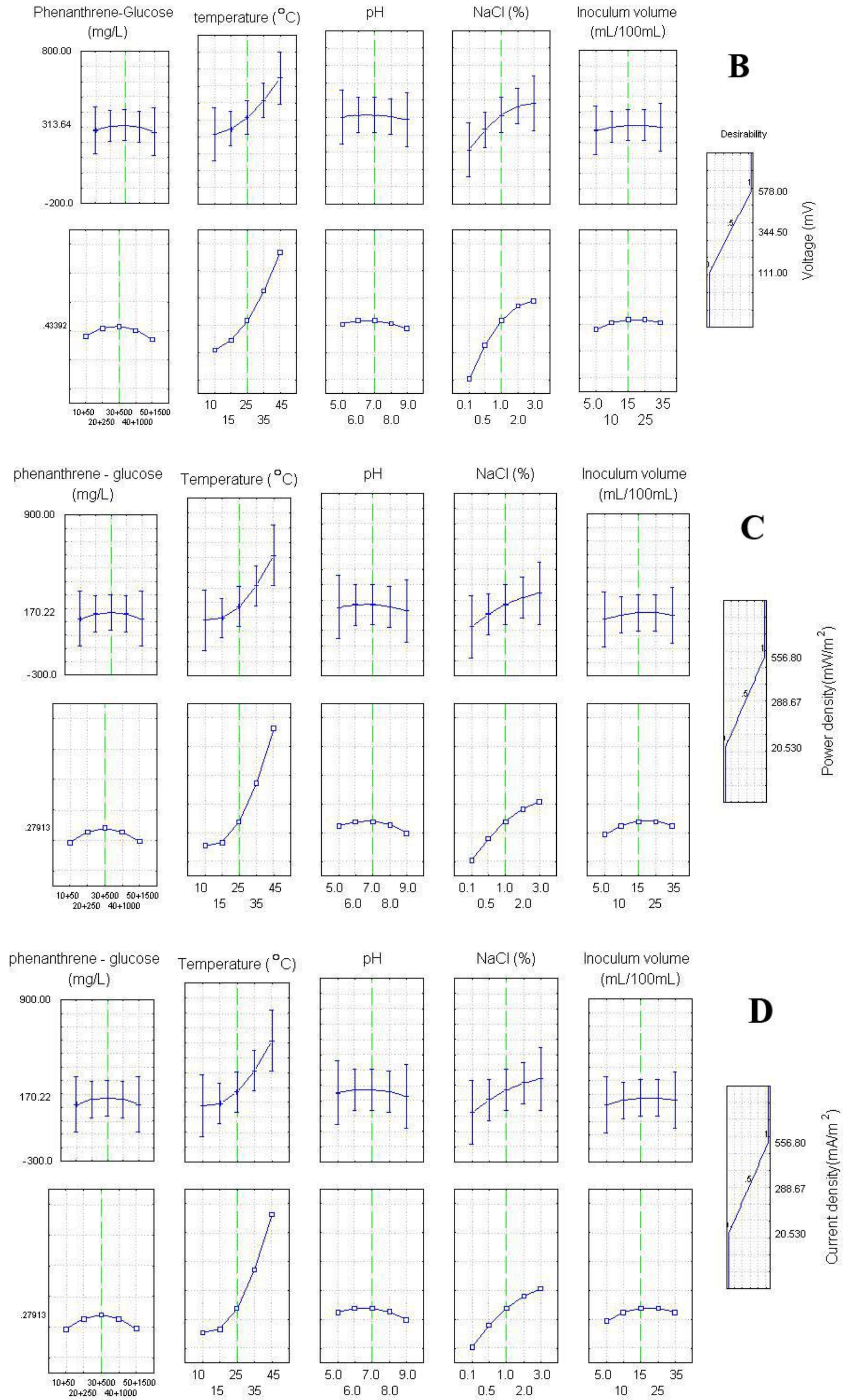


Fig. 1: Time course of voltage (mV), power density (mW/m^2), current density (mA/m^2), phenanthrenedegradation (%) and COD removal (%), which have been produced on the basal experiment in MFC.

Table 2: The Box–Wilson statistical designfor five independent factors: phenanthrene– glucose concentration (A), temperature (B), pH (C), NaCl concentration (D) and inoculum volume (E). And the experimental results for the phenanthrenedegradation(PD), voltage(VO), power density(POD), current density(CD), coulombic efficiency(CE) and COD removal(COD).

	Factors						The experimental results					
Run	A	B	C	D	E		PD (%)	VO (mV)	POD (mW/m^2)	CD (mA/m^2)	CE (%)	COD (%)
1	1.00	1.00	-1.00	1.00	1.00		42.0	196.0	64.02	.32	6.0	23.0
2	1.00	-1.00	1.00	1.00	1.00		51.0	211.0	74.20	.35	11.0	36.0
3	-1.00	1.00	1.00	1.00	-1.00		93.0	413.0	284.28	.68	17.0	87.0
4	1.00	1.00	1.00	-1.00	-1.00		76.0	236.0	92.82	.39	12.0	61.0
5	1.00	1.00	-1.00	-1.00	-1.00		91.0	277.0	127.88	.46	14.0	83.0
6	1.00	-1.00	-1.00	-1.00	1.00		63.0	167.0	46.48	.27	5.0	27.0
7	-1.00	-1.00	-1.00	1.00	-1.00		77.0	143.0	34.08	.23	5.0	51.0
8	-1.00	-1.00	1.00	-1.00	1.00		81.0	187.0	58.28	.31	13.0	42.0
9	-1.00	1.00	-1.00	1.00	1.00		91.0	391.0	254.80	.65	21.0	82.0
10	1.00	-1.00	1.00	1.00	-1.00		94.0	503.0	421.68	.83	22.0	85.0
11	-1.00	1.00	1.00	-1.00	1.00		86.0	402.0	269.34	.67	19.0	71.0
12	-1.00	-1.00	-1.00	-1.00	-1.00		83.0	273.0	124.21	.45	10.0	55.0
13	-1.86	0.00	0.00	0.00	0.00		91.0	311.0	161.20	.51	14.0	73.0
14	1.86	0.00	0.00	0.00	0.00		72.0	267.0	118.81	.44	8.0	61.0
15	0.00	-1.86	0.00	0.00	0.00		55.0	210.0	73.50	.35	7.0	43.0
16	0.00	1.86	0.00	0.00	0.00		100.0	578.0	556.80	.96	23.0	91.0
17	0.00	0.00	-1.86	0.00	0.00		93.0	299.0	149.00	.49	13.0	72.0
18	0.00	0.00	1.86	0.00	0.00		97.0	317.0	167.48	.52	20.0	61.0
19	0.00	0.00	0.00	-1.86	0.00		41.0	111.0	20.53	.18	4.0	23.0
20	0.00	0.00	0.00	1.86	0.00		98.0	412.0	282.90	.68	21.0	90.0
21	0.00	0.00	0.00	0.00	-1.86		92.0	310.0	160.16	.51	15.0	83.0
22	0.00	0.00	0.00	0.00	1.86		91.0	299.0	149.00	.49	11.0	67.0
23 (C)	0.00	0.00	0.00	0.00	0.00		87.0	293.0	143.10	.48	13.0	79.0




B
Phenanthrene-Glucose (mg/L)
temperature (°C)
pH
NaCl (%)
Inoculum volume (mL/100mL)
Desirability
Voltage (mV)
578.00
344.50
111.00
800.00
313.64
-200.0
.43392
C
phenanthrene - glucose (mg/L)
Temperature (°C)
Power density(mW/m2)
556.80
288.67
20.530
900.00
170.22
-300.0
.27913
D
Current density(mA/m2)
10+50 20+250 30+500 40+1000 50+1500
10 15 25 35 45
5.0 6.0 7.0 8.0 9.0
0.1 0.5 1.0 2.0 3.0
5.0 10 15 25 35

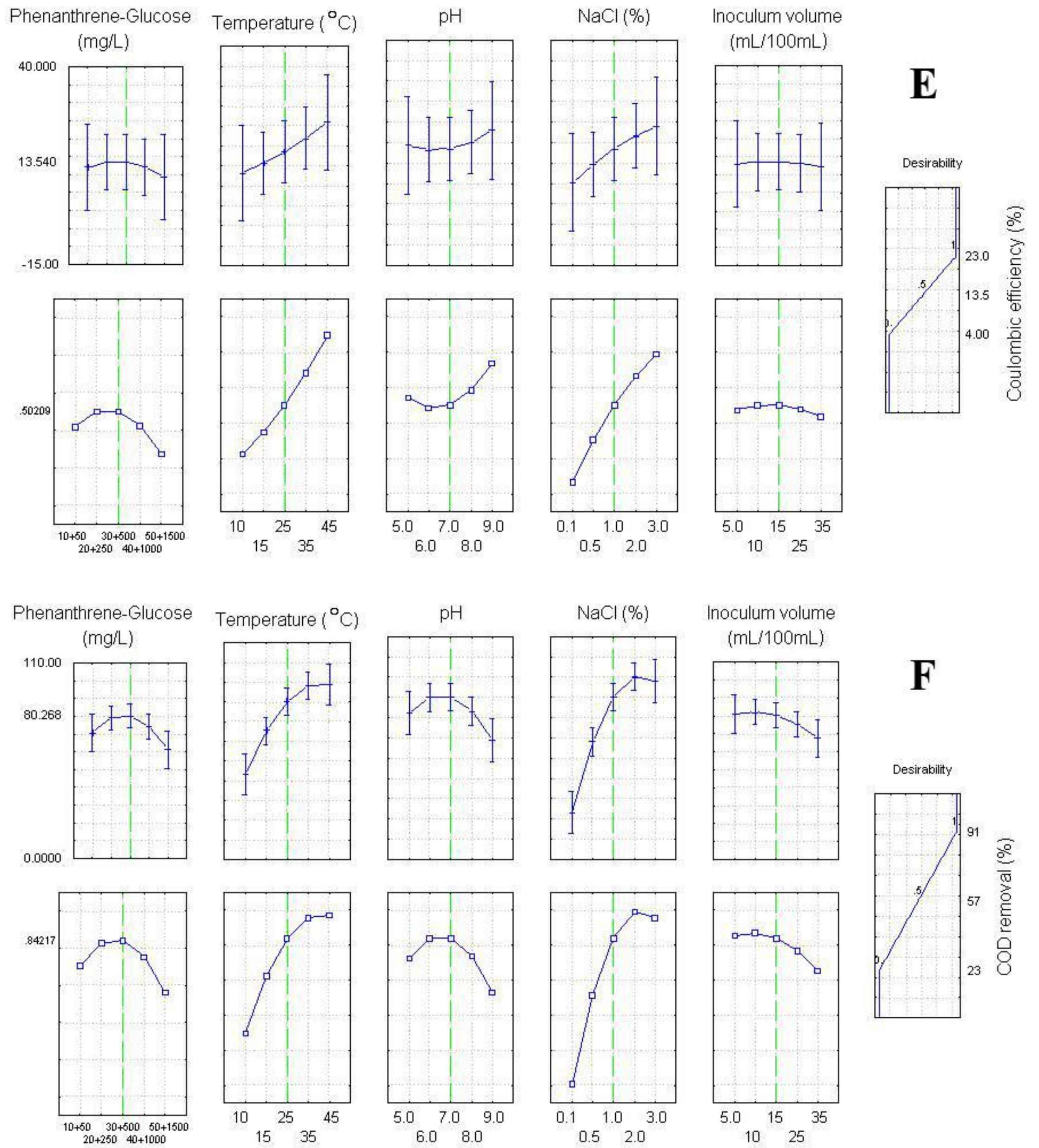
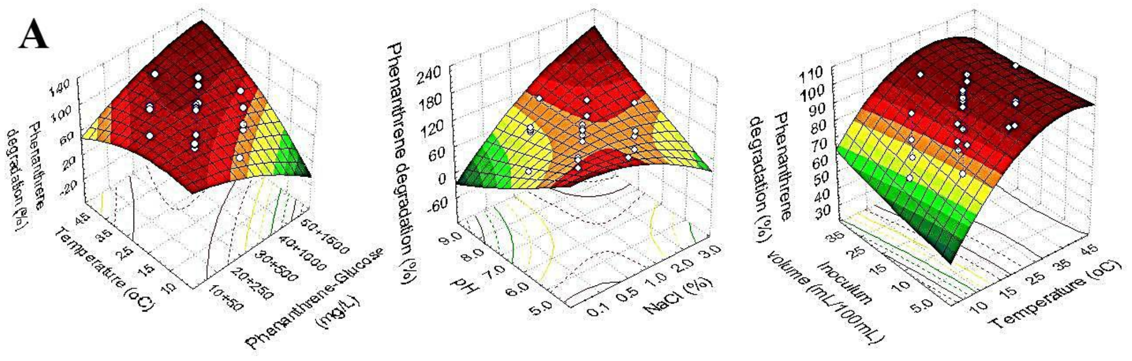


Fig. 2. The relationship between the responses (phenanthrenedegradation - A, voltage - B, power density - C, current density - D, coulombic efficiency -E and COD removal - F), and the different independent factors, showing the predicted optimal values based on the Box – Wilson statistical design results.



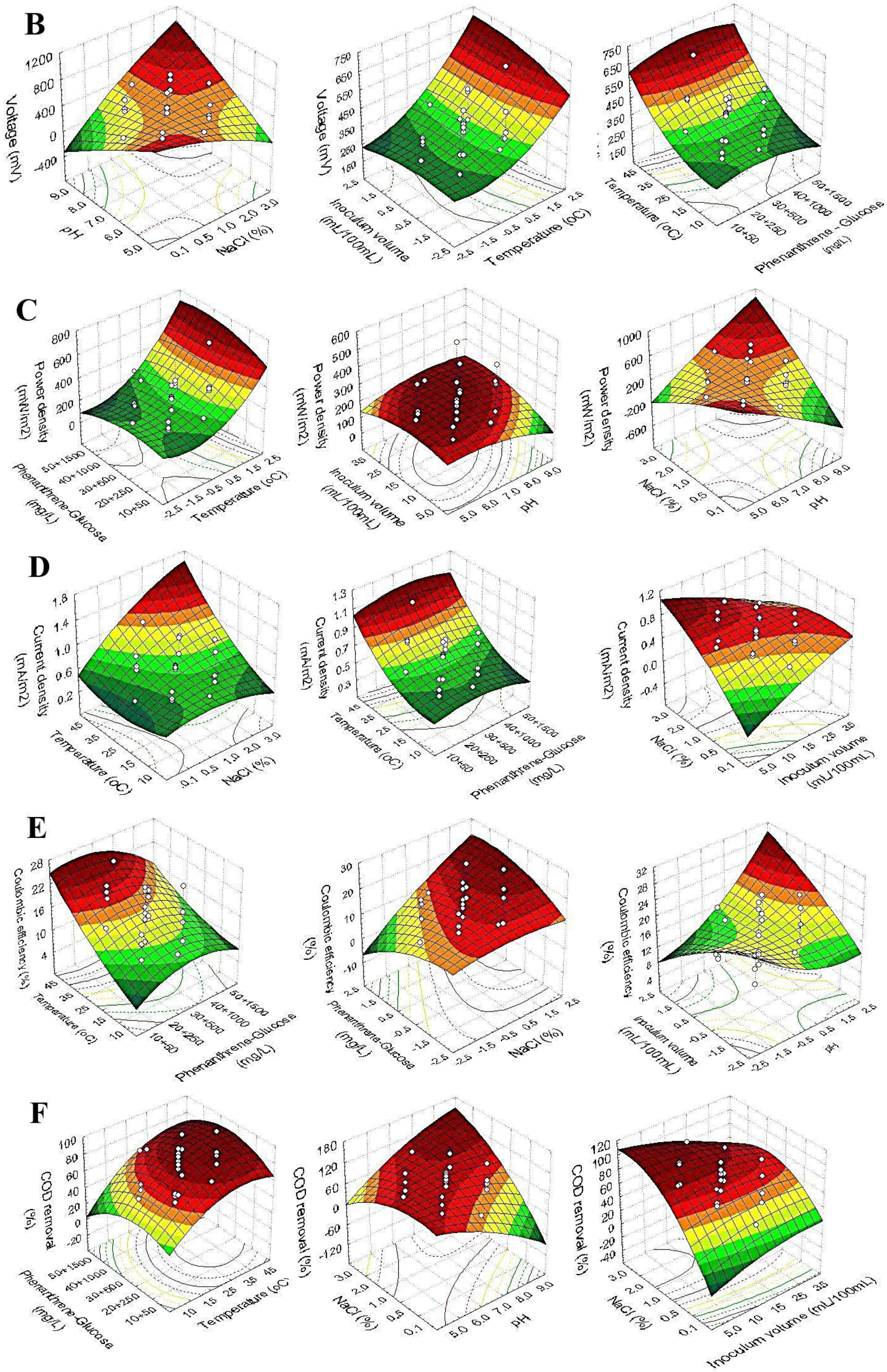


Fig. 3. Countour plot of the responses: phenanthrenedegradation - A, voltage - B, power density - C, current density - D, coulombic efficiency - E and COD removal - F, showing interaction between the different independent factors(phenanthrene– glucose concentration, temperature, pH, NaCl concentration and inoculum volume).

Table 3: Analysis of variance (ANOVA) for phenanthrenedegradation.

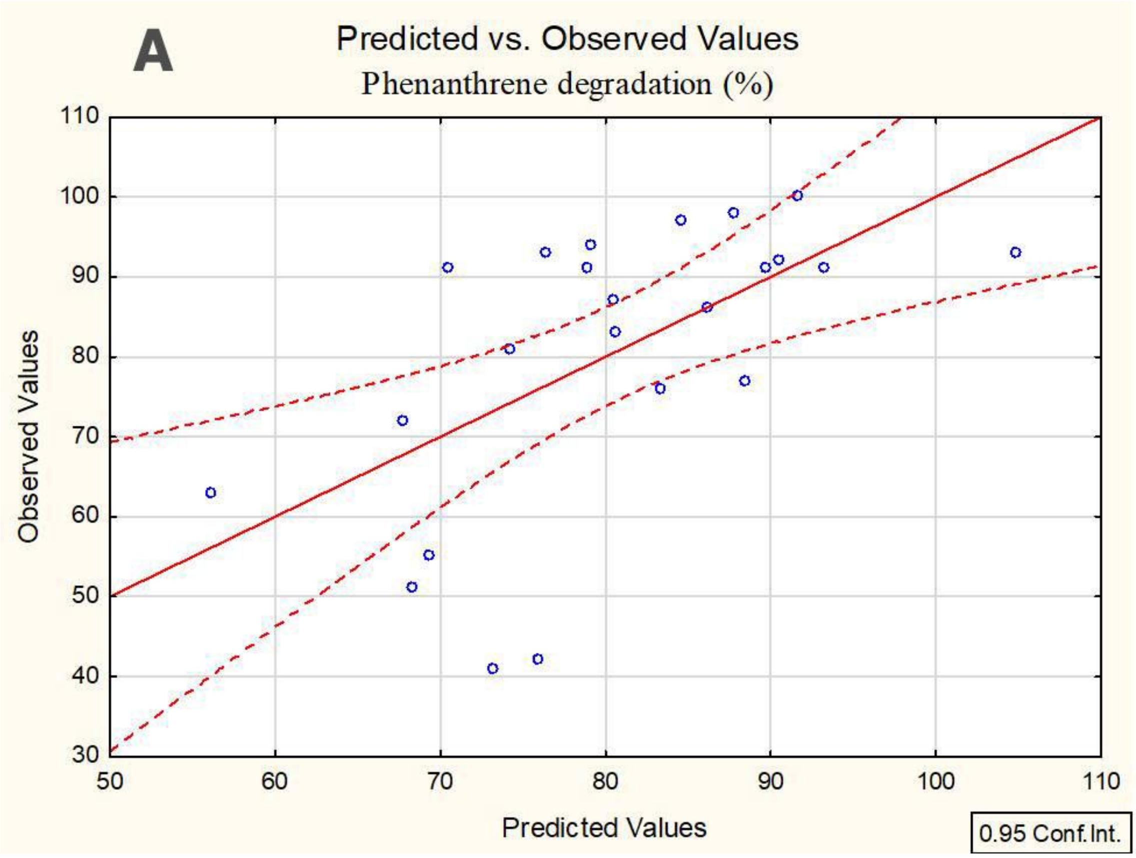
Source	Sum of squares	Degree of freedom	Mean of square	*F*-value	Prob>*F*	Remarks
Model	1761.11	9.1	181.01	126.26	<0.0001	Significant
Residual	16.72	9.4	1.19			
Cor total	1013.13	19.3				

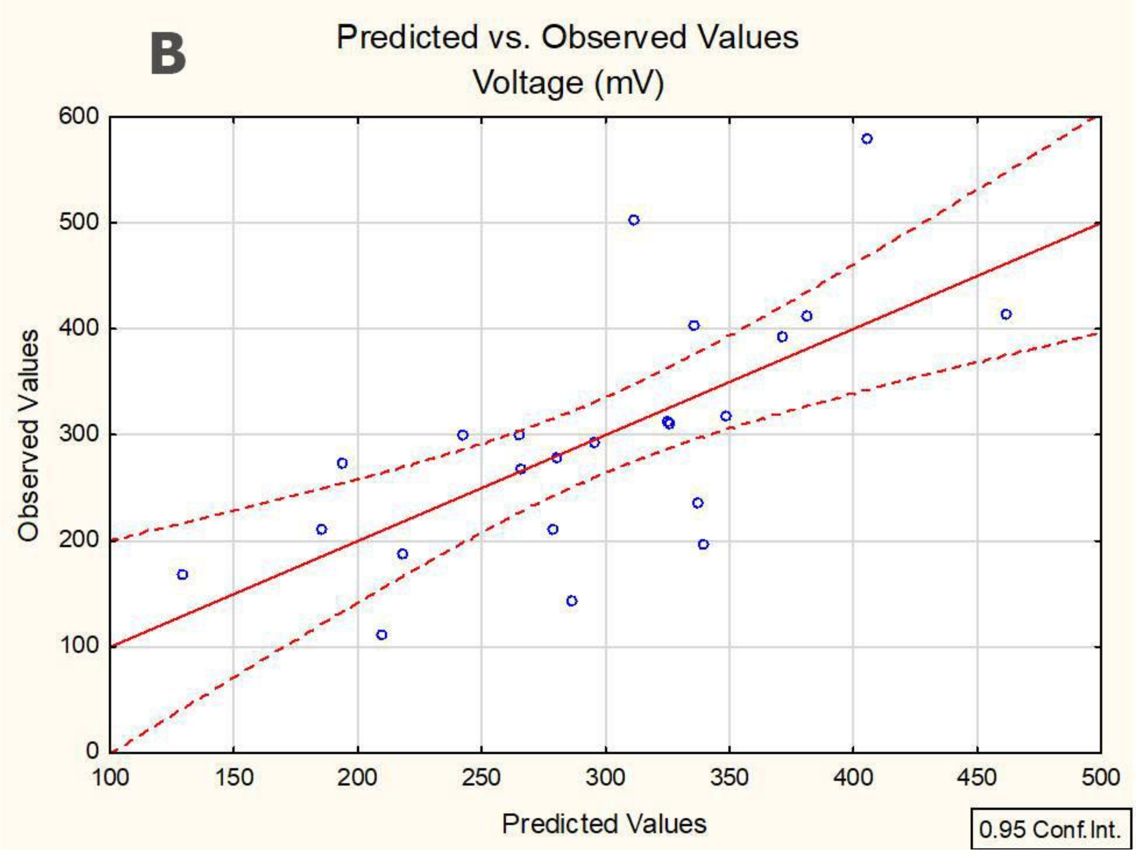
Table 4: Analysis of variance (ANOVA) for power density.

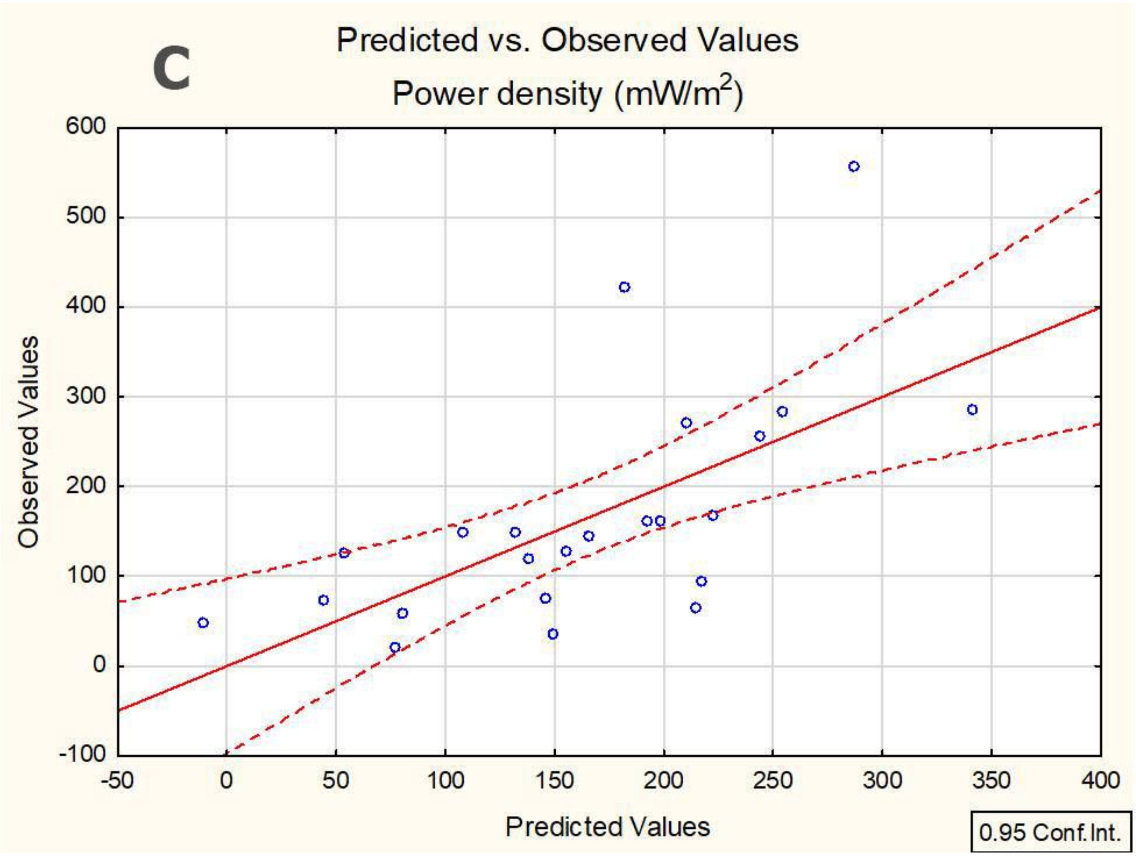
Source	Sum of squares	Degree of freedom	Mean of square	*F*-value	Prob>*F*	Remarks
Model	148.20	9.6	39.02	142.76	<0.0001	Significant
Residual	6.29	9.2	3.11			
Cor total	227.82	19				

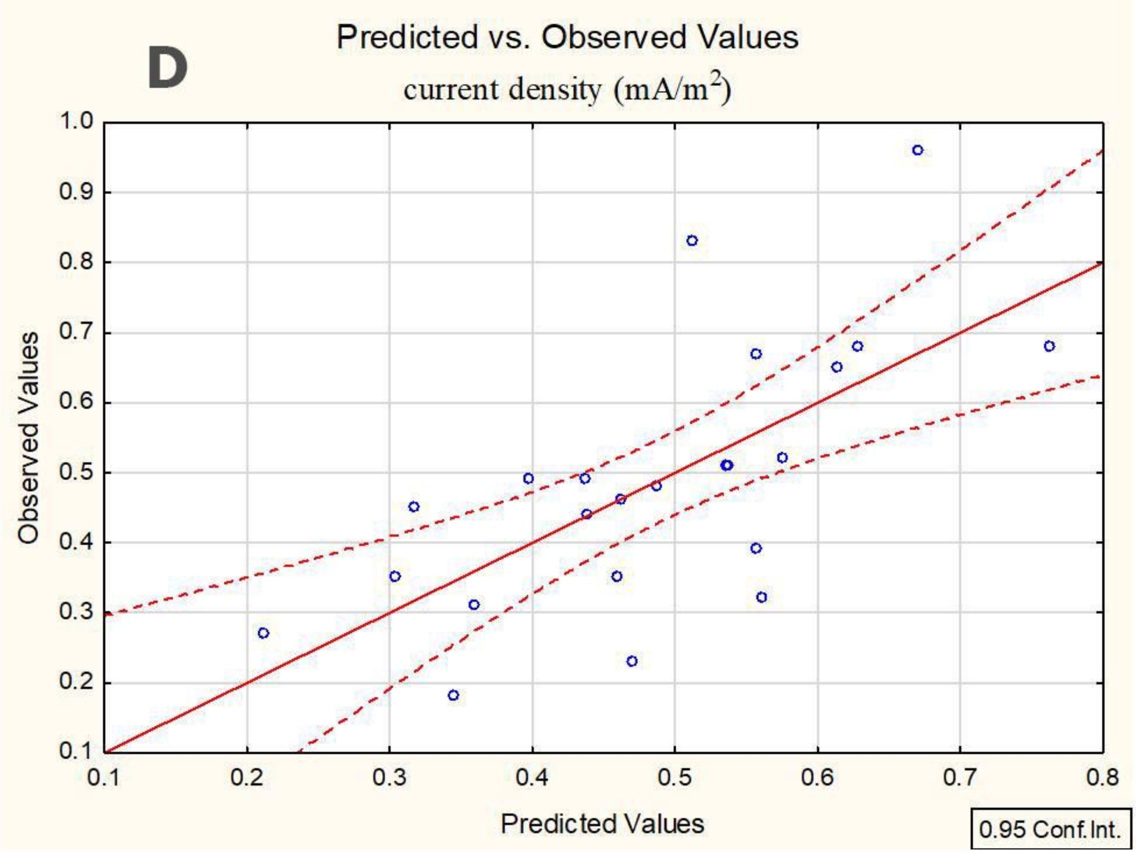
Table 5: Analysis of variance (ANOVA) for coulombic efficiency.

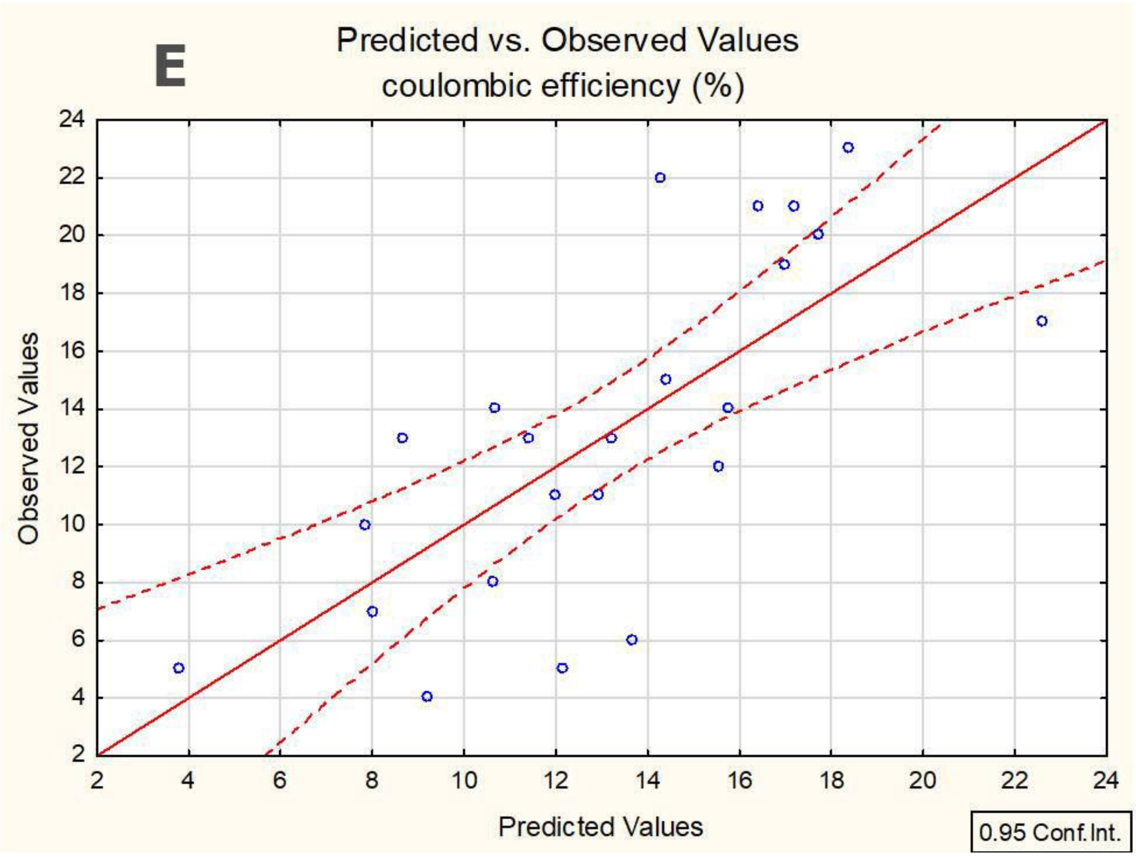
Source	Sum of squares	Degree of freedom	Mean of square	*F*-value	Prob>*F*	Remarks
Model	3940.77	9.8	536.97	136.21	<0.0001	Significant
Residual	109.31	9.1	91.10			
Cor total	1742.64	19				




B
Predicted vs. Observed Values
Voltage (mV)
Observed Values
Predicted Values
0.95 Conf.Int.
600
500
400
300
200
100
0
100
150
200
250
300
350
400
450
500


C
Predicted vs. Observed Values
Power density (mW/m2)
Observed Values
Predicted Values
0.95 Conf.Int.
600
500
400
300
200
100
0
-100
-50
0
50
100
150
200
250
300
350
400


D
Predicted vs. Observed Values
current density (mA/m2)
Observed Values
Predicted Values
0.95 Conf.Int.


E
Predicted vs. Observed Values
coulombic efficiency (%)
Observed Values
Predicted Values
0.95 Conf.Int.

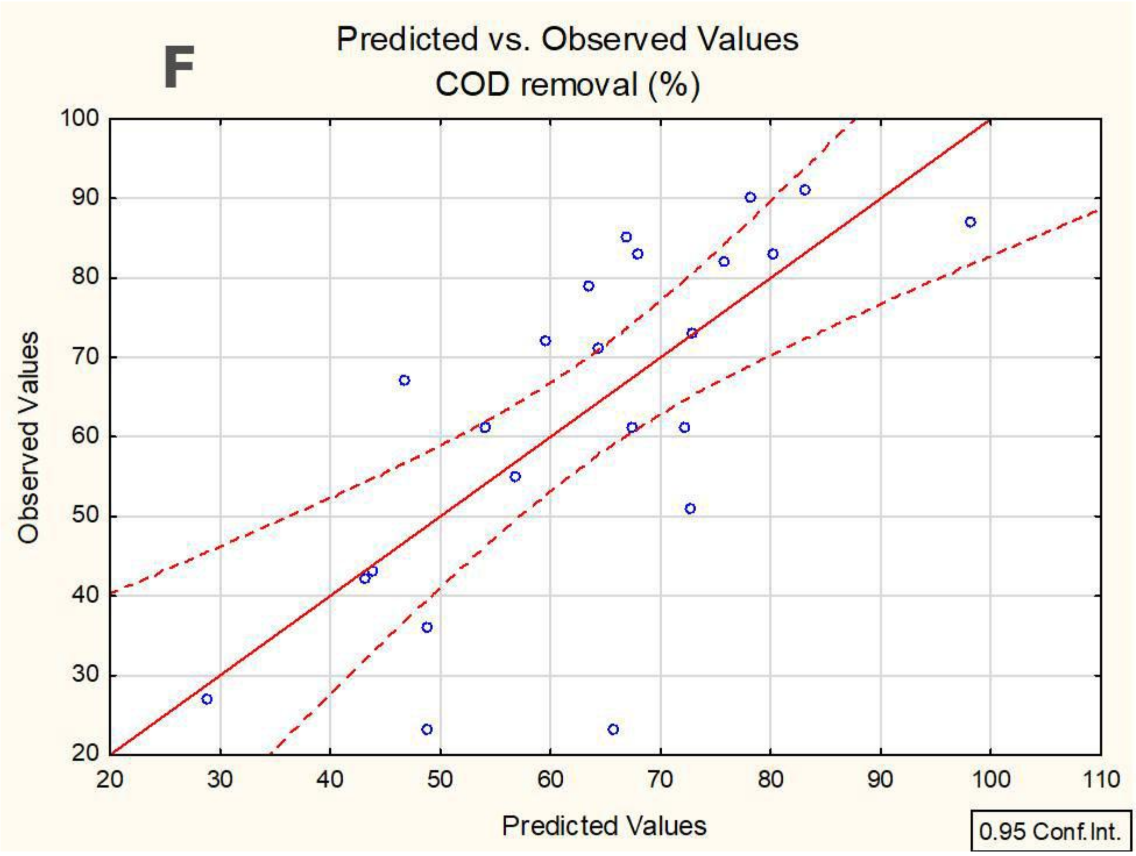


Fig. 4. Parity plot showing observed vs. predicted values for modeled responses (phenanthrenedegradation - A, voltage - B, power density - C, current density - D, coulombic efficiency - E and COD removal – F).

References

[1]. Adelaja O.A., Keshavarz T., Kyazze G. (2014) Enhanced biodegradation of phenanthrene using different inoculum types in a microbial fuel cell, Eng. in Life Sci. 14: 218–228.

[2]. Adour L., Arbia W., Amrane A., Mameri N. (2008) Combined use of waste materials-recovery of chitin from shrimp shells by lactic acid fermentation supplemented with date juice waste or glucose. J. Chem. Tech. Biotech. 83: 1664-1669.

[3]. Akhir S.M., Abd-Aziz S., Salleh M.M., Rahman R.A., Illia R.M., Hassan M.A. (2009) Medium optimization of chitinase enzyme production from shrimp waste using *Bacillus licheniformis*TH-1 by response surface methods. Biotechnol. 8(1): 120-125.

[4]. Al-Ahmadi K.J., Yazdi M.T., Najafi M.F., Shahverdi A.R. (2008) Optimization of medium and cultivation conditions for chitinase production by the newly isolated *Aeromonas*sp. Biotechnol. 7(2): 266-272.

[5]. Alalayah W.M., Kalil M.S., Kadhum A.A.H., Jahim J., Zaharim A., Alauj N.M., El Shafie A. (2010) Applications of the Box-Wilson Design model for bio-hydrogen production using *Clostridium saccharoperbutylacetonicum* N1-4(ATCC13564). Pak. J. of Biol. Sci. 13(14): 674 – 682.

[6]. Alshehri A.N.Z. (2015a) Generation of renewable power from biodegradation of anthracene in a microbial fuel cell reactor using different bacterial inocula. Int J ApplSciBiotechnol. 3(2): 151-161.

[7]. Alshehri A.N.Z. (2015b) Employment of microbial fuel cell technology to biodegrade naphthalene and benzidine for bioelectricity generation. Int. J. Curr. Micobiol. App. Sci. 4(1): 134-149.

[8]. Alshehri A.N.Z. (2015c) Statistical optimization of pentachlorophenol biodegradation and electricity generation simultaneously in mediator - less air cathode microbial fuel cell. J. Environ. Appli. Biores. 3(1): 06-15.

[9]. Alshehri A.N.Z. (2015d) Employing a central composite rotatable design to define and determine significant toxic levels of heavy metals on *Shewanellaputrefaciens* in microbial fuel cell. Arab. J. Sci. Eng. 40(1):93–100.

[10]. Alshehri A.N.Z., Ghanem K.M., Algarni S.M. (2011) A comparative study for electricity generation in microbial fuel reactor with and without mediators. Sci. Res. Essays 6(29): 6197 – 6202.

[11]. Alshehri A.N.Z., Ghanem K.M., Algarni S.M. (2013) Statistical optimization of medium components to enhance bioelectricity generation in microbial fuel cell. Arab. J. Sci. Eng. 38:21–27.

[12]. Alshehri A.N.Z., Ghanem K.M., Algarni S.M. (2016) Application of a five level central composite design for optimization of operating conditions in microbial fuel cell. J. Taibah. Univ. Sci. 10: 797–804

[13]. Antonopoulou G., Stamatelatou K., Bebelis S., Lyberatos G. (2010) Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell. Biochem. Engine. J. 50: 10 – 15.

[14]. Auta M., Hameed B.H. (2011) Optimized waste tea activated carbon for adsorption of Methylene Blue and Acid Blue 29 dyes using response surface methodology. Chem. Engin. J. 175: 233–243.

[15]. Bezerra M.A., Santelli R.E., Oliveira E.P., Villar L.S., Escaleira L.A. (2008) Response surface methodology (RSM) as a tool for optimization in analytical. Talanta. 76: 965–977.

[16]. Box G.E.P., Wilson K.B. (1951) On the experimental attainment of optimum conditions. J. Royal Stat. Soc. Ser. 13(1): 1 – 38

[17]. Caldwell D. R. (1989) Effects of methanol on the growth of gastrointestinal anaerobes. Can. J. Microbiol. 35: 313–317.

[18]. Chae K.J., Choi M.J., Lee J.W., Kim K.Y., Kim I.S. (2009) Effect of different substrate on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. Bioresou. Technol. 100: 3518 – 3525.

[19]. Chandrasekhar K., Mohan S.V. (2012) Bioelectrochemical remediation of real field petroleum sludge as an electron donor with simultaneous power generation facilitates biotransformation of PAH: Effect of substrate concentration. Bioresour. Technol. 110: 517–525.

[20]. Coates J.D., Chakraborty R., McInerney M.J. (2002) Anaerobic benzene biodegradation a new era. Res. Microbiol. 153: 621–628.

[21]. Cooper E.M., Heather M.S., Cole W.M., Richard T.D., Andrew J.S. (2010) Ultraviolet treatment and biodegradation of dibenzothiophene: identification and toxicity of products. Environ. Toxicol. Chem. 29: 2409–2416.

[22]. DC M. (2001)Design and analysis of experiment. 5ed. New York: John Wiley and Sons.

[23]. Foght J. (2008) Anaerobic biodegradation of aromatic hydrocarbons: pathways and prospects. J. Mol. Microbiol. Biotechnol. 15: 93–120.

[24]. Gan C.Y., Latiff A.A. (2011) Optimization of the solvent extraction of bioactive compounds from Parkiaspeciosa pod using response surface methodology. Food Chem. 124: 1277–1283.

[25]. Ghanem K.M., Al-fassi F.A., Farsi R.M. (2011a) statistical optimization of cultural conditions for chitinase production from shrimp shell fish waste by *Alternaria alternate*. Afr. J. microbial. Res. 5(13):1649 -1659.

[26]. Ghanem K.M., Al-Garni S.M., Al-Shehri A.N. (2009) Statistical optimization of cultural conditions by surface methodology for phenol degradation by a novel *Aspergillusflavus*isolate. Afr. J. Biotechnol. 8(15): 3576-3583.

[27]. Ghanem K.M., Al-garni S.M., Biag A.K. (2011b.) Statistical optimization of cultural conditions for decolorization of methylene blue by mono and mixed bacterial consortium. Afr. J. microbial. Res. 5(15): 2187 – 2197.

[28]. Ghani Z.A, Khamil I.N.M., Isa M.R.M, Jamaluddin M.A., Ishak M.A.M., Ismail K. (2011) Pyrolysis of Jatrophacurcas l. Husk: optimization solid, liquid and gas yield by using response surface methodology (RSM). In: Proceedings of 3rd international symposium & exhibition in sustainable energy & environment (ISESEE). The Research and Innovations in Sustainable Energy (RISE) Center of Institute of Science (IOS) UniversitiTeknologi MARA (UiTM).

[29]. Ghani Z.A., Ishak M.A.M., Ismail K. (2010) Direct liquefaction of MukahBalingianlowrank Malaysian coal: optimization using response surface methodology. Asia Paci. J. Chem. Engine. 6: 581 - 588.

[30]. Gil G.C., Chang I.S., Kim B.H., Kim M., Jang J.K., Park H.S., Kim H.J. (2003) Operational parameters affecting the performance of a mediator-less microbial fuel cell. BiosonserBioelecton. 18: 327 – 334.

[31]. Guerrero A.L., Scott K., Head I.M. (2010) Effect of temperature on the performance of microbial fuel cells, Fuel 89: 3985–3994.

[32]. Halim S.F.A., Kamaruddin A.H., Fernando W.J.N. (2009) Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: optimization using response surface methodology (RSM) and mass transfer studies. Bioresou. Techno. 100: 710 -716.

[33]. Hawkes F.R., Kim J., Kyazze G., Premier G.C., Guwy A. (2010) Feedstocks for BES conversions, in: K. Rabaey, L.T. Angenent, U. Shroeder, J. Keller (Eds.), Bioelectrochemical Systems: From Extracellular Electron Transfer to Biotechnological Application, IWA Publishing, London. pp. 369–388.

[34]. Huang L., Zeng R.J., Angelidaki I. (2008) Electricity production from xylose using a mediator-less microbial fuel cell. Bioresou. Technol. 99: 4178 – 4184.

[35]. Juana B.E., Sarina J.E., Daniel P.Y.C., Edward D.S. (1998) Bioremediation Principles McGraw. Hill publisher. pp. 122-135.

[36]. Kermanshahi pour. A., Karamanev D., Margaritis, A. (2005) Biodegradation of petroleum hydrocarbons in an immobilized cell airlift bioreactor. Water Res. 39: 3704–3714.

[37]. Khanal S.K. (2008) Anaerobic biotechnology for bioenergy production: principles and application. John Wiley and Sons, Inc. ISBN: 978 – 0 – 813 – 82346 – 1.

[38]. Khayet M., Zahrim A.Y., Hilal N. (2011) Modelling and optimization of coagulation of highly concentrated industrial grade leather dye by response surface methodology. Chem. Eng. J. 167: 77-83.

[39]. Kim J.R., Jung S.H., Regan J.M., Logan B.E. (2007) Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. Bioresou.Technol. 98: 2568 – 2577.

[40]. Lefebvre T., Zi K., Shailesh Y., Ng How. (2012) Effect of increasing anodic NaCl con- centration on microbial fuel cell performance. Bioresour. Technol. 112: 336–340.

[41]. Liu H., Logan B.E. (2004) Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane. Environ. Sci. Technol. 38:4040 – 4046.

[42]. Liu H., Logan B.E. (2004) Electricity generation using an air–cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. Environ. Sci. Technol. 38: 4040–4046.

[43]. Logan B.E. (2008) Microbial Fuel Cells. John Wiley and Sons. New Jersey.

[44]. Lopes M.A., Gomes D.S., Bello-Koblitz M.G., Pirovani C.P., Cascardo J., Goes-Neto A., Michell, F. (2008) Use of response surface methodology to examine chitinase regulation in the basidiomycete*Moniliophthoraperniciosa*. Mycol. Res. 112: 399-406.

[45]. Luo Y., Zhang R., Liu G., Li J., Li M., Zhang C. (2010) Electricity generation from indole and microbial community analysis in the microbial fuel cell. J. Haz. Mat. 176: 759 – 764.

[46]. Meckenstock R.U., Michael K.J., Stefan B.H. (2005) Anaerobic degradation of benzene, toluene, ethylbenzene, and oxylene in sediment-free iron-reducing enrichment cultures. Appl. Environ. Microbiol. l71: 3355–3358.

[47]. Morris J.M., Jin S. (2008) Feasibility of using microbial fuel cell technology in bioremediation of hydrocarbons in groundwater. J. Environ. Sci. Health Part A. 43: 18–23.

[48]. Morris J.M., Jin S. (2012) Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells. J. Hazard. Mater. 474–477.

[49]. Morris J.M., Jin S., Crimi B., Pruden A. (2009) Microbial fuel cell in enhancing anaerobic biodegradation of diesel. Chem. Eng. J. 146 161–167.

[50]. Myers R.H., Montgomery D.C. (2002) Response surface methodology: Process end product optimization using designed experiments. 2nd ed. John Wiley and Sons, New York.

[51]. Niessen J., Schroder U., Scholz F. (2004) Exploiting complex carbohydrates for microbial electricity generation a bacterial fuel cell operating on starch. Electroch. Com. 6: 955 – 958.

[52]. Patil S.A., Surakasi V.P., Koul S., Ijmulwar S., Vivek A., Shouche Y.S., Kapadnis B.P. (2009) Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. Bioresour. Technol. 100: 5132–5139.

[53]. Prasad K.N., Hassan F.A., Yang B., Kong K.W., Ramanan R.N., Azlan A. (2011) Response surface optimization for the extraction of phenolic compounds and antioxidant capacities of underutilized MangiferapajangKosterm. Peels. Food Chem. 128: 1121–1127.

[54]. Rao M.S., Mu_noz J., Stevens W.F. (2000) Critical factors in chitin production by fermentation of shrimp biowaste. Appl. Micro. Biotech. 54: 808-813.

[55]. Rozendal R., Hamelers H.V.M., Rabaey K., Keller J., Buisman C.J.N. (2008) Towards practical implementation of bioelectrochemical wastewater treatment, Trends in Biotechnol. 26: 450–459.
[56]. Tan I.A.W., Ahmad A.L., Hameed B.H. (2008) Optimization of preparation conditions for activated carbons from coconut husk using response surface methodology. Chem. Engine. J. 137:462 - 470.
[57]. Tehrani D.M., Minoui S., Herfatmanesh A. (2009) Effect of salinity on biodegrada- tion of polycyclic aromatic hydrocarbons (PAHs) of heavy crude oil in soil. Bull. Environ. Contam. Toxicol. 82: 179–184.
[58]. Tsai J.C., Kumar M., Lin J.G. (2009) Anaerobic biotransformation of fluorene and phenanthrene by sulfate-reducing bacteria and identification of biotransformation pathway. J. Hazard. Mater. 164: 847–855.
[59]. Vargas A.M.M., Garcia C.A., Reis E.M., Lenzi E., Costa W.F., Almeida V.C. (2010) NaOH – activated carbon from flamboyant (Delonixregia) pods: Optimization of preparation conditions using central composite rotatable design. Chem. Engin. J. 162: 43–50.
[60]. Wadhwani T., Desai K., Patel D., Lawani D. (2009) Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. Int. J. Microbiol. 7: 1.
[61]. Wu C.H., Lai C.Y., Lin C.W., Kao M.H. (2013) Generation of power by microbial fuel cell with ferricyanide in biodegradation of benzene. Clean Soil Air Water 41: 390–395.
[62]. Zhang C., Mingchen L., Guangli L., Haiping L., Renduo Z.(2009) Pyridine degradation in the microbial fuel cells. J. Haz. Mate. 172: 465–471.
[63]. Zhang T., Gannon S. M., Nevin K. P., Franks A. E. (2010) Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. Environ.Microbiol. 12: 1011–1020.
[64]. Zhu X., Ni J. (2009) Simultaneous processes of electricity generation and *p*-nitrophenol degradation in a microbial fuel cell. Electroch. Com. 11: 274 – 277.

Alshehri A.N.Z. "Using Box–Wilson statistical design to optimize biodegradation of phenanthrene and generation of sustainable power in microbial fuel cell simultaneously." IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 12.4 (2017): 43-59.