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Research article

Optimization of co-culture inoculated microbial fuel cell performance using response surface methodology

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ABSTRACT

Microbial fuel cells (MFCs) are considered as promising technology to achieve simultaneous wastewater treatment and electricity generation. However, operational and technological developments are still required to make it as a sustainable technology. In the present study, response surface methodology (RSM) was used to evaluate the effects of substrate concentration, co-culture composition, pH and time on the performance of co-culture (*Klebsiella variicola* and *Pseudomonas aeruginosa*) inoculated double chamber MFC. From the statistical analysis, it can be seen that the performance of MFC was not influenced by the interaction between the initial COD and time, pH and time, pH and initial COD, time and initial COD. However, the interaction between the inoculum composition and time, pH and the inoculum composition, initial COD and inoculum composition significantly influenced the performance of MFC. Based on the RSM results, best performance (power density and COD removal efficiency) was obtained when the inoculum composition, initial COD, pH and time were about 1:1, 26.690 mg/L, 7.21 and 15.50 days, respectively. The predictions from the model were in close agreement with the experimental results suggesting that the proposed model could adequately represent the actual relationships between the independent variables generating electricity and the COD removal efficiency.

1. Introduction

The exhaustion of fossil fuels and excessive wastewater generation are driving intensive efforts towards developing new technologies for the sustainable treatment and utilization of wastewater (Karim et al., 2018; Yousuf et al., 2017). The production of electricity from wastewater using microbial fuel cell (MFC) can offer a solution for both the energy and clean water demands (Islam et al., 2017b; Xu et al., 2018). MFC is considered as an emerging technology where the microbes are used to convert chemical energy in wastewater to electricity through the microbial metabolism (Islam et al., 2016; Zhou et al., 2018). Several crucial factors influence the performance of MFCs such as physical (configurations, electrode material, etc.), chemical (oxidizing agent) and biological (microbial community) etc. (Estrada-Arriaga et al., 2018; Ortiz-Martínez et al., 2015). In last few years, a number of studies have been conducted to optimize the afore-mentioned aspects (Pendyala et al., 2016; Wang et al., 2015a). However, the development of MFC in terms of practically applicable energy recovery from wastewater fed MFCs is still limited due to some bottleneck issues.

Until now, in MFCs, the common sources of inocula are the laboratory grown pure cultures or environmentally derived mixed consortia (Chang et al., 2017; Islam et al., 2017b; Sotres et al., 2016). In MFCs, it is common for investigators to use pure cultures of electrochemically active species, presumably for experimental clarity, but recently, there has been a move towards using mixed, undefined cultures derived from environmental samples such as wastewater and marine sediments to inoculate MFCs (Islam et al., 2017a). For large scale application of MFCs, mixed cultures particularly anaerobic sludge (AS) are generally preferred as inoculum source because they are readily available in bulk quantities and are more tolerant to environmental conditions (Nimje et al., 2009). However, in most cases, the mixed cultures driven MFCs could not be able to achieve significant performance because, the methanogens that are ubiquitously present in the AS suppresses the growth of electrogenic bacteria (Rajesh et al., 2015). Moreover, the inter-microbial interactions can be synergistic, antagonistic or neutral but it is practically impossible to identify them

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because there are numerous microorganisms in the AS (Nimje et al., 2009). It is important to note that the synergistic interactions within the microbes especially which are involved in the electron transfer could positively influence the performance of MFCs (Wang et al., 2015b) whereas the antagonistic interactions between the bacteria may negatively influence the performance of MFCs.

Therefore, in recent years, selective co-culture or mixed culture inoculums have been renewed attention in researchers community since it could solve the above mentioned difficulties (Islam et al., 2018a). For instance, Venkataraman et al. (2011) reported that the fermentation product (2,3-butanediol) produced by *Enterobacter aerogens* stimulated the production of mediator by *P. aeruginosa* that boosted up the current density by 14-fold in co-culture MFC compared to their monocultures. In another study, Wang et al. (2015b) reported that the metabolite enabled mutualistic interaction between *S. oneidensis* and *E. coli* helped to achieve higher power generation in coculture MFC compared to the monocultures in MFC. However, the performance of selective co-cultures not only depends on the type of microbes but also associated with other parameters. For instance, Madani et al. (2015) reported that the electrolyte pH significantly influences the bacterial cell growth and physiology; therefore plays an important role in the power generation of MFCs. On the other hand, some studies reported that the pH of anode can decrease over time and that in turn reduces the metabolic activity of bacteria as well as the performance of MFCs (Tang et al., 2014; Wang et al., 2010). However, Borole et al. (2008) reported that the operation of MFCs at low anodic pH boosted the proton transfer rates and thereby achieved high availability of protons at the cathode region.

Besides, the substrate concentration also influences the growth rate of microbial community (Islam et al., 2018c) and is directly proportional to the performance of MFC (Pant et al., 2010). But, based on the inoculum used, different microbial communities can be established and the optimal values of substrate concentration can vary, thus makes it very difficult to determine an optimal range for this parameter (Ali Shah et al., 2014). For example, Ghoreyshi et al. (2011) reported that the optimum concentration to achieve maximum performance using the glucose and date syrup was around 3 g/L in MFCs inoculated with *Saccharomyces cerevisiae* and that high concentrations may have a significant role on the performance of MFC. Therefore, these important parameters need to be optimized in order to get the maximum performance by co-culture MFC.

Optimizing the operational parameters in MFC could reduce the cost of operation and simultaneously enhance the performance of MFC. Recently, in biological operating systems, the statistical optimization has emerged as a popular technique due to the increasing impact of the parameters. In addition, it helps to search within a wide experimental area with a least runs and can provide the contribution of each factor and its share on the different responses (Rajendhran et al., 2002). Furthermore, the interactions among the different variables can also be evaluated using this technique. The influence of a certain factor can be analyzed at different levels of the other factors, therefore the conclusions are more accurate over the total experimental space (Madani et al., 2015). In MFCs, several operational parameters such as temperature, pH, substrate concentrations, have been optimized so far (Jia et al., 2014; Madani et al., 2015; Pendyala et al., 2016; Sadabad and Gholikandi, 2017). For example, Sadabad and Gholikandi (2017) optimized the operational parameters of pH and temperature in MFCs and achieved the maximum performance when the temperature and pH were about 35 °C and 7.0, respectively. In another study, Madani et al. (2015) optimized the buffer concentrations and pH in MFCs and achieved the highest power (461 $\mathrm{mW/m^2}$) at the pH and buffer concentration of 6.3 and 82 mM, respectively. However, optimization of key parameters which determines the performance of MFCs such as inoculum composition along with substrate concentration, pH and operational time has rarely been addressed.

In the present study, optimization of the operational parameters such as inoculum composition, pH, substrate concentration and time was carried out using Box-behnken design to achieve the maximum performance in MFC.

2. Methodology

2.1. MFC fabrication and operation

The MFCs were fabricated with a cubic plexi glass (Shanghai, Sunny Scientific, China) with a dimension of 10 cm × 10 cm x 10 cm and a total working volume of 20 mL. Carbon felt (3 × 0.9 × 3 cm) was used as both anode and cathode electrode in all the experiments. The electrodes were cleaned with 1.0 M sodium hydroxide followed by 1.0 M hydrochloric acid after each experiment and stored in distilled water before use. A cation exchange membrane (Nafion 117, Dupont Co., USA) was used to separate the anode and cathode compartments of MFC. Prior to use, the Nafion membrane was pre-treated using dilute H_2SO_4 for 1 h followed by washing with de-ionized water several times. Thereafter, the whole MFC set up was tightened up with the screws. The anode compartments were filled with 20 mL of sterilized 50% POME and subsequently inoculated with the pure cultures or co-cultures (1 mL) while the cathode chamber was filled with $KMnO_4$ solution, as an oxidizing agent. The whole experiment was carried out for 11 days at room temperature. The detailed methodologies about sample collection, inoculum preparation, performance evaluation and electrochemical analysis have been presented in Fig. SP1-SP4.

2.2. Experimental design and data analysis

In order to establish a mathematical model, the power density and COD removal efficiency were considered as dependent variables and the inoculum composition, pH, initial COD and time were considered as predictor variables. A sequential approach using design of experiments was carried where each regressor was coded as follows (Eq. (1)):

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \left(\sum_{i=1}^{n} b_{ii} x_i\right)^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n} b_{ij} x_i x_j \tag{1}$$

In the equation, Y is predicted response, b_i is linear coefficient, b_o is constant coefficient, b_{ii} is quadratic coefficient, b_{ij} is the interaction of coefficient, x_i, x_j coded values of MFC.

2.3. Statistical analysis

The data obtained from experimental runs were analyzed by Design-Expert Version 6.0.4 (Stat- Ease Inc., Minneapolis, MN, USA) and in order to diagnose the fitted models, the model adequacy and error independency were used for each of the variables. In addition, different statistics were used to judge the adequacy of a fitted model and the coefficient of determination was used to evaluate the goodness of the fitted model, R^2. Furthermore, the ratio of the signal to noise was measured by adequate precision statistic (Madani et al., 2015). The Cook's distance statistics was used to test how well the model fitted with the *i*th point and how far that point was from the rest of the data. The large distance data (greater than unity) needs to be analyzed with more care since the point is more influential than the others (Madani et al., 2015). The normality of the errors was checked by normal probability plot of the residuals. The fairness of variances was examined by plotting the residuals versus the time sequence of the runs, each independent variable and predicted values. While discrepancies were observed, the data transformation on the response was used to ease the issues. The replicates of the centre points were added to the factorial designs to examine the adequacy of the model for capturing the curvature expressed in the response. The analysis of variance (ANOVA) with Fisher's statistical test (P-value $<$ 0.05) was used to evaluate the significance of a fitted model. The predicted response for optimum value and their