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Optimization of process parameters for the bioconversion of activated sludge by *Penicillium corylophilum*, using response surface methodology

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Abstract

The optimization of process parameters for the bioconversion of activated sludge by *Penicillium corylophilum* was investigated using response surface methodology (RSM). The three parameters namely temperature of 33°C, agitation of 150 r/min, and pH of 5 were chosen as center point from the previous study of fungal treatment. The experimental data on chemical oxygen demand (COD) removal (%) were fitted into a quadratic polynomial model using multiple regression analysis. The optimum process conditions were determined by analyzing response surface three-dimensional surface plot and contour plot and by solving the regression model equation with Design Expert software. Box-Behnken design technique under RSM was used to optimize their interactions, which showed that an incubation temperature of 32.5°C, agitation of 105 r/min, and pH of 5.5 were the best conditions. Under these conditions, the maximum predicted yield of COD removal was 98.43%. These optimum conditions were used to evaluate the trail experiment, and the maximum yield of COD removal was recorded as 98.5%.

Key words: optimization; response surface methodology; *Penicillium*; activated sludge; domestic wastewater sludge

Introduction

Optimum process conditions are required to significantly enhance the bioconversion in liquid-state bioconversion (LSB) process for the treatment of sludge from domestic wastewater treatment plant (DWTP) (Alam *et al*., 2003a). Several authors have conducted single-factor optimization to evaluate the optimum carbon source (wheat flour) and the optimum process conditions of temperature (33°C), initial pH (5.5), inoculum size (2% v/v), and agitation rate (150 r/min) for fungal treatment of DWTP sludge in shake flask under controlled conditions (Alam *et al*., 2003a, b). However, single-variable optimization methods are not only tedious but also can lead to misinterpretation of results, especially because the interaction between different factors is overlooked (Wenster-Botz, 2000). Therefore, at present, the multivariable optimization methods have been chosen by the researchers.

Statistical approaches are the ideal means for process optimization studies in biotechnology (Haaland, 1989; Gupta *et al*., 2002). Response surface methodology (RSM) is now being routinely used for optimization studies (for multivariables) in several biotechnological and industrial processes (De Coninck *et al*., 2000; Beg *et al*., 2002; Puri *et al*., 2002).

RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching for optimum conditions of factors for desirable responses (Li *et al*., 2002; Lee *et al*., 2003). It has been extensively applied in many areas of biotechnology such as optimization of media and cultivation conditions (Rao *et al*., 1993; Chen, 1996; Hujanen *et al*., 2001; Lai *et al*., 2003; Elibol, 2004), biotechnological conditions (Vasconcelos *et al*., 2000; Triveni *et al*., 2001), lipase-catalyzed reaction conditions (Kiran *et al*., 1999; Murthy *et al*., 2000; Krishna *et al*., 2001; Soo *et al*., 2004), pyrene oxidation (Launen *et al*., 1999), xylitol production (Silva and Roberto, 2001; Rodrigues *et al*., 2003), citric acid production fermentation (Kılıç *et al*., 2002 ; Kumar *et al*., 2003), and lactic acid fermentation (Kiran *et al*., 2000). It has also been used to determine the optimal values for processing parameters such as pH, temperature, and aeration (Harris *et al*., 1990) and to optimize feeding rates (Bazaraa and Hassan, 1996).

Recently, RSM has been used to optimize the process parameters for extracellular polysaccharide and mycelial biomass by *Boletus* spp. ACCC 50328 in submerged fermentation (Wang and Lu, 2005). It has been used for bioprocess design and optimization (Kalil *et al*., 2000), for the production of α-amylase by *Aspergillus oryzae* (Francis *et al*., 2003), and for the optimization of the

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medium components (Vohra and Satyanarayana, 2002).

This study was conducted to optimize the process parameters for the fungal treatment of activated sludge with *Penicillium corylophilum* (Mannan *et al*., 2005) using Box-Behnken design technique under RSM.

1 Materials and methods

1.1 Sample collection and preparation

The domestic activated sludge with 0.7%–1.00% (w/w) total suspended solids (TSS), initial pH 6.65–6.9, was collected from the DWTP, Indah Water Konsortium (IWK), Kuala Lumpur, Malaysia. The required concentration of sludge of 1.0% (w/w) of TSS was prepared by adjusting the moisture of the original sludge collected. The sludge of 1% (w/w) concentration (TSS) was used throughout the experiment to determine the optimum process conditions for microbial treatment of activated (DWTP) sludge (Mannan *et al*., 2005).

1.2 Microorganism

Penicillium corylophilum WWZP1003 (IMI385277) was used in the study. This strain was obtained from the laboratory stock of Biochemical Engineering Lab, University Putra Malaysia (UPM), Malaysia. *Penicillium corylophilum* was isolated from the relevant sources (wastewater and sludge cake) (Alam *et al*., 2001). Its potential on the bioconversion of the domestic wastewater sludge in LSB process was optimized under controlled (Alam *et al*., 2003; 2004) and natural conditions (Mannan *et al*., 2005). The culture was maintained on potato dextrose agar (Merck, Germany) slants. Subculturing was carried out once every month and stored at 4°C.

1.3 Inoculum (cultured inoculum)

Spore suspension was prepared according to the method of Fakhru'l-Razi *et al*. (2002). After recording its concentration (9.55×10^6 spores/ml of *Penicillium corylophilum*) with a hemocytometer, cultured inoculum was prepared for treatment of domestic sludge according to the methods of Mannan *et al*. (2005). In this procedure, 2% (w/v) wheat flour in sterilized distilled water was inoculated with 2% (v/v) spore suspension and then processed for 48–72 h in a rotary shaker at 150 r/min at optimum temperature of 33°C for pellet formation (Alam *et al*., 2003a, b). The pellet cultured was used as an inoculum at 10% (v/v) (Mannan *et al*., 2005) to determine the process conditions for the bioconversion of activated domestic sludge in LSB process under natural conditions. The concentration of cultured inoculum of *Penicillium corylophilum* was 14.357 g/L.

1.4 Experimental design and procedure

RSM has been widely used in optimization study, in which some of the popular choices include the Plackett-Burman design (Plackett and Burman, 1946; Kalil *et al*., 2000; Viswanathan and Surlikar, 2001), the Box-Behnken design (Box and Behnken, 1960; Francis *et al*., 2003; Wang and Lu, 2005), the central composite design (Sunitha and Lee Jung-Kee, 1999; Dey *et al*., 2001; Vohra and Satyanarayana, 2002; Beg *et al*., 2003; Soo *et al*., 2004) and the Graeco-Latin square design (Haltrich *et al*., 1994). These statistical experimental designs have by now been established as a convenient method for optimizing various processes (Wang and Lu, 2005).

Box-Behnken design only has three levels (low, medium, and high, coded as –1, 0, and +1) and need fewer experiments; this design is more efficient and easier to arrange and interpret in comparison with others (Bosque-Sendra *et al*., 2001). Therefore, this statistical technique was used in this study. A total of 17 runs were used to optimize the process parameters namely temperature, agitation, and pH. The level and code of variables considered in this study are shown in Table 1. The average from two replicated values of each run was taken as dependent variables or response or yield (chemical oxygen demand (COD) yield).

Table 1 Level and code of variables for Box-Behnken design

Variables	Symbols	Coded levels		
		–1	0	+1
Temperature (°C)	A	30	33	36
Agitation (r/min)	B	100	150	200
pH	C	3	5	7

The software Design Expert (Version 6.0.11, State-Ease Inc., Minneapolis, USA) was used for the experimental design, data analysis, quadratic model buildings, and graph (three-dimensional response surface and contour) plotting.

The experiments were conducted in a 250-ml Erlenmeyer flask containing 100 ml of sludge samples. The inoculated and uninoculated samples were incubated for 3 d (Mannan *et al*., 2005) in a rotary shaker (Innova 4000, New Brunswick Scientific Co. Inc., Edison, USA) under various conditions of temperature, agitation, and pH (Table 2). The initial pH (6.65–6.90) of the sludge samples was adjusted by sulfuric acid.

Table 2 Box-Behnken design matrix along with the experimental and predicted values of COD removal (%)

Std.	Temperature (°C)	Agitation (r/min)	pH	COD removal (%)	
				Experimental	Predicted
1	0	0	0	98.18	98.22
2	+1	–1	0	96.34	96.14
3	–1	+1	0	79.78	79.97
4	+1	+1	0	80.44	80.40
5	–1	0	–1	75.03	75.01
6	+1	0	–1	75.09	75.31
7	–1	0	+1	81.99	81.77
8	+1	0	+1	79.80	79.82
9	0	–1	–1	90.58	90.56
10	0	+1	–1	75.71	75.53
11	0	–1	+1	97.98	98.16
12	0	+1	+1	79.19	79.21
13	0	0	0	83.97	83.30
14	0	0	0	83.31	83.30
15	0	0	0	82.99	83.30
16	0	0	0	83.25	83.30
17	0	0	0	82.99	83.30

1.5 Analytical methods

Specific resistance to filtration (SRF) test was used for dewaterability test of fungal-treated and untreated sludge. The detail procedure (Carman, 1938) was followed according Alam *et al*. (2003a). The filtration was carried out using a 90-mm-diameter Whatman #1 filter paper at an applied vacuum pressure of 300 mmHg (Alam *et al*., 2003). The filtrate of the treated and untreated (control) samples was collected for COD analysis. This analysis was done according to the standard methods (APHA, 1999). SRF test data were not included in this experiment. Only COD removal (%) or COD yield after 2 d of fungal treatment (Mannan *et al*., 2005) was used to determine the optimum process conditions for fungal treatment. The SRF filtrate was used to compare the previous study (COD removal efficiency for fungal treatment; Mannan *et al*., 2005).

2 Results and discussion

A Box-Behnken design under RSM was used to analyze the interactive effect of temperature, agitation, and pH and to arrive at an optimum. The base points for the design were selected from a single-parameter study in LSB process under control conditions (Alam *et al*., 2003a).

2.1 Optimization of chemical oxygen demand (COD) removal

The design matrix of the variables in the coded units is shown in Table 2, along with the predicted and experimental values of response (COD removal or COD yield). The predicted values of responses (COD yield) were obtained from quadratic model fitting techniques using the software Design Expert. The statistical model was developed by applying multiple regression analysis methods on using the experimental data for the removal of COD in the treatment, which can be given as:

$$Y = -13.33799 + 8.89863A - 1.05477B + 16.16533C - 0.13927A^2 + 0.0026542B^2 - 1.01810C^2 + 0.00417AB - 0.094071AC - 0.009805BC \quad (1)$$

where Y (yield) is the COD removal (%); A is the temperature (°C); B is the agitation (r/min) and C is the pH.

The statistical model was checked by F-test, and the analysis of variance (ANOVA) for the response surface quadratic model is summarized in Table 3. In Table 3, the Model F-value of 795.03 implies that model is highly significant. There is only a 0.01% chance that a Model F-value this large could occur due to noise. There is a very low probability value (P model, $F < 0.0001$). Values of "Prob>F" less than 0.0500 indicate that model terms are significant. Another evidence is the lack-of-fit F-value. The lack-of-fit F-value of 0.51 implies the lack of fit is not significant relative to the pure error. There is a 69.60% chance that a lack-of-lit F-value this large could occur due to noise. Nonsignificant lack of fit is good, and in this case, all the model coefficients, namely A, B, C, A^2, B^2, C^2, AB, AC, BC are significant (Table 3). The goodness of the model can be checked by the determination coefficient R^2 and the adjusted R^2 (multiple correlation coefficient R). The value of adjusted R^2 (0.9978) for Eq. (1) suggests that the total variation of 99.78% for COD yield is attributed to the independent variables and only about 0.22% of the total variation cannot be explained by the model. The closer the values of adjusted R^2 to 1 are, the better is the correlation between the experimental and predicted values (Pujari and Chandra, 2000; Wang and Lu, 2005). Here, the predicted R^2 of 0.9946 is in reasonable agreement with the adjusted R^2 of 0.9978 between the experimental and predicted values of COD yield. "Adeq precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 85.421 of the Model indicates an adequate signal (Table 3). This model can be used to navigate the design space.

Table 3 ANOVA for the response surface quadratic polynomial model

Source	Sum of squares	d*f*	Mean square	F-value	Probability (P) > F
Model	898.24	9	99.80	795.03	<0.0001
A	1.37	1	1.37	10.88	0.0131
B	577.49	1	577.49	4600.25	<0.0001
C	63.55	1	63.55	506.26	<0.0001
A^2	6.61	1	6.61	52.69	0.0002
B^2	185.39	1	185.39	1476.79	<0.0001
C^2	69.83	1	69.83	556.25	<0.0001
AB	1.57	1	1.57	12.47	0.0096
AC	1.27	1	1.27	10.15	0.0154
BC	3.85	1	3.85	30.63	0.0009
Lack of fit	0.24	3	0.081	0.51	0.6960
Pure error	0.64	4	0.16		
Cor.total	899.12	16			

R^2=0.9990; adjusted-R^2=0.9978; predicted-R^2=0.9946 and Adeq precision=85.421.

The fitted response surface plot was generated by statistically significant above model by Design Expert program to understand the interaction of the parameters required for optimum yield (COD yield). The plots are shown in Figs.1, 2, and 3. Eq. (1) was then used to facilitate plotting of three-dimensional surface and contour plots at a time. Two parameters of each model were plotted at any one time on the X and Y axes with the yield in Z axis. The other one remaining parameters set at their center point values (i.e. temperature of 33°C, agitation of 150 r/min, and pH of 5) automatically by the software to make each plot.

The shape of the contour plots (circular or elliptical) indicates whether the mutual interactions between variables are significant or not. A circular contour plot indicates that the interactions between related variables are negligible. An elliptical contour plot indicates that the interactions between related variables are significant (Muralidhar *et al*., 2001). By analyzing plots (Figs.1, 2, and 3), the predicted yield of COD removal is observed as 98.02% and lies in the following ranges of the examined variables: temperature 32.5–33.5°C, agitation 100–105 r/min, and pH 5–6.

By solving the Eq. (1) using above-mentioned software, the optimum values of the test variables were temperature 33.12°C, agitation 102.45 r/min, and pH 5.46. Under this

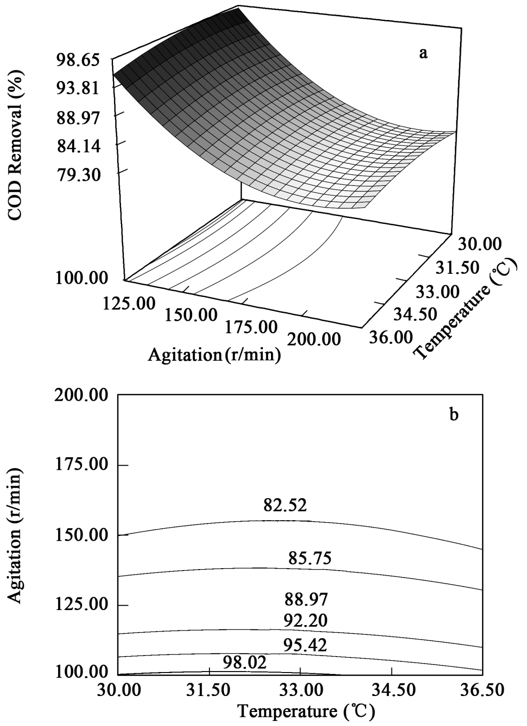

Fig. 1 Three-dimensional surface plot (a) and corresponding contour plot (b) of COD removal (%) vs. agitation (r/min) and temperature (°C).

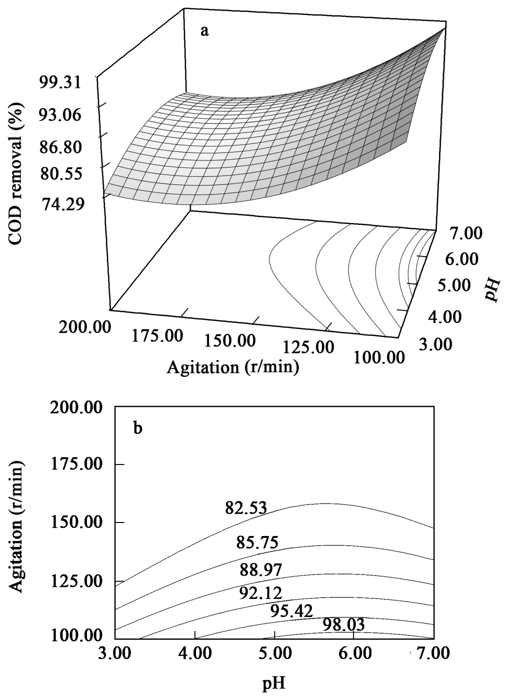

Fig. 2 Three-dimensional surface plot (a) and corresponding contour plot (b) of COD removal (%) vs. agitation (r/min) and pH.

condition, the maximum predicted yield of COD removal was observed with 10% (v/v) inoculum dose of *Penicillium corylophilum* for sludge treatment. It was about 98.43%.

2.2 Validation of the models

The trail experiments were conducted under optimized process conditions. The optimum process conditions

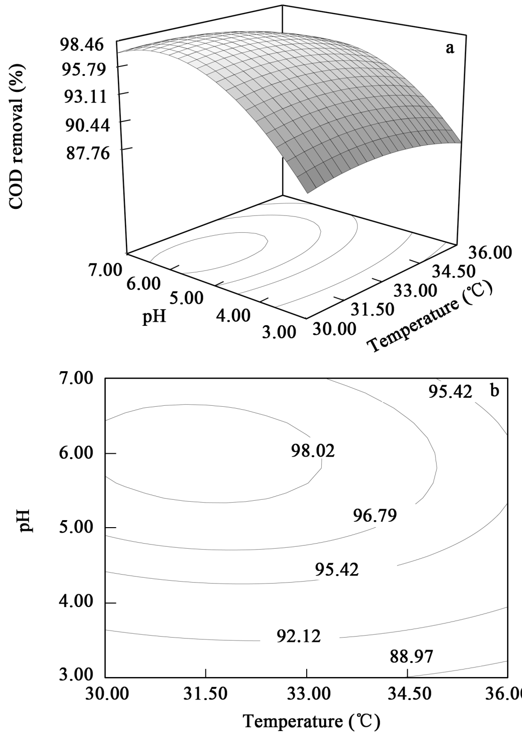

Fig. 3 Three-dimensional plot (a) and corresponding contour plot (b) of COD removal (%) vs. pH and temperature (°C).

namely temperature of 33.5°C, agitation of 105 r/min, and pH of 5.5 were used for the treatment of activated domestic sludge (1% w/w) with 10% (v/v) inoculum dose of *Penicillium*. The result was found as 98.5% of COD removal by 10% (v/v) inoculum dose of *Penicillium* (data not shown). These results were better than the COD removal (94.40%) observed under preliminary process conditions of temperature of 33°C, agitation of 150 r/min, and pH of 5.5 by *Penicillium* (Mannan *et al*., 2005).

3 Conclusions

Conventional processes of optimization are usually time consuming and expensive. Single-variable optimization methods are monotonous and they can also lead to misinterpretation of results that are used to select the precise factors that influenced the process. The single-factor optimization cannot explain the actual interactions of the parameters of the experimental data because the interaction between different factors is overlooked, leading to a misinterpretation of the results. In this study, optimization of multiple factors yielded more accurate results from which one could choose the value of factors.

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