Optimization of Process Factors for The Biodegradation Of DWTP Sludge in Large Scale Using Low Mixing Rates

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Abstract— **This paper investigates the optimization of biodegradation of DWTP sludge by mixed strains of selected Fungi using response surface methodology (RSM). Three numerical factors of aeration rates (vvm), and mixing rate (rpm) with three levels was carried out to evaluate the linear and interaction effects on effective biodegradation of DWTP sludge. The experimental data on chemical oxygen demand (COD) removal (%) were fitted into a quadratic polynomial model using multiple regression analysis with Design Expert software. 3-level factorial design technique under RSM was used to optimize their interactions, which showed that The optimum aeration rate (vvm) and mixing rate (rpm) were observed to be (0 vvm), and (10 pm), respectively to obtain maximum predicted COD response of 98.7% in fungal treated sludge by LSB under natural conditions in pilot scale.**

Index Terms— **RSM, LSB Process, biodegradation process, mixed culture of Fungi**

I. INTRODUCTION

Optimum process conditions are required to significantly enhance the bioconversion in liquid-state bioconversion (LSB) process for the treatment of domestic wastewater treatment plant (DWTP) sludge [2]. single-factor optimization has been conducted to evaluate the optimum carbon source (wheat flour) as a co-substrate supplementation and the optimum process conditions of temperature (33ºC), 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 sterilized controlled conditions [2]. Response surface methodology (RSM) is now being routinely used for optimization studies (for multivariable) in several biotechnological and industrial processes [5-6, 13]. It has been also conducted to determine the LSB process optimal parameters such as pH, temperature, and agitation rate for the fungal treatment of activated sludge with single culture using RSM in shake flasks under non-sterilized conditions [11].

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II. EXPERIMENTAL MATERIALS AND METHODS REVIEW STAGE

A. Sample Collection

Domestic wastewater treatment plant (DWTP) sludge of (TSS 0.7~1.3% w/w, pH 6.2-6.9) samples were collected. Adding distilled water and reducing the supernatant of original domestic sludge concentration prepared the justification of the sludge concentrations required throughout the study of 1% w/w. All sludge samples were used as fresh samples.

B. Microorganisms

The microorganisms used in the present study as mixed strains (The equal volume of 5.5×10^5 and 3.25×10^5 spore/mL of *Aspergillus niger* and *Penicillium corylophilum* inoculum mixed together and used when and whatever necessary).

C. Inoculum (Spore Suspension) Preparation

The inoculum of cultured spore suspensions was used for the sporulating strains mixed culture (equal volume) of *Aspergillus niger* and *Penicillium corylophilum* in this study [7-8].

D. Experimental Procedures and Analysis

A 300 L Pilot scale with 100 L working volume was used throughout this study Process conditions of 1% w/w TSS, 5% v/w inoculum size, and 11 hrs inoculum feeding intervals without co-substrate supplementations were used with DWTP sludge at pH of 6.5±3 monitored but not adjusted, and process temperature was recorded to be 30±2 °C along the 2 days of treatment period. All experiments were carried out using mixed culture (equal volume of 5.5×10^5 and 3.25×10^5 spore/mL of *Aspergillus niger* and *Penicillium corylophilum* inoculum) (section II.C). In this study, the levels of each independent numerical variable (parameter) of mixing rate (rpm) and aeration rate (vvm) were used for the design of experiments to study the effect of mixing and aeration rates on the performance of the mixed culture of selected under natural conditions (Non sterilized and non controlled conditions) . pH and temperature were recorded (but not justified) throughout the study. The optimum process factors were discussed in terms of biodegradability for microbial treatment of DWTP sludge

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under Natural conditions. The levels of independent variables are given in Table I. The statistical software package "Design –Expert", [version 6.0.4 for windows, Stat-Ease, Inc.,] was used to organize the experimental design. The experiments were designed using Response Surface Methods (3-level factorial design); all experiments were designed with combination of three levels of all parameters with three replications for 13 sets of experiments (39 treated samples and 39 control samples) for 2 days of treatment. The response surface method (RSM) (3-level factorial design) of all experiments was presented in Table II. All parameters were determined in terms of maximum COD removal (%) in microbial treatment of DWTP sludge under natural conditions.COD of supernatant were determined within 5 minutes after switching of the pilot according to the standard mentioned [3]. All the data obtained were an average of 3 replicates of each experiment.

TABLE I
PARAMETERS LEVELS OF PROCESS CONDITIONS FOR RESPONSE SURFACE METHOD (3-LEVEL FACTORIAL DESIGN OF EXPERIMENTS

Experiments	Parameter for Optimization	Levels of parameters/code		
		-1	0	+1
Numerical parameters	Mixing rate (rpm)	0	5	10
	Aeration rate (vvm)	0	0.75	1.5

TABLE II
DATA MATRIX FOR THE EXPERIMENTAL DESIGN BY RESPONSE SURFACE METHOD (3-LEVEL FACTORIAL DESIGN) FOR THE PROCESS PARAMETERS

Std	Run	Numerical Variables			
		Aeration Rate		Mixing Rate	
		Coded	Actual (vvm)	Coded	Actual (rpm)
1	7	-1	0	-1	0
2	13	0	0.75	-1	0
3	5	1	1.5	-1	0
4	12	-1	0	0	5
5	3	0	0.75	0	5
6	4	1	1.5	0	5
7	6	-1	0	1	10
8	9	0	0.75	1	10
9	2	1	1.5	1	10
10	8	0	0.75	0	5
11	1	0	0.75	0	5
12	10	0	0.75	0	5
13	11	0	0.75	0	5

III. Results and Discussion

Optimum process conditions of aeration, agitation and pH for the bioconversion of activated sludge by single strain of *Penicillium corylophilum* was studied using response surface methodology (RSM) under non-sterilized condition with modified cultured inoculum [11]. Whereas this study was conducted to show the statistical significance of the process parameters of aeration rate (vvm), and mixing rate (rpm) without co-substrate supplementation in terms of biodegradability for the microbial treatment of DWTP sludge using mixed strains as in section II.C under natural conditions leading to 13 sets of experiments by 3-Level Factorial design [under response surface methodology (RSM)] with three replicates. The low, middle and high levels of each parameter were given in Table I. The statistical model was developed by regression analysis using the experimental data for the increased COD removal (%)without co-substrate supplementation using selected fungi in the treatment of DWTP sludge which shown as below.

$$\text{COD Removal (\%)} = +91.82815 + 4.32872\times \text{Aeration} - 0.31596\times\text{mixing} - 5.37389\times \text{Aeration}^2 + 0.10029 \times \text{mixing}^2 - 0.37673\times \text{Aeration} \times \text{mixing} \quad (1)$$

Where, Y (response) is the COD-response

The coefficient of determination (R^2) was calculated to be 0.9976 for the COD removal (%) (COD response), (Table III). R^2 represents the proportion of variations in the response (data) that is explained by the predictor model, which means, R^2 value provides a measure of how much variability in the observed response values those can be explained by the experimental factors and their interactions. The range of R^2 is between 0 and 1. The closer the R^2 value is to 1, the model can be said a stronger and better model which can predict the response [9-11, 14]. R^2 can be represented by percentage thus is interpreted as the percent variability in the response expressed by the statistical model. That means only 0.0024 % for COD-response samples variation of total variation were not explained by this model which ensure a satisfactory adjustment of the quadratic model to the experimental data. When testing the significance of the regression models it was found that p-values obtained were small, 0.0001 for the model as indicated in Table IV, and, Compared to a desired significant level, 0.05. This indicates that the regression models were significant to the COD removal (%).

Table III
THE COEFFICIENT OF DETERMINATION (R-SQUARED) AND ADEQ PRECISION OF REGRESSION MODEL FOR RESPONSE (EQUATION 1)

Values	COD removal (%) (COD-response)
R-Squared	0.9976
Adjusted R-Squared	0.9958
Predicted R-Squared	0.9850
Adeq Precision	86.859

Table IV
ANNOVA FOR THE RESPONSE SURFACE QUADRATIC MODEL ANALYSIS FOR COD REMOVAL (%)

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	170.1215	5	34.02431	574.098	< 0.0001
A	106.4372	1	106.4372	1795.93	< 0.0001
B	24.52686	1	24.52686	413.846	< 0.0001
A^2	25.23658	1	25.23658	425.821	< 0.0001
B^2	17.36133	1	17.36133	292.941	< 0.0001
AB	7.98345	1	7.98345	134.706	< 0.0001
Residual	0.41486	7	0.059266		
Lack of Fit	0.22514	3	0.075047	1.58226	0.3260
Pure Error	0.18972	4	0.04743		
Cor Total	170.5364	12			

By means of observation, based on Table IV, it was found that the Model F-value of 574.10 implies the model is significant.There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant.In this case A, B, A^2, B^2, and AB are significant model terms.Values greater than 0.1000 indicate the model terms are not significant, If there are many insignificant model terms (not counting those required to support hierarchy), so model reduction may improve your model [12].The "Lack of Fit F-value" of 1.58 implies the Lack of Fit is not significant relative to the pure error. There is a 32.60% chance that a "Lack of Fit F-value" this large could occur due to noise.Non-significant lack of fit is good.The "Pred R-Squared" of 0.9850 is in reasonable agreement with the "Adj R-Squared" of 0.9958. An “Adeq Precision” measure the signal to noise ratio.A ratio greater than 4 is desirable.Your ratio of 86.859 indicates an adequate signal. This model can be used to navigatethe design space.

The all conditions for this model may have implied a satisfactory presentation of the process by the model Equations 1. The 3-Level factorial design matrixes alone with the experimental and predicted values of COD removal (%) are presented in Table (V) for models understanding. The three dimensional response surface and contour plots were plotted by statistically significant model to understand the interaction of the predictors required for the COD-response. Equation (1) was used to facilitate plotting of surface and contour at a time. The factors interaction with each other (two at a time) on the X-axis and Y-axis with the COD-response on the Z-axis as clearly presented in Figure 1.

The interactive effect of two variables aeration rate and mixing rate is presented in Figures 1 for the COD-response. By means of observation, it was found that The COD response decreased as aeration rate increases from its minimum value of 0 vvm and as mixing rate decreases from its maximum value of 10 rpm as shown in Figures (1a). COD response was predicted to be 98 % and above at range of aeration rate and mixing rate of 0-0.4 vvm, and 9.6-10 rpm, respectively after 2 days of treatment (Figure 1b).

From the above observation it might be recorded that minimum mixing rate can provide a proper mixing of air into substrate, which maintain the COD reduction at the maximum level with minimum level of aeration rate for better growth. The fungal growth was declined at high mixing rate and aeration rate and as a result it might affect the removal rate under unsuitable mixing and aeration since the fungal mycelia in treated sludge might be damaged due to shear stress from high mechanical mixer, which might affect its growth [1]. It was reported that maximum COD removal was observed at low rate of air flow (0.25-0.5) vvm in bioconversion process in batch fermenter under sterilized controlled conditions [1]. Bioconversion process was suppressed at higher rates of agitation since it damage of fungal mycelia in the microbial treatment using Fungi [1, 4,11].

TABLE V
3-LEVEL FACTORIAL DESIGN MATRIX ALONG WITH THE EXPERIMENTAL AND PREDICTED VALUES OF COD REMOVAL (%) AND SRF DECREASED (%) IN PILOT PLANT

Std	Aeration Rate	Mixing Rate	COD Removal (%)	
	(vvm)	(rpm)	Experimental Values	Predicted Value
1	-1	-1	91.70	92.11
2	0	-1	92.30	91.87
3	1	-1	86.11	86.14
4	-1	0	93.83	93.28
5	0	0	91.74	91.64
6	1	0	84.30	84.51
7	-1	1	98.80	98.94
8	0	1	95.80	95.90
9	1	1	87.60	87.36
10	0	0	91.62	91.64
11	0	0	91.33	91.64
12	0	0	91.80	91.64
13	0	0	91.40	91.64

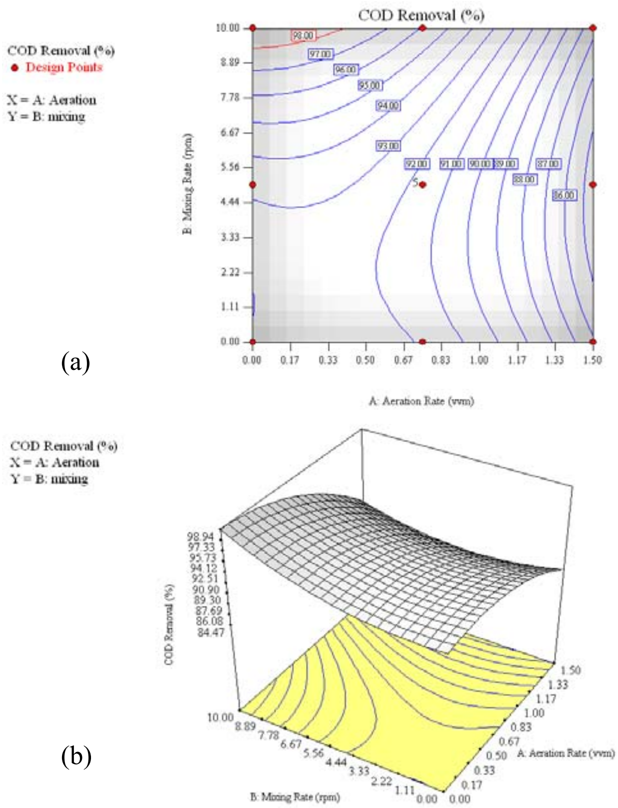

Fig. 1. The interaction between aeration rate and mixing rate COD-response (COD removal %), in pilot scale under natural conditions; (a) surface plot curves, and (b) contour plot curve.

IV. CONCLUSION

It was statistically predicted that the maximum COD response values of 98.7 % was recorded at optimum process parameters of aeration rate and mixing rate of 0 vvm and 10 rpm, respectively. The fungal growth was declined at high mixing rate and aeration rate.

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