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Optimization of Pyrene Biodegradation Rate by Response Surface methodology

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ABSTRACT

The application of Response Surface Methodology (RSM) in the biodegradation process can enhance removal, reduced process variability, decrease development time and overall costs. In this study, the non-linear response behavior was analyzed using the center points and star points runs. The response was the biodegradation rate of pyrene; a 4-ring of polycyclic aromatic hydrocarbon by fungi that identified as *Rhizoctonia zeae* SOL3 in batch experiments. Factors like glucose concentration, NaCl concentration, initial pyrene concentration, pH, temperature and agitation were investigated to determine the optimum parameters for the biodegradation rate of pyrene. The quadratic model forecasted that the higher biodegradation of pyrene was 44.7% in 15 days, when the factors glucose concentration, temperature, NaCl concentration and initial pyrene concentration were 19.6 g/l, 28°C, 9.9 g/l and 24 mg/l respectively. Validation experiment was made in duplicate to verify the forecasted results. The biodegradation was 44% in 15 days under the optimal medium conditions, which was in good match with the forecasted values. Keywords: Response Surface Methodology, Optimization, Biodegradation, Pyrene

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Introduction:

Response Surface Methodology (RSM) is a group of statistical and mathematical techniques. It is useful for improving, developing and optimizing processes. It can be used to evaluate the interactions and relative significance of several affecting factors (1). The applications of RSM in the biodegradation process can enhance removal, reduced process variability, decrease development time and overall costs (2).

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Polycyclic Aromatic Hydrocarbons (PAHs) are organic compounds composed of two or more benzene rings fused together. EPA has identified sixteen substances of PAHs in the priority contaminated list (3) as a results of its mutagenic and carcinogenic effects (4, 5). Methods such as adsorption, photo-degradation ,phytoremediation and biotransformation are used to eliminate PAHs (6, 7, 8). Among these methods, the biotransformation is favorable considering ecological and economical aspects. In biotransformation process,the microorganisms' enzymes can alter the molecules' structure of pollutants that entered to the environment (9). High molecular weight-polycyclic aromatic hydrocarbons (HMW-PAHs) such as pyrene resist the microorganisms' degradation (10). However, it has been proved that HMW-PAHs can be degraded by some species of fungi (11), which indicate their potential as environmental bio-remediators. *Rhizoctonia* is a species of fungi that do not produce spores. It is a soil inhabitant fungus with many species and wide host range.

RSM was applied to optimize the pollutants degradation rate (1). In this study, the non-linear response behavior was analyzed using the center points and star points runs. Variables with three coded levels (+1, 0 and -1) were included. In order to obtain a good estimation of the experimental error, the center point runs were reduplicate many times. The experimental runs were conducted in randomized order to reduce the bias of unexpected elements during the experimental runs. The response was the biodegradation rate of pyrene by fungi that were isolated and identified as *Rhizoctonia zeae* SOL3 in batch experiments (12). Factors like glucose concentration, NaCl concentration, initial pyrene concentration, pH, temperature and agitation were investigated to determine the optimum parameters for the biodegradation rate of pyrene.

Experimental design: In this research, three sets of experiments were made to study the effect of temperature, pH, glucose concentration, salt concentration, initial pyrene concentration and agitation to optimize the biodegradation rate of pyrene by fungi. Throughout the experiments, NaCl was used as a representative for salty medium, while agitation was set at zero for fixed culture and 80 rpm for shaken culture.

Optimize the biodegradation rate of pyrene by *Rhizoctonia zeae* SOL3. The experiments were conducted by *Rhizoctonia zeae* SOL3 with the following parameters: glucose concentration, temperature, salt concentration and initial pyrene concentration. The range and level of experimental variables investigated in this research are presented in Table 1. A total of twenty-nine experimental runs were carried out in Box Behnken.

Table 1. Range, level and unit of the experimental variables used to optimize the biodegradation of pyrene in Box Behnken

Factor	Unit	Lower value	Upper value
A: glucose concentration	g/l	0	20
B: temperature	°C	20	37
C: salt concentration	g/l	0	48
D: pyrene concentration	mg/l	20	60

Results and Discussion

In this experiment, the independent variables were glucose (A), temperature (B), salt concentration (C) and initial pyrene concentration (D). The ANOVA showed that the model is highly significant at *p-value* <0.0001. This model exhibits significant non-linearity for glucose, temperature, salt and pyrene concentration with the *p*-values of <0.0001, 0.0004, <0.0001 and <0.0001, respectively. The interactions are significant between glucose concentration and salt concentration with the *p*-value of 0.003, glucose and pyrene concentration with the *p*-value of 0.0048, temperature and pyrene concentration with the *p*-value of 0.0313. While the interactions are not significant (*p*-values >0.05) between glucose concentration and temperature the *p*-value of 0.4846, temperature and salt concentration with the *p*-value of 0.6396, salt concentration and pyrene concentration with the *p*-value of 0.6396. The *p*-value for the LOFT is not significant i.e. at 0.2248. This implies that the analytical understanding of the model is statistically accurate. The plot is shown in Fig. 1 reveals that the actual values are distributed relatively near to or around the straight line of the mathematical model. The coefficient value of the model R_2 was found to be 98.3%, which implied that this model unclarified 1.7% of variation only. The higher value of R_2 indicates a better representation capability of the full quadratic equation under the given experimental domain. Adequate precision of 26.17 shows a suitable signal to noise ratio (> 4 is more desirable). As well as the reliability and high precision of the experiments can be made from the coefficient of variation (CV of 10.25 %). For these reasons, this model can be used to navigate the design space. The value of the adjusted determination coefficient (adjusted R_2 96.6%) indicated that the model was meaningful, and it was a good match between the forecasted and experimental values of biodegradation.

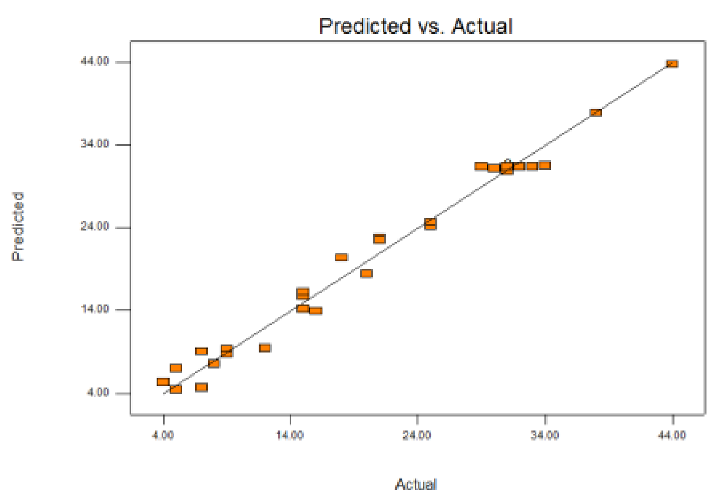

Figure 1. Predicted versus actual data for biodegradation rate

To predict the response, a 2nd order polynomial equation was used to correlate the independent and dependent variables. The best statistical model that can be used to represent the response of biodegradation of pyrene in this research is given in Equation 1

$$\text{Biodegradation rate \%} = 31.4 + 10.5 \times A + 2.75 \times B - 3.92 \times C - 9.17 \times D - 5.03 \times A^2 - 9.16 \times B^2 - 6.66 \times C^2 - 5.78 \times D^2 + 0.75 \times A \times B - 3.75 \times A \times C - 3.5 \times A \times D - 0.5 \times B \times C - 2.5 \times B \times D - 0.5 \times C \times D \quad \ldots(1)$$

Where; Y: Biodegradation rate %, A: glucose concentration; B: temperature; C: salt concentration and D: pyrene concentration. Fig. 2 shows the surface scheme that represent the effect of varying glucose concentration and temperature at a fixed salt concentration at 9.33 g/l, pyrene concentration at 24 mg/l and fixed culture. It can be noticed that increasing or the glucose concentration at moderate temperature, has caused an increase in the biodegradation rate. The contour plot in Fig. 2-A shows the highest maximum biodegradation rate at 44.7%. The point that shows the highest biodegradation rate is shown in Fig. 2-B.

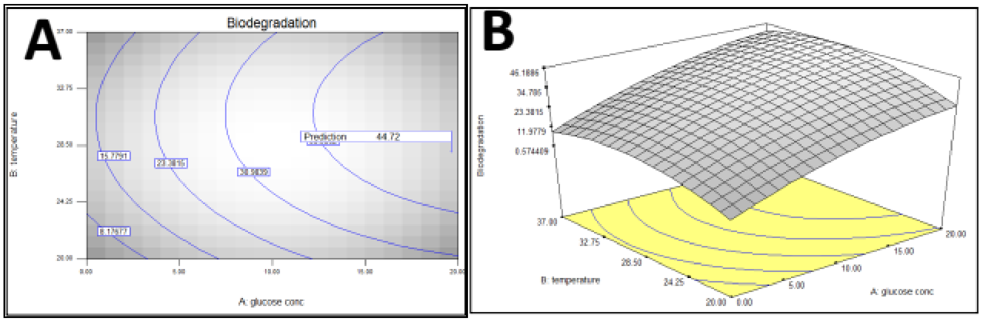

Figure 2. Contour (A) & three-dimension response surface scheme (B) representing relationship between glucose, temperature and biodegradation rate.

Fig. 3-A illustrates the biodegradation rate as a response in a contour and response surface scheme for the interaction between varying glucose and salt concentration. The interaction was observed at a constant temperature of 28°C and initial pyrene concentration of 24 mg/l. It is noticed that the effect of glucose concentration behavior was similar as in Fig. 2. The best biodegradation has occurred when the glucose concentration was between 15 and 20 g/l, while the salt concentration was from 0 to 12 g/l; outside of these ranges, the biodegradation has decreased.

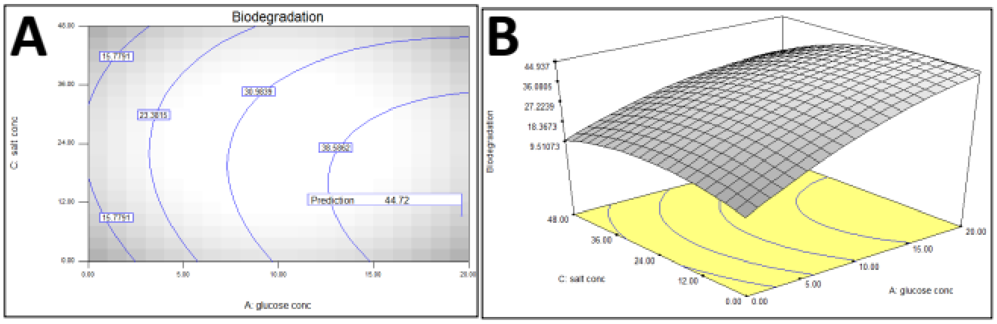

Figure 3. Contour (A) & three-dimension response surface scheme (B) representing relationship between the glucose, salt concentration and biodegradation rate.

Fig. 4 illustrates the biodegradation rate as a response in a contour and response surface scheme for the interaction between varying glucose and initial pyrene concentration.

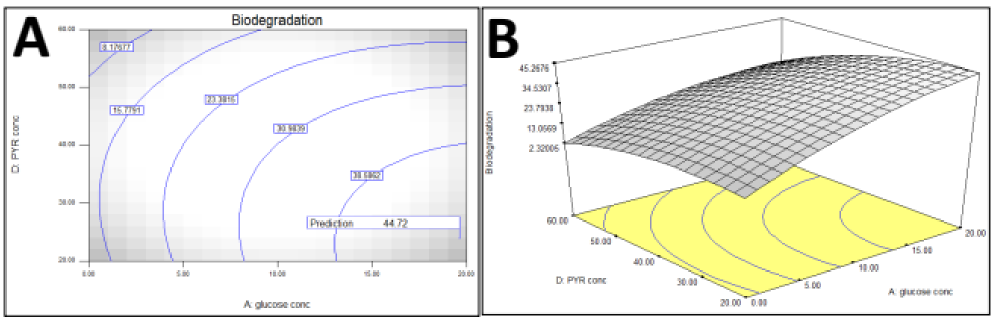

Figure 4. Contour (A) & three-dimension response surface scheme (B) representing relationship between glucose, pyrene concentration and biodegradation rate.

The interaction was observed at a constant temperature of 28°C and salt concentration at 9.3 g/l. The degradation has decreased with increasing pyrene concentration. The highest degradation occurred between 20 and 35 mg/l of pyrene.

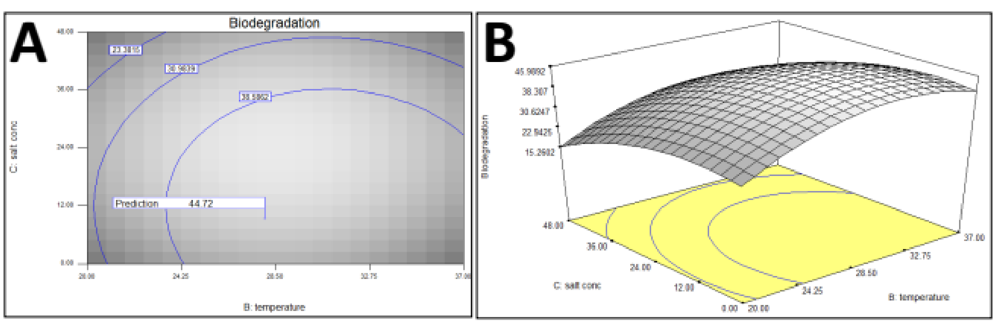

Figure 5. Contour (A) & three-dimension response surface scheme (B) representing relationship between temperature, salt concentration and biodegradation rate.

Fig. 5 illustrates the biodegradation rate as a response in a contour and response surface scheme for the interaction between temperature and salt concentrations. The interaction was observed in the initial pyrene concentration at 24 mg/l and static condition. The best biodegradation has occurred at temperatures between 26 and 32°C while the biodegradation decreased with increasing salt concentration. The best degradation has occurred between 0 and 24 g/l of salt. Fig. 6 illustrates the biodegradation rate as a response in a contour and response surface scheme for the interaction between temperature and initial pyrene concentration. The interaction was observed in salt concentration at 9.3 g/l and fixed culture. The best biodegradation was occurred at temperature between 26 and 32°C, while the degradation decreased with increasing pyrene concentration. The best degradation occurred between 20 and 25 mg/l of pyrene.

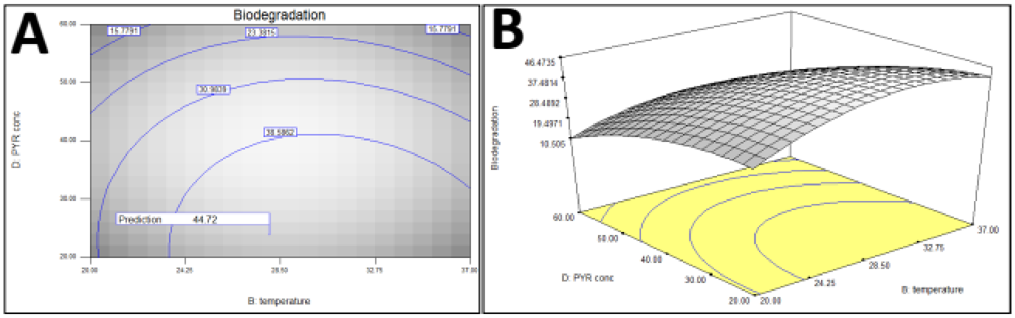

Figure 6. Contour (A) & three-dimension response surface scheme (B) representing relationship between temperature, pyrene concentration and biodegradation rate.

Fig. 7 illustrates the biodegradation rate as a response in a contour and response surface scheme for the interaction between varying salt concentrations and initial pyrene concentration. The interaction was observed at a temperature of 28°C and fixed culture. The best biodegradation was between 0 and 35 g/l of salt, while the degradation decreased with increasing pyrene concentration. The best degradation occurred between 20 and 25 mg/l of pyrene.

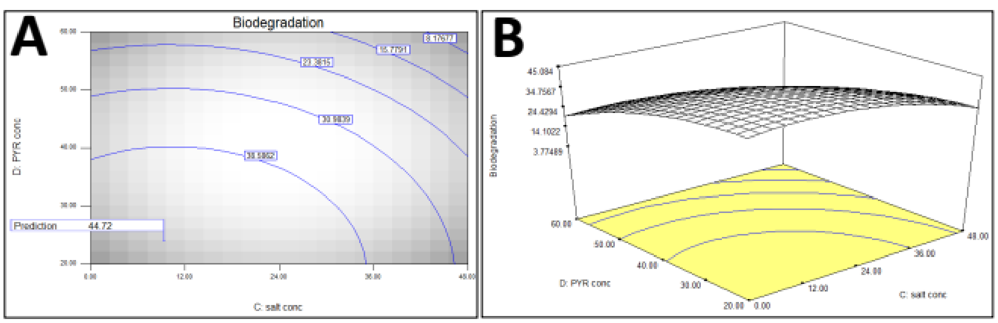

Figure 7. Contour (A) & three-dimension response surface scheme (B) representing relationship between NaCl concentration, pyrene concentration and biodegradation rate

The quadratic model forecasted that the higher biodegradation of pyrene was 44.7% in 15 days, when the factors A (glucose concentration), B (temperature), C (salt concentration) and D (initial pyrene concentration) were 19.6 g/l, 28°C, 9.9 g/l and 24 mg/l respectively as shown in Table 2. Validation experiment was made in duplicate to verify the forecasted results. The biodegradation was 44% in 15 days under the optimal medium conditions, which was in good match with the forecasted values.

Table 2. Predicted biodegradation rate

No.	glucose conc.	Temperature	NaCl conc.	Initial pyrene conc.	Biodegradation rate %	Desirability
1	19.6	28.0	9.9	24	44.7	1.00
2	18.4	29.8	12.3	20	42.43	1.00
3	19.0	29.2	7.9	20	42.43	1.00

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Conflicts of Interest: None.