The optimization of desulfurization conditions by response surface methodology

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Dibenzothiophene and its derivatives are components of high-sulfur crude oil, and these sulfides would reduce the oil quality and increase sulfur dioxide emissions when burned, which will decline environmental quality. Compared with the traditional desulfurization method, biodesulfurization has many advantages, but how to improve the biological desulfurization efficiency has been an important issue. In this study, culture conditions and desulfurization conditions were optimized by response surface methodology (RSM) to improve the biological desulfurization efficiency. Based on the single-factor experiments, Plackeet-Burman method (PB) was used to determine the main factors which would influence the efficiency of desulfurization. And the three main factors were temperature, pH and DBT concentration. Response surface optimization experiments show that when temperature was 29.70°C, initial pH was 7.43, and DBT concentration was 105.47mg/L, the desulfurization rate would be maximum, theoretical maximum degradation rate were 72.32%, and actual degradation rate were 74%. The interaction between the three key factors was significant, the influence degree was DBT concentration> pH> temperature. This study provides a method to improve the biological desulfurization efficiency by optimizing culture conditions and desulfurization conditions.

Keywords: Biodesulfurization; Dibenzothiophene; Response Surface Methodology.

1. Introduction

The sulfides in petroleum, such as dibenzothiophene and its derivatives, will reduce the quality of the oil product, and the combustion products will cause serious pollution to the air[1-4]. In view of this, more and more strict oil sulfur standards have been promulgated and implemented, and now refining industry in China is facing an unprecedented severe challenge to reduce the sulfur content of oil. The traditional desulfurization methods are chemical and physical methods. Sulfur-containing heterocyclic aromatic hydrocarbons existed in petroleum are difficult to be removed by conventional methods, but it can be done with biodesulfurization technology[5-8]. Bio desulfurization is a method to

† Work partially supported by "the Fundamental Research Funds for the Central Universities"

selectively convert the sulfur content of oil into water soluble sulfur compounds by microbial enzymes, and then through the oil and water separation method, the purpose of oil desulfurization will be achieved. Compared with catalytic desulfurization, bio desulfurization has many advantages, such as less investment, lower operating cost, cleaner and easier removal of thiophene compounds and so on, but it also has the following shortcomings at the same time, for example, flexibility is low, the process is not easy to control, and more stringent requirements are needed, due to the growth of microorganisms requires a certain condition[9-12]. It is a very important thing to create a good growth condition for the microorganism to improve the desulfurization efficiency. In this study, we provide a method, response surface methodology (RSM), to improve the biological desulfurization efficiency by optimizing culture conditions and desulfurization conditions.

2. Materials and Methods

2.1. *Materials*

Dibenzothiophene (DBT) were purchased from SIGMA, America.

2.2. *Bacteria strain and cultivation*

Rhodopseudomonas sp. Strain AS-21 which can remove dibenzothiophene (DBT) effectively was used in this study. DBT-acetone solution: DBT was dissolved in acetone (10/L) and thenstored in the refrigerator. Sulfur-free medium (BSM): 2.44g of KH_2PO_4, 14.03g of $Na_2HPO_4 \cdot 12H_2O$, 0.36g of $MgCl_2 \cdot 6H_2O$, 1mg of $CaCl_2 \cdot 2H_2O$, 1mg of $FeCl_3 \cdot 6H_2O$, 4mg of $MnCl_2 \cdot 4H_2O$, 10g of glycerin, 2.00g of NH_4Cl, dissolved in 1l distilled water. Liquid selective medium (BSDM): 100mL BSM adding an appropriate amount of DBT-acetone solution as the sole source of sulfur.

2.3. *Analytical methods*

Dibenzothiophene was analyzed by GC, and Cell mass concentration was detected by UV (OD620).

2.4. *Detection of DBT content in culture medium*

Bacteria were cultured in BSDM (containing 100mg/L DBT) for 2 days. 25ml of bacterial culture solution was taken to separating funnel, and then it was acidified to pH 2 by the addition of HCl. The remaining dibenzothiophene in the culture solution was extracted with equal volume of n-hexane. Collect n-hexane

extract after 20min. The extract was centrifuged for 10min (10000r/min). The obtained n-hexane extract was used for gas chromatography analysis.

2.5. *Detection of cell mass concentration*

Bacterial suspension has characteristic absorption peak at 620nm. After determination of the absorbance of the bacterial suspension in OD620, cell mass concentration in culture medium could be obtained by the standard curve of the relationship between the absorbance and the mass concentration of the strains.

3. Results and Discussion

3.1. *Plackeet-Burman (PB) experiment*

Plackeet-Burman experiment was based on the single factor experiment which was done before this study. The results of the single factor experiment were shown in Table 1.

Table 1. The results of the single factor experiment.

carbon sources	glycerol concentration	nitrogen sources	NH_4Cl concentration	temperature	pH	DBT concentration
glycerol	10g/L	NH4Cl	2g/L	30°C	7.5	100mg/L

Based on the results of the single factor experiment, experimental design table using Plackeet-Burman method (PB) could be made. The experimental results could be analyzed by Design-Expert software, shown in Table 2.

Table 2. Partial regression coefficients and significant impact factor analysis.

factor	Regression coefficients	Standard error	E(x1)	Sum of squares	Contribution value
A- temperature	-0.046	0.021	-0.095	0.032	12.46
B-pH	-3.80E-02	0.021	-0.072	0.015	8.85
C- glycerol concentration	0.014	0.021	0.025	1.88E-03	1.08
D- NH_4Cl concentration	-1.50E-03	0.021	-5.00E-03	7.50E-05	0.04
E- DBT concentration	0.014	0.021	0.028	2.41E-03	11.32

The three factors, which showed the maximum contribution value, are temperature, pH and DBT concentration. According to the Plackeet-Burman method (PB), these three factors were the main factors affecting desulfurization efficiency. The E(x1) value shows that, based on the single factor experiment, desulfurization efficiency can be improved when temperature and pH were reduced appropriately or the DBT concentration was increased.

3.2. *Response surface optimization experiment*

The response surface optimization experiment was designed based on the PB experiment. Temperature, pH and DBT concentration were the independent variables, the desulfurization rate was the dependent variable. The results were shown in Table 3.

Table 3. BBD experimental designs and results

Run	T(°C)	pH	DBT (mg/L)	Desulphurization rate(%)	cell mass (g/L)
1	25.00	8.00	120.00	42.23	1.593
2	30.00	7.50	100.00	71.67	2.351
3	35.00	8.00	120.00	41.78	1.697
4	30.00	7.50	133.64	68.01	2.176
5	30.00	7.50	100.00	73.18	2.373
6	30.00	7.50	66.36	52.56	2.053
7	25.00	8.00	80.00	39.12	1.428
8	38.41	7.50	100.00	31.79	0.458
9	30.00	7.50	100.00	74.87	2.348
10	30.00	8.34	100.00	47.78	1.636
11	30.00	7.50	100.00	72.13	2.381
12	25.00	7.00	120.00	44.29	1.744
13	35.00	8.00	80.00	38.21	1.482
14	30.00	7.50	100.00	70.57	2.219
15	35.00	7.00	80.00	46.01	1.652
16	30.00	6.66	100.00	49.55	1.979
17	35.00	7.00	120.00	45.78	1.798
18	25.00	7.00	80.00	43.78	1.789
19	21.59	7.50	100.00	38.69	1.37
20	30.00	7.50	100.00	68.95	2.068

The results were analyzed by Design-Expert software. The model was determined according to the results of variance analysis by selecting different models. The results of the analysis of variance are shown in Table 4 and Table 5.

Table 4. ANOVA for response surface quadratic model.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob>F	
Model	3851.26	9	427.92	29.25	< 0.0001	significant
X_1-Temperature	6.26	1	6.26	0.43	0.5278	
X_2-pH	33.84	1	33.84	2.31	0.1592	
X_3-DBT concentration	79.47	1	79.47	5.43	0.0420	
X_1 X_2	3.23	1	3.23	0.22	0.6487	
X_1 X_3	9.800E-003	1	9.800E-003	6.700E -004	0.9799	
X_2 X_3	5.12	1	5.12	0.35	0.5672	
X_1^2	2743.21	1	2743.21	187.53	< 0.0001	
X_2^2	1180.41	1	1180.41	80.70	< 0.0001	
X_3^2	351.98	1	351.98	24.06	0.0006	
Residual	146.28	10	14.63			
Lack of Fit	125.24	5	25.05	5.95	0.1362	not significant
Pure Error	21.04	5	4.21			
Cor Total	3997.54	19				

Table 5. Analysis Table

R-Squared	Adi-Squared	Pred-Squar	Adeq Precisior
0.9634	0.9305	N/A	14.85

Table 4 indicate that this model is significant (Model "Prob>F" less than 0.0500). The "Lack of Fit F-value" of 5.95 and "Lack of Fit "Prob>F"" of 0.1362 imply that the Lack of Fit is not significantly relative to the pure error. All these indicate that this model can be used.

"Adeq Precision" measures the signal to noise ratio. In this model, the "Adeq Precision" of 14.85 indicates a high reliability. This model can be used to predict the desulfurization efficiency of strain AS-21.

The influence degree was DBT concentration> pH> temperature based on "p-value".

A Second-degree polynomials equation was obtained using Design-Expert software, shown in Eq. (1).

$$Y=72.02-0.68X_1-1.57X_2+2.41X_3-0.63X_1X_2 -0.035X_1X_3+0.80X_2X_3-13.80X_1^2-9.05X_2^2-4.94X_3^2 \quad (1)$$

Where Y represents the desulfurization rate, X_1, X_2 and X_3 represent temperature, pH and DBT concentration respectively.

Optimization solutions were obtained under the condition of the maximum response value, according to this model. Results show that when temperature was 29.70°C, initial pH was 7.43, and DBT concentration was 105.47mg/L, the desulfurization rate would be maximum and theoretical maximum degradation rate would be 72.32%. Experiments were carried out under the above optimal

desulfurization conditions and growth conditions, and the actual degradation rate was 74%, which was close to the theoretical value.

4. Conclusions

This study provide a method to improve the biological desulfurization efficiency by optimizing culture conditions and desulfurization conditions. Response surface optimization experiments show that the desulfurization rate would be maximum when temperature was 29.70°C, initial pH was 7.43, and DBT concentration was 105.47mg/L. Theoretical maximum degradation rate were 72.32%, and actual degradation rate were 74% under these conditions.

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