

# Optimization of the effect of substrate concentration, pH and temperature on bio-desulphurization using regression model

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## Abstract:

This study investigates the effect of initial substrate concentration, pH, and temperature on the bio-desulphurization process. Box Wilson experimental design method was used to design the various combinations of independent variables such as initial substrate concentration ( $X_1$ ), pH ( $X_2$ ), and temperature ( $X_3$ ) and how it varies with extent of bio-desulphurisation ( $Y$ ). The extent of bio-desulphurisation was determined for all 15 experimental runs and the results are presented which showed that the maximum extent of bio-desulphurization is 84.5% which is the result obtain from experimental run 9 when initial substrate concentration is 150 mg/l, pH is 6.75 and temperature is 35°C. The minimum extent of bio-desulphurization is 50.4% which the results for experimental runs 6 and 8 which only differ in terms of pH (i.e., pH for run 6 is 7 and pH for run 8 is 6.5). The empirical model is therefore given as:  $Y = -30.91 + 0.073X_1 + 7.07X_2 + 0.135X_3 - 0.000243X_1^2 + 2.5 \times 10^{-05}X_1X_2 + 6.25 \times 10^{-7}X_1X_3 + 5 \times 10^{-5}X_2X_3$ . The empirical model developed had an R-squared value of 0.9991 which implies that the model can be used predict the extent of bio-desulphurization with 99.91% accuracy given the initial substrate concentration, pH and temperature.

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## 1. Introduction

A vast amount of research is performed on the development of aerobic microbiological desulphurization. Dibenzothiophene (DBT) is the key heterocyclic sulphur compound used in most bio-desulphurization studies. One of the aerobic pathways is the Kodama. The transformation of DBT in the 'Kodama' pathway results in ring cleavage of one of the aromatic DBT rings, while the sulphur is not released [1]. [2] In their bio-desulphurization experiment, the organism was suspended in a sulphur free phosphate medium and the kerosene. It is easier for the organism to utilize carbon in glucose which is in aqueous state in which the organism is also suspended if available than kerosene which is oil. Thus, it was concluded that the bio-desulphurization of thiophene and 2, 5 - dimethyl thiophene took place via a sulphur-specific degradation pathway. The GC analysis revealed that the kerosene contained 6.955 mg/l of thiophene and 41.724 mg/l of 2, 5 - dimethyl thiophene. No benzothiophene and dibenzothiophene were found in kerosene. It showed that Desulfobacterium indolicum steadily desulphurised the benzothiophene decreasing its concentration to 1.72 mg/l at the end of 72 hours.

To reduce sulphur-related air pollution, the level of sulphur in fuels is regulated, and to meet these regulations sulphur must be removed from fuels during the refining process. The availability of low-sulphur crude has decreased over the last decade as a consequence of the

increasing reserves of heavy crude [3]. The sulphur compounds may even precipitate. This strategy is more efficient if the sulphur compounds are oxidized. Before the ionic liquids can be recycled, the water must be removed. Water evaporation from ionic liquids is a crucial step in this process in terms of energy consumption. The multi-stage evaporation process (four-stage evaporation) at different temperatures and pressures was proposed to save energy [4].

Literally hundreds of papers on ODS appeared in the past 2 years, indicating that this is a field of considerable interest at present. The most common industrial application of ODS, is sweetening. Sweetening is a refining process that is employed for the conversion of thiols into disulphides [5]. It is possible 30 to sulphurise crude oil directly by selecting appropriate microbial species [6]. Fossil fuels are the most widely used sources of energy in the world. Although the percentage of energy obtained from fossil fuels declined over recent years, the share of world energy from fossil fuels is still over 82%, half of which comes from petroleum [7]. At high biomass concentration, downstream processing is complicated, because proteins originating from the biomass emulsify the oil/water mixture.

In addition, mixing efficiency and O<sub>2</sub> availability is less optimal in emulsions with high biomass concentration. Furthermore, diluted sulphate is formed as the end product of the aerobic route that must also be removed, while the H<sub>2</sub>S that is formed in anaerobic route can be treated with

existing refinery desulphurization plants (e.g., Claus process). In the aerobic sulphur specific route oxygen molecules are added to the hydrocarbon skeleton. This is not desirable, because 2-hydroxybiphenyl is involved in the formation of viscous oil sludge (gum) in the fuel. Furthermore, in product inhibition, 2-hydroxybiphenyl formed in the cells will eventually diffuse back to the oil phase, but the phenolic molecule is a well-known biocide this as reported by [8] it become necessary to control biomass concentration for effective biodesulfurisation [9]. isolated novel strain capable of more BDS rate and optimization of DBT removal by both growing and resting cells.

Response surface Methodology (RSM) was applied for evaluating the interactive effects of three independent factors including DBT concentration, temperature and pH. The three factors Box Benken design with three centre points was performed to generate the optimum condition for DBT removal by growing cells in aqueous medium and resting cells in biphasic medium. Among the isolated bacteria from oil contaminated soil, a gram positive, non-spore forming isolate designated PD1 showed the high BDS rate and capable to convert the DBT to 2hydroxybiphenyl (2HBP) as the final product.

Analysis of variance (ANOVA) demonstrated that all of the studied parameters in the growing cells system showed significant effect on BDS rate, while in the resting cells effect of pH was not significant ( $P > 0.05$ ). Maximum 2HBP production (0.21 mM) by growing cells of PD1 strain was obtained at 0.38 mM initial DBT concentration, pH 6.88 and temperature of 27.57°C. For resting cells, maximum BDS activity of PD1 strain was determined as 0.46  $\mu$ M 2HBP/min g DCW at optimum pH 6.29, temperature of 26.13°C and DBT concentration of 7.73 mM. The BDS efficiency of Rhodococcus erythropolis PD1 (NCBI Gene Bank Accession no. JX625154) was increased by setting each factor at the optimum level [9].

The pH is an effective parameter that controls the bacterial growth rate [10]. However, because of the dependence of the 3D shape of enzymes on the pH enzymes are affected by the pH variations in the system. The impact of pH variation is not devoted to only the shape of enzymes. However, it may also affect the shape or electrical charge properties of the substrate in such a way that the substrate cannot bind to the enzyme active site or it cannot undergo catalysis [11]. Previous studies showed that the highest DBT bio-desulphurization by the bacterial strains were obtained at pH near to neutral conditions [12]. The optimum pH in growing condition was 6.88 and 2HBP production was reduced by decreasing or increasing of pH. Optimum pH was obtained as 6.29 for BDS of DBT in model oil system. In contrast with the growing cells, the pH factor and interactions between pH and temperature were not significant for BDS by resting cells in biphasic system. Also, it has been indicated that the purified 4Spath way enzymes such as flavin reductase have higher activities at the pH interval of 6 to 8 [13].

Maximum BDS activity of PD1 resting cells was determined as 0.46  $\mu$ M 2HBP/min g DCW at optimal conditions. [14] Reported that newly isolated Brevibacillus invocatus C19 relative to R. erythropolis IGTS8 removed

66.85 and 50% of the initial 1000 ppm DBT and produced 31.98 and 31.34 ppm 2HBP, respectively. Isolated PD1 strain in this study showed higher ability to removal of DBT mainly from oil model thus could be used for petroleum DBT bio-desulphurization.

In particular, temperature should be studied as an interactive factor, because it affects all chemicals and biochemical processes [15]. It has been indicated that in bio-desulphurization by Mycobacterium phlei WUF1, conversion of DBT to DBTO2 would be stopped at high temperatures while the conversion of DBTO2 and other intermediate compounds would continue at the same conditions. It can be concluded that the activity of the first enzyme in the 4Spathway which oxidizing DBT to DBTO2 is more sensitive to temperature variations and is considered as the BDS rate limiting enzyme [16]. Therefore, the aim of the study is to determine the optimum substrate concentration, pH and temperature on bio-desulphurization using regression model

## 2. Materials and Method

### 2.1 Experimental Design

Box Wilson experimental design method was used to design the various combinations of independent variables such as initial substrate concentration ( $X_1$ ), pH ( $X_2$ ), and temperature ( $X_3$ ) and how it varies with extent of bio-desulphurization ( $Y$ ). The following is the governing empirical model for the experimental design:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

Where  $\beta_i$  and  $\beta_{ij}$  are constant parameters to be estimated by regression analysis. Each independent variable consists of central points, a high and a low point, an axial high and an axial low point. The axial high and low points ( $\pm \alpha$ ) is determined using Equation 2

$$\alpha = C_p \pm \frac{H_p - L_p}{\sqrt{k}} \quad (2)$$

Where  $C_p$  is the Centre point,  $H_p$  is the high point,  $L_p$  is the low point,  $k$  is the number of independent variables. Table 2 shows all the possible experimental runs for determining the extent of bio-desulphurization using Box Wilson experimental design method.

Table 1: Parameter Values of Initial Substrate Concentration, pH and Temperature for Box Wilson Design of Experiments

Independent Variable	Centre Point (0)	High Point (1)	Low Point (1)	Axial High ( $\alpha$ )	Axial Low ( $-\alpha$ )
Initial Substrate Concentration (mg/l)	150	170	130	173.09	126.91
pH	6.75	7.00	6.50	7.04	6.46
Temperature (°C)	35	45	25	46.54	23.45

The total number of experimental trials are given by;

$$-N = 2^k + 2k + 1 = 2^3 \times 3 + 1 = 15 \quad (3)$$

Table 2: Runs for Determination of Extent of Bio-desulphurization Using Box Wilson Design Method:

Run	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>
1	170	7.00	45
2	170	7.00	25
3	170	6.50	45
4	170	6.50	25
5	130	7.00	45
6	130	7.00	25
7	130	6.50	45
8	130	6.50	25
9	150	6.75	35
10	150	6.75	46.54
11	150	6.75	23.45
12	150	7.04	35
13	150	6.46	35
14	173.09	6.75	35
15	126.91	6.75	35

## 2.2 Procedure for Data Analysis

A result of Experimental runs was analysed using Microsoft Excel 2016. Graphical plots were used for the presentation of experimental data while regression analysis used to estimate empirical model parameters.

## 3. Results and Discussion

The extent of bio-desulphurization was determined for all 15 experimental runs and the results are presented in Table 3.

Table 3: Results of Experimental Runs

Run	X <sub>1</sub>	X <sub>2</sub>	X <sub>3</sub>	Y
1	170	7	45	0.559
2	170	7	25	0.514
3	170	6.5	45	0.558
4	170	6.5	25	0.513
5	130	7	45	0.549
6	130	7	25	0.504
7	130	6.5	45	0.548
8	130	6.5	25	0.504
9	150	6.75	35	0.845
10	150	6.75	46.54	0.635
11	150	6.75	23.45	0.566
12	150	7.04	35	0.810
13	150	6.46	35	0.808
14	173.09	6.75	35	0.732
15	126.91	6.75	35	0.715

Table 3. showed that the maximum extent of bio-desulphurization is 84.5% which is the result obtain from experimental run 9 when initial substrate concentration is 150 mg/l, pH is 6.75 and temperature is 35°C. The minimum extent of bio-desulphurization is 50.4% which the results for experimental runs 6 and 8 which only differ in terms of pH (i.e., pH for run 6 is 7 and pH for run 8 is 6.5). The study sought to determine the effect of each of these independent variables on the extent of bio-desulphurization. Since the number of independent variables is 3 with 1 dependent variable, this will require a 4-dimensional plot for proper data visualization. However, even with modern technology, a 4-dimensional plot is quite tedious to achieve because entire universe is modelled spatially in 3-dimension.

### 3.1 Effect of Initial Substrate Concentration

Figure 1 shows how initial substrate concentration varies with the extent of bio-desulphurization. The bar chart showed that a decrease in initial substrate concentration is accompanied by a corresponding decrease in the extent of reaction. Thus, to achieve higher extent of bio-desulphurization, the initial substrate concentration must be high as well.

### 3.2 Effect of pH

Figure 2 shows how pH varies with the extent of bio-desulphurization. The bar chart showed that an increase in pH from 6.5 to 7.0 does not seem to alter the extent of bio-desulphurization. This could be because the pH range used for this study is not long as all pH values fall within the neutral zone, hence the microorganism can be able withstand changes in the pH. A wider pH range might give significant variations in the extent of bio-desulphurization.

### 3.3 Effect of Temperature

Figure 3 shows how temperature varies with the extent of bio-desulphurization. The bar chart showed that a decrease in temperature from 45° to 25°C is accompanied by a decrease in the extent of bio-desulphurization.

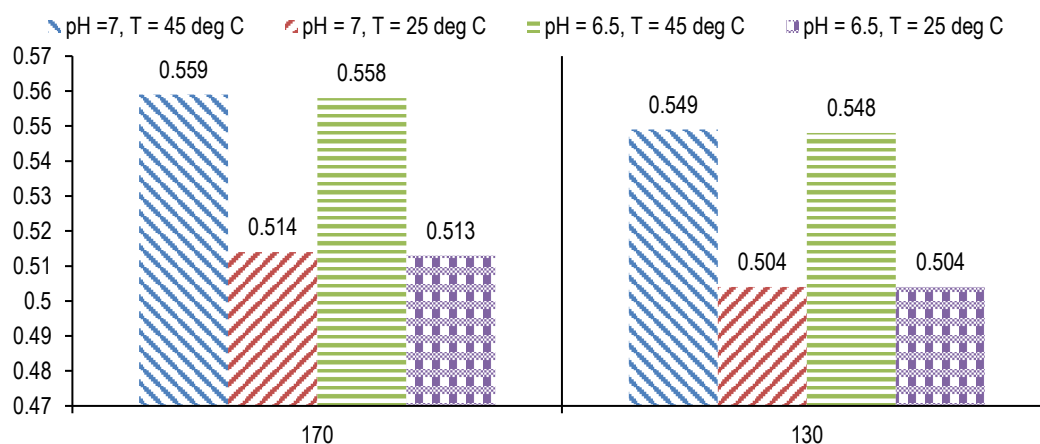


Figure 1: Effect of Initial Substrate Concentration on Bio-desulphurization

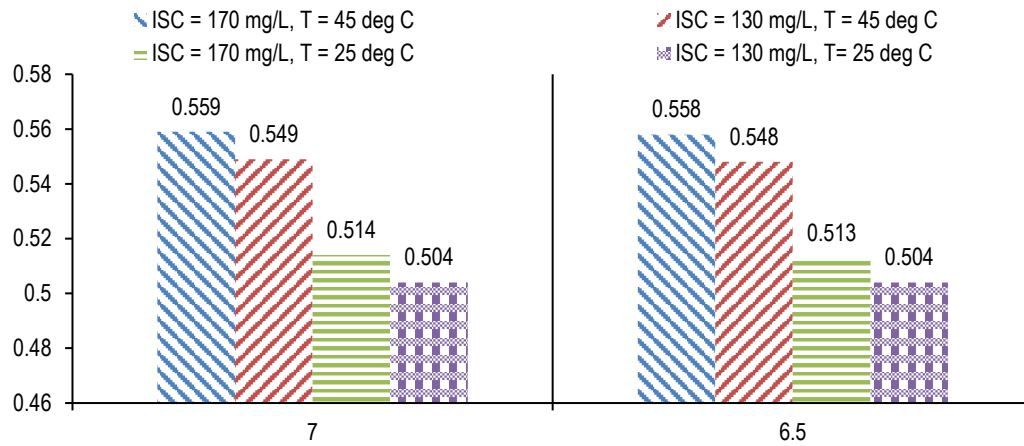


Figure 2: Effect of pH on Bio-desulphurization

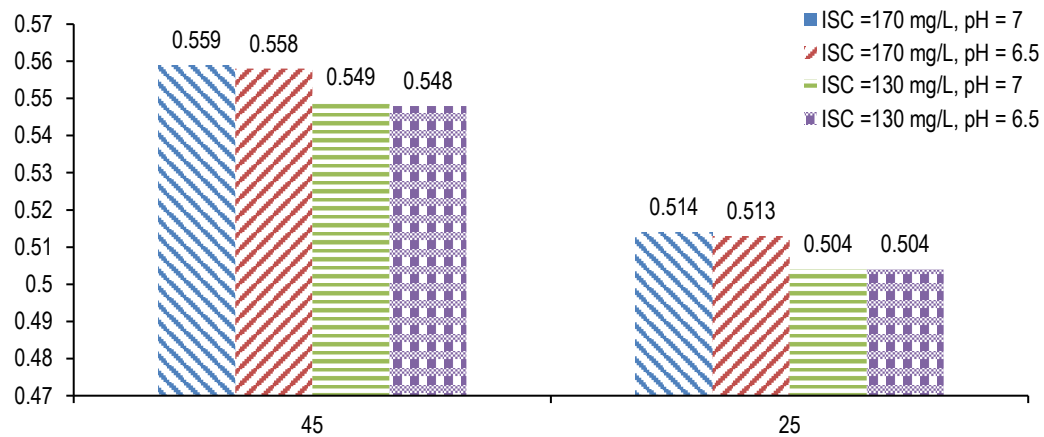


Figure 3: Effect of Temperature on Bio-desulphurization

### 3.4 Regression Analysis of Empirical Model

The empirical model for this research as presented in equation 1 is given by

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

The coefficients or parameters i.e.,  $\beta$ -values for Equation above were determine by carrying out regression analysis in Excel 2016. Figure 4 show a screenshot of the result of the analysis

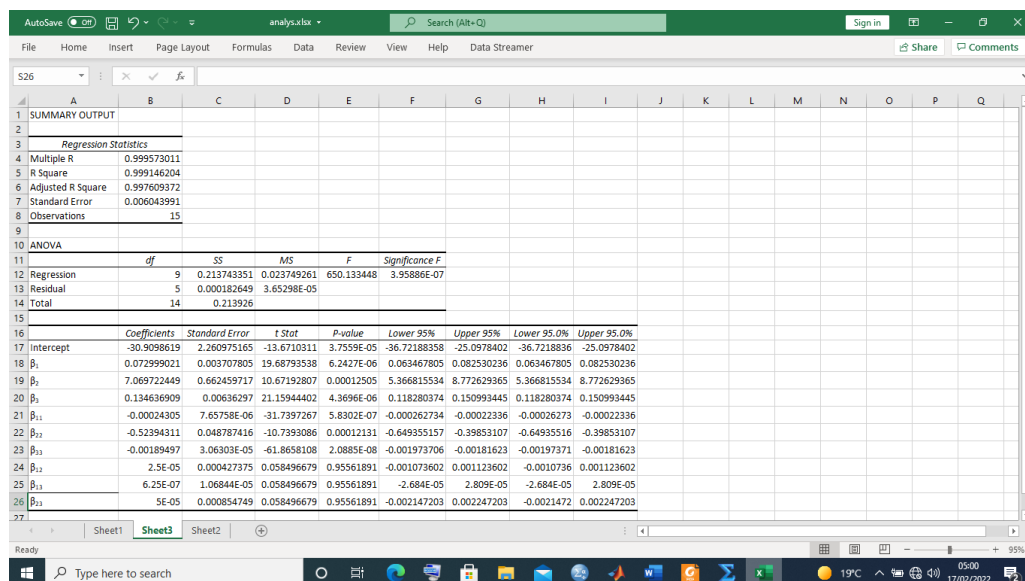


Figure 4: Regression Analysis of Empirical Model

From Figure 4, it can be seen that the R-squared value is 0.9991 which implies that the three independent variables i.e., initial substrate concentration, pH and temperature account for 99.91% of the variations in the extent of bio-desulphurization. In more clearer terms, the proposed empirical model is 99.91% accurate in predicting the extent of bio-desulphurization when given these three operation conditions and parameters. The p-value of the ANOVA table symbolized by significance  $F = 3.95886 \times 10^{-7}$  showed that the variations in the model output are very significant and it is due to the independent variables. Thus, initial substrate concentration, pH and temperature have a very significant effect on the extent of bio-desulphurization. A closer look at the p-values of the  $\beta$ -parameters showed that the p-values of the  $\beta$ -parameter for each independent variables and their squares is less than 0.05 which implies a high level of significance, while p-value of  $\beta$ -parameters for interaction of independent variables were higher than 0.05 which implies that interaction of independent variables does not have significant impact on the extent of bio-desulphurization. The empirical model is therefore given as:

$$Y = -30.91 + 0.073X_1 + 7.07X_2 + 0.135X_3 - 0.000243X_1^2 + 2.5 \times 10^{-5}X_1X_2 + 6.25 \times 10^{-7}X_1X_3 + 5 \times 10^{-5}X_2X_3$$

#### 4. Conclusion

Conclusively, an increase in the initial substrate concentration leads to an increase in the extent of bio-desulphurization when the other independent variable was held constant. Similarly change in the pH did not show any significant change in the extent of bio-desulphurization when other variable was held constant. This could be due the fact that the pH range used for this experiment i.e., 6.5 to 7.0 was in the neutral range hence has no significant impact on microbial activity and thus extent of bio-desulphurization. Also increase in the temperature from 25°C to 45°C leads to a significant increase in the extent of bio-desulphurization provided that the other independent variables are held constant. The empirical model developed had an R-squared value of 0.9991 which implies that the model can be used predict the extent of bio-desulphurization with 99.91% accuracy given the initial substrate concentration, pH and temperature. Interactions between independent variable have no significant effect on the extent of bio-desulphurization. Finally, it can be concluded that at the optimum pH, temperature and DBT concentration, cell efficiency increases and the highest rates of bio-desulphurization activity can be obtained by setting all these effective factors at the optimum levels [9].

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