

# KINETICS OF BIODESULFURIZATION OF DIESEL USING IMMOBILIZED CELLS

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## ABSTRACT

Today, as a result of sulphur compound pollutants emanating from combustion of fuel containing sulphur, the environmental protection agencies have imposed stringent limits for sulphur levels in fuel. The current industrial method for removal of sulphur from fuels is hydro desulfurization (HDS), which is costly and energy-intensive for deep desulfurization. Furthermore, some sulphur compound are resistance to HDS process, thus biodesulfurization (BDS), which operates under ambient temperature and pressure, is expected to be a complement and a promising alternative to HDS. In this study, the bacterium *Pseudomonas stutzeri* was isolated and immobilized on sodium alginate and subsequently used to desulfurize diesel. The desulfurization was modeled and the obtained simulated results were compared to the experimental ones. Kinetics parameters such as maximum rate constant ( $k_1$ ), Michaelis-Menten constant ( $K_m$ ), absorption constant ( $n$ ) and mass transfer co-efficient ( $k_L$ ), were determined experimentally. It was observed from the biodesulphurization of diesel that there is high desulfurization affinity of benzothiophene, BT compared with dibenzothiophene, DBT. The DBT was desulfurized from 161.02 mg/L to 22.45 mg/L (86%), and theoretically from 161.02 mg/L to 29.27 mg/L while BT from 9.57 mg/L to 1.72 mg/L (82%), and theoretically from 9.57 mg/L to 2.34 mg/L. The equation obtained from the model development shows better agreement with the desulfurization of DBT than BT.

**Key words:** Biodesulfurization, Immobilized cells, *Pseudomonas*, Kinetics

## 1 INTRODUCTION

Sulphur is the third most common element in the crude oil composition after carbon and hydrogen, it is present both in inorganic and organic forms. The presence of Sulphur in fuels causes severe environmental pollutions. Combustion of petroleum based fuels containing sulphur components will release oxides of sulphur ( $\text{SO}_x$ ) gases to the atmosphere leading to acid rain, corrosion problems and as pollutant to the environment. Consequently, deep desulfurization of the crude oil is compulsory to prevent the production of such pollutants [1]. However, the effect of these pollutants has called the attention of environmental regulation bodies to impose the emission limit of these poisonous gases. The refineries are expected to adhere to this regulation by reducing the sulphur contents in their refined products to as low as 100 ppm. For hydrodesulfurization to achieve this objective it would require large reactor volume, longer processing time, substantial hydrogen and energy input, deposition of carbonaceous coke and lastly the hydrogen sulphide generated poison the catalyst as inhibitors. All of these requirements are capital intensive [2]. The major challenges of oil refineries are to increase fuel quality standard and reduce pollutant emissions from fossil fuels [3]. Some of the organic sulfur are not easily removed by conventional desulfurization treatment of hydrodesulfurization (HDS). In [4] oxidative desulfurization (ODS) technology was recognized as one of the most effective methods for the removal of sulphur in fuel oils, due to the very mild operation conditions it requires. The ODS methods was divided into  $\text{H}_2\text{O}_2$  oxidation method, organic oxidation method, photochemical oxidation method, as well as those involving the use of plasma or ultrasound based on the oxidants involved. Recently, oxidative desulphurization of jet and diesel fuels

using hydrogen peroxide generated in-situ by catalytic air oxidation has been examined [5]. In [6] a comparative study of desulphurization potentials of two metal oxides-activated manganese dioxide and activated zinc oxide, including the kinetic analyses of the adsorption process was carried out. Also in [7] oxidative desulfurization of crude oil incorporating sulphone decomposition by alkaline earth metal oxides was investigated. They explored a novel approach to desulphurization of whole crude oil where thiophenic sulphur compounds were oxidized to sulphones followed by sulphone decomposition into sulphur-free hydrocarbons. Biodesulphurization(BDS) presents a complementary role to HDS. It can be carried out at ambient conditions like other bioreactions, additionally, it is substrate specific but generally is a slow process. To improve the rate of biodesulphurization, the process must be enhanced, one method of enhancement is immobilizing the cells of the microorganism carrying out the reaction. Immobilized microbial cells create opportunities in a wide range of sectors including environmental pollution control. Compared with suspended microorganism technology, cell immobilization shows many advantages, such as resistance to toxic chemicals. The ability of the immobilized spores of *Aspergillus flavus* cultured in increasing concentrations of sodium metabisulfite to remove sulphur from diesel oil was investigated [8]. Immobilization of cells containing specific enzymes has further advantages such as elimination of long and expensive procedures for enzymes separation and purification and it is vital to expand their application by enabling easy separation and purification of products from reaction mixtures and efficient recovery of catalyst [9]. In the case of the immobilization of microbial cells, their field of application spreads from industrial to environmental process. Microorganisms retained on a carrier can be used in continuous and semi-continuous production processes allowing for significant cost decrease, as the biocatalyst does not need to be refilled [10]. Recently, immobilized whole cell has been regarded as an alternative method to enzyme immobilization but the process is tedious and time consuming. Also the cost of isolation and purification of enzymes is high which is not necessary in whole cell immobilization. From [11] the main advantage that immobilization confers to whole cells are low sensitivity to temperature and pH changes when compared to free enzymes. From [12] it was discovered that immobilized microbial cells are able to maintain the microbial population in carriers without any loss during continuous operations of laboratory scale bioreactors. In [13] Kinetic model for DBT desulfurization by resting whole cells of *Pseudomonas putida* on model oil was studied and in [14] biodesulfurization of dibenzothiophene by immobilized cells of *Pseudomonas stutzeri* still on model oil was carried out without studying the kinetic. Similarly, from [1] studied the kinetics of biodesulfurization of diesel and kerosene but not on immobilised cells. Also in [8] a worked on microbial desulfurization of crude oil using *Aspergillus flavus* was carried out. In this work, the bacterium, *Pseudomonas stutzeri* was isolated and immobilized on sodium alginate and subsequently used to desulfurize diesel.

## 2 MATERIALS AND METHOD

Soils contaminated with diesel were collected from diesel store, administrative block generator room and Computer Centre generator room, all in Modibbo Adama University of Technology, Yola, Nigeria. All the samples were kept in sterile polythene bags. The diesel sample was purchased from a local filling station along Numan – Mubi highway in a sterilized plastic container. The microorganism was isolated and identified as previously described by [15]. The cells were immobilized by direct cell entrapment into polymer matrices of sodium alginate (SA). 3 g of Sodium alginate was dissolved in 100 ml deionized water at 100°C and allowed the temperature of agar gel solution cooled to 45°C, five grams cell suspension was added to the solution and thoroughly mixed. This mixture was then put into 4% (w/w) CaCl<sub>2</sub> solution to form the immobilized cells beads. The immobilized cells were then washed with deionized water and stored [14]. The biodesulphurization experiment was carried out by mixing the immobilized cells (10g of bead containing 0.125 g of dry cells in 100ml of diesel) with 9 mL of sulphur-free phosphate buffer of pH 7 in 250 mL Erlenmeyer flask containing 2% w/v glucose solution. The flask was then immediately placed into Gallenkamp rotary shaker incubator for 48 hours. The temperature of the shaker was 35°C and operated at a speed of 120 rad/min. The experiments were conducted for 6, 12, 18, 24, 30, 38, 42 and 48 hours. Thereafter, the separation of the oil and cell suspension was done by centrifugation for 30 minutes. 10 ml of the oil phase was removed with a syringe and was taken for analysis of the sulphur content. The concentration of dibenzothiophene and benzothiophene were analyzed by Varian

gas chromatography (Model 3800) using a WCOT FUSED SILICA Colum and a flame ionization detector. The column oven temperature was varied from 100 to 280°C, and the temperature of injector and detector were 320°C. N<sub>2</sub> and air were the carrier gas. Then the change in concentration of these sulfur-containing compounds in diesel with time was extrapolated. The values were plotted against time [14]. Kinetic model that was used to describe the process was adopted from [15]. Kinetics parameters were also obtained as outlined by [15].

### 3 RESULTS AND DISCUSSION

The DNA of the isolated microorganism was extracted and gel electrophoresis was conducted to confirm the presence of DNA which was viewed using UV illuminator and later amplified by sequencing with biocatalyst 16S Rrna genes using sequence of the forward primer (16SF) 5-AGAGTTTGATCCTGGCTCAG. The amplified segment was matched in NCBI Genesbank database where it was confirmed as *pseudomonas strutzeri*. Figure 1, shows the extent of conversion for the biodesulfurization of dibenzothiophene and benzothiophene in diesel using immobilized cells. It was observed that from zero to thirty five hours both substrate shows equal conversion rate from then the influence of higher concentration of substrate favoured Dibenzothiophene to achieved high extent of desulfurization than Benzothiophene . Similarly, Figures 2 and 3 show the concentration versus time for experimental and theoretical results for DBT and BT. Theoretically the mass transfer rate shows high significant effect in DBT and very low in BT which might be as a result of low concentration of BT in the diesel. In theory, higher concentration of reactants implies faster reactions; consequently, equal rates can only imply parallel reactions. DBT was desulfurized steadily from its initial concentration of 161.02 mg/L to 22.54 mg/L in 48 hours of desulfurization while theoretically from 161.02 mg/L to 29.27 mg/L as shown in figure 2. This translates to about 86% desulfurization. The plot for BT in figure 3 showed that the substrate desulfurized steadily from its initial concentration of 9.57 mg/L to 1.72 mg/L in 48 hours of desulfurization. This translates to over 82% desulfurization while theoretically from 9.57 mg/L to 2.34 mg/L. In [14] *Pseudomonas stutzeri* immobilized in sodium alginate beads and achieved 95 % desulfurization on DBT in model diesel. However for this research conducted on real diesel it achieved 86% conversion, this might be as a result of high mass transfer resistance in real diesel than the model diesel. Immobilization method reduces the cost of downstream process but the product inhibition by 2-hydroxybiphenyl might play a role of reducing the biodesulfurization activity by adhering at surface of the bead which seriously affect the adsorption of substrate into the bead. From [16] *P.putida* and *R.erythropolis* were used on model oil (16MI Hepes buffer containing 10um of DBT) and achieved 96% conversion which shows that the microorganisms are more effective in model oil than real diesel. Similarly for the kinetic, since the kinetics of interest is disappearance of substrate not the product formed therefore lumping, the rate equations to obtain the overall rate equation to get rate constant and Michael-Mentis constant is far more better than to obtained the kinetic parameters of each intermediate as done in [12]. Eadie-Hofstee, Lineweaver-Burk and Hanes plots were used in [1] to obtain rate constant k and the Michaelis – Menten constant K<sub>M</sub>. The results showed that, the three methods have little difference from one another. For this research work, integral method was used to linearized Michaelis – Menten equation which is more precise. The results showed that Michaelis – Menten constants were smaller compared with that obtained in [1], this might be as a result of difference in microorganism used. In theory the lower the Michaelis–Menten constants the higher the affinity of biodesulfurization. It is pertinent to examine the extent to which the theoretical model proposed agrees with the experimental result. Levenspiel shows that the concentration-time behaviour of Michaelis–Menten equation has three dynamic behaviour that is first order behaviour which is curve shape follow by shift point at C<sub>A</sub>=K<sub>M</sub> and lastly zero order behaviour which is a straight line. In Figures 2 both theoretical and experimental results showed first order behaviour and Figures 3 showed zero order behaviour theoretically and first order behaviour experimentally, this means that for zero order behaviour, the rate of desulfurization is independent of concentration. The results of the experimental and theoretical of the biodesulfurization were presented in Figures 2 and 3.

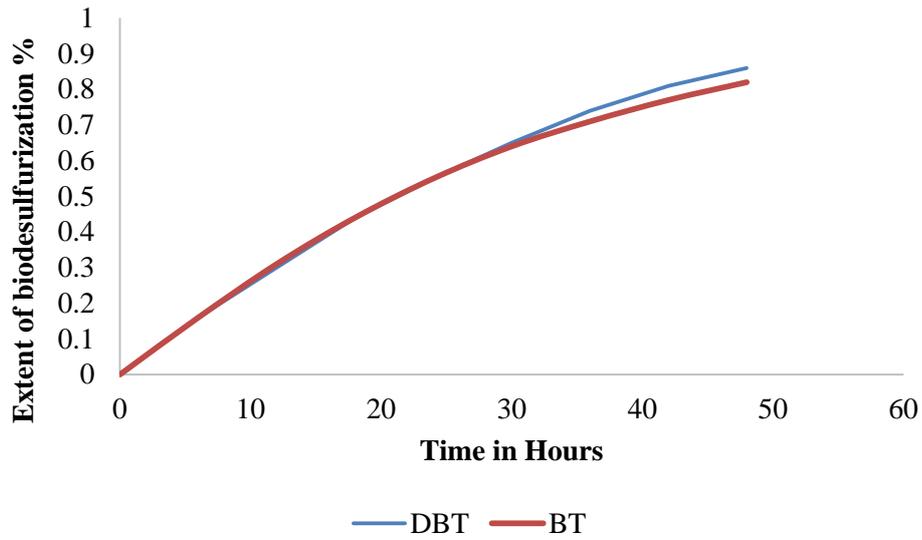


Figure 1. Conversion profile against time for Dibenzothiophene and Benzothiophene

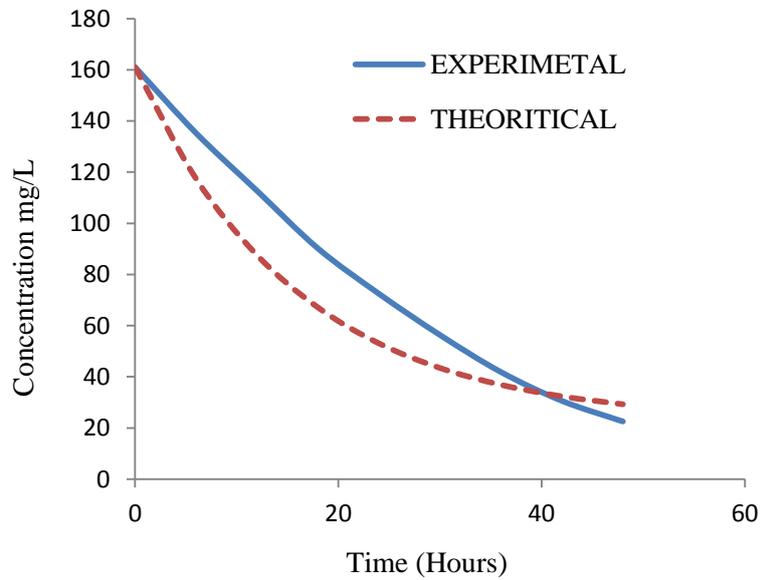


Figure 2. Concentration profile of Dibenzothiophene with Time.

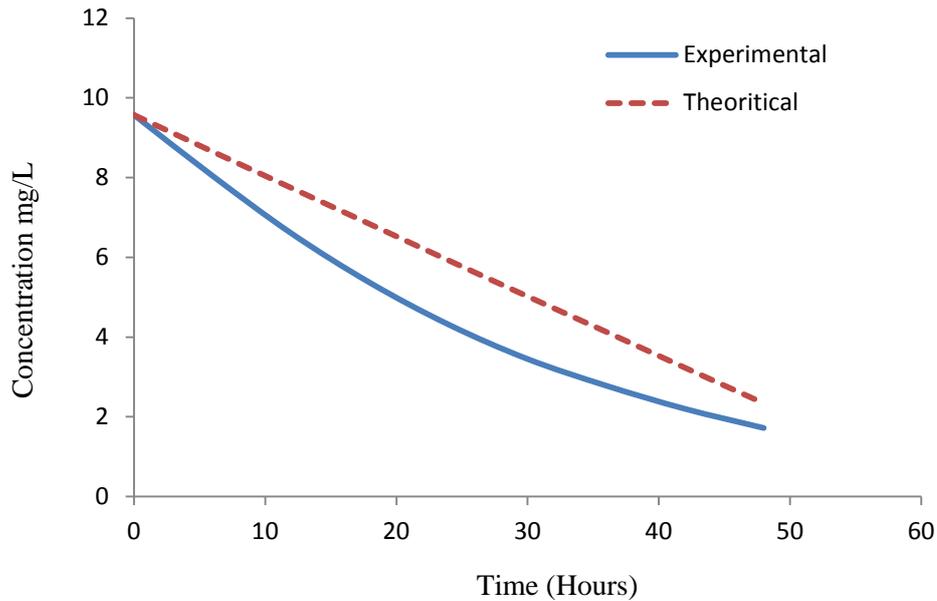


Figure 3. Concentration profile of Benzothiophene with Time

The kinetic parameters used in the developed model equation were obtained from the work in [15], to obtain the theoretical values for both DBT and BT, these were presented in Figures 2 and 3 respectively. The six kinetics parameters were determined for the two substrates and were presented in Table 1. The relationship between concentration and Michaelis-Menten constant, showed that when  $C_A = K_M$  half of the enzymes are in free form the others half combined, when  $C_A \gg K_M$  most of the enzymes are tied up as complex and lastly when  $C_A \ll K_M$  most of the enzymes are in free form. Considering the initial concentration of DBT and BT  $C_A \gg K_M$  which means that most of the enzymes are tied up as complex with high desulfurization activity.

Table 1. Parameters of the Model Equation

	$K_{La}$ (mg/Lh)	$K_M$ (mg/L)	$k$ (mg/Lh)	$k_f$ (mg/Lh)	$C_s$ (mg/Lh)	$n$	$n_i$
DBT	-0.0634	69.017	1.036	0.096	22.54	0.58	1.1746
BT	-0.0624	4.437	0.053	0.802	1.72	15.77	0.0634

From [1] it was reported that the Michaelis-Menten constant, for Benzothiophene by *Desulfobacterium aniline* and *Desulfobacteriumindolicum* was 18.050 (mg/L) and 19.837 (mg/L) respectively, and 1182.27 (mg/L) and 192.7827 (mg/L) for Dibenzothiophene which was higher compare with one obtained by *pseudomonas strain* which is 4.437 mg/L and 69.017 mg/L as presented in Table 1. This shows that *pseudomonas* has high desulfurization affinity than *Desulfobacteriumanilini* and *Desulfobacteriumindolicum*. That might be one of the reasons *pseudomonas* achieved 86% and 82% conversions for both DBT and BT in forty eight hours. Comparing this with model developed in [13] this research was a little different in which the influence of mass transfer on biodesulfurization was taken into consideration. The volumetric mass transfer coefficient was determined as the intercept by plotting dependent variable against time [14]. The volumetric mass transfer coefficient for both DBT and BT was found almost the same; these implied that mass transfer coefficient for both substrates in diesel was the same. However, the rate of mass transfer was difference because of difference in substrates concentration and volumetric mass transfer coefficients  $k_{La}$  which were time dependent. One of the useful models for isothermal adsorption is the Freundlich model, equation 1.

$$\ln q = \frac{1}{n} \ln C + \ln k_f \tag{1}$$

Where  $k_f$  and  $n$  are constants based on the characteristics of the particular adsorption system. If adsorption is favorable, the value of  $n$  is greater than 1. For  $n$  less than 1, the adsorption process is not favorable. The result presented in Table 1 showed that the value of  $n$  in BT is higher than DBT which ascribe high adsorption in BT than DBT on biodesulfurization process. The kinetics parameters were a key that can be used to properly describe mass transfer operations and reaction. The kinetics parameters are not valid if they do not agree with experimental data. The root mean square error (RMSE) of BT is less than that of DBT. One reason for this conflicting result is that the concentration of DBT is very high compared to BT hence deviations in DBT are small compared to BT when calculated as a percentage of the initial concentrations in both cases. The RMSE for BT and DBT accounts for 12.12% and 9.48% respectively. Thus the kinetics shows better agreement with the experimental data of DBT than that of BT, this deviation was as a result of temperature changes during the experiment. As stated earlier, the initial concentrations of the substrate are not the same, as a result, the standard error computed for both sets of data have nothing in common. If the RMSE deviation must be used, both data must be converted to values with a similar basis. One common method is to calculate the RMSE deviation of the conversion rather than concentration [17]. This will convert all the different concentrations to percentage or decimal fraction with the same initial value of zero ( $X_{A0}=0$ ). This could be the reason that in [9] conversion values were used to determine standard deviation rather than concentration values. The result showed that this kinetics has a better agreement with DBT than BT, which may be due to the higher concentration of DBT. From basic knowledge of the factors affecting the rate of reaction, the higher concentration implies faster reaction rates, making mass transfer to be rate controlling. This seems to suggest that mass transfer is the rate controlling step, since DBT has a very high mass transfer coefficient and a lower reaction rate constant. Table 2 gives the summary of the root mean square error for the kinetics. The third column of the table expresses the RMSE deviation as a percentage of initial substrate concentration.

Table 2. Root Means Square Error of the Experiment

	$C_{A0}$ (mg/L)	RMSE	Percentage (%)
DBT	161.02	15.27	9.48
BT	9.57	1.16	12.12

#### 4 CONCLUSION

The isolated microorganism was *Pseudomonas strutri*; it was found to be capable of biodesulfurizing the two selected sulfur compounds DBT and BT as substrate. It was observed that there was high desulfurization affinity in DBT compared with BT., the *Pseudomonas* achieved 86% conversion in Benzothiophene and 82% in Dibenzothiophene while in model diesel it achieved 95% desulfurization as reported in literatures. The determined kinetic parameters showed better agreement with DBT than BT. Kinetics parameters obtained represent a valuable pool of information that can be used for reactor designs that will take the technology to the market. To overcome the limitation biocatalysts capable of desulfurizing a broader range of sulfur compounds should be screened or developed by using genetic engineering. The production of 2-hydroxybiphenyl has to be reduced by modifying the oxidative pathway because it serves as an inhibitor to microbial growth at high concentration and also it increases the oil viscosity which is one of the critical parameters for diesel quality.

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