IMPACT OF MAGNETIC NANOPARTICLES ON THE KINETICS OF BIODESULFURIZATION OF DIESEL

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ABSTRACT

The need to reduce sulfur content has become imperative. The sulfur content of hydrodesulfurized fuel are still high because of the presence of some sulfur compounds that are either recalcitrant or are refractory. The regulatory agencies are mounting pressure because the combustion of sulfur containing fuel produce substances that are harmful to the environment. Biodesulfurization readily comes handy as a complimentary method to hydrodesulfurization, however, biodesulfurization like other bioreactions is slow. For biodesulfurization to play the complimentary role effectively, deliberate attempt must be made to make it faster. This study in an attempt to do that has synthesized a magnetic nanoparticle, characterized it using Telescopic Electron Microscope, TEM and X-ray Electronic Microscope and then coat the synthesised particle with an isolated Pseudomonas stutzeri. The impact of the nanoparticle on the biodesulfurization activity of Pseudomonas stutzeri on diesel was investigated. The synthesized magnetic nanoparticles have a size range of predominantly between 9.75 and 10.25 nm. The results from this work showed a high level of desulfurization of 88% for coated bacteria as compare to 68% for uncoated bacteria. A kinetic model describing the desulfurization was also developed. In all cases, the coated organism desulfurise better than the uncoated one. The simulated data from the developed model were found to fit well to all the experimental data.

Keywords: *Biodesulfurization; Diesel; Magnetic; Nanoparticles; Kinetics*

1. INTRODUCTION

Researchers have shown that alkylated dibenzothiophenes are the major sulfur component in the conventional hydrodesulfurized treated oil fraction due to the fact that they are highly recalcitrant to chemical catalysts. According to Moheballi and Ball (2016), biodesulfurization technology should be viewed as complementary technology to remove recalcitrant molecules present in hydrodesulfurized-treated oils. In line with the above statement, more researches need to be carried out on desulfurization of petroleum fractions in order to meet up with the lower sulfur level (15-10ppm) as regulation proposed by United state environmental protection agency (Mayank *et al*, 2016).

Magnetic nanoparticles play a vital role in our industries and in the field of biosciences due to the fact that they have finite size effects such as high surface-to-volume ratio, different crystal structure and low toxicities. According to Ansari *et al*, (2009), the rate of biodesulfulrization activities shown by bacteria coated with nanoparticles increase as compared to uncoated bacteria. Karimi *et al*, (2016) investigated the application of magnetic nanoparticles on the rate of biodesulfurization of dibenzothiophene (DBT) using *rhodococcus erythropolis* IGTS8 and found that the coated cells had higher desulfurization activities as compared to uncoated cells. Likewise, Bardania *et al*, (2013), investigated the desulfurization activity and reusability of magnetite nanoparticle-coated *rhodococcus* bacteria and found that the coated cells had little impact on the rate of biodesulfurization of DBT.

In biotechnological processes, kinetic equations which describe the activity of microorganisms or an enzyme on a particular substrate are very important in understanding many phenomena. Description of a true behavior of a system can be done by obtaining an accurate estimate of the kinetic parameters in the models (Olsen, 2006). Kinetic studies of the four reactions of the 4S route have been reported in the literature, not as a reaction network but as single reactions. Several works have shown that desulfurization kinetics is only described as the DBT disappearance rate. Others researchers also used Michaelis–Menten equation as a model equation for biodesulfurization process (Mayank *et al*., 2016).

In this work, *Pseudomonas stutzeri* was isolated from the soil samples as a bacterium that feed on sulfur, this

work also synthesized magnetic nanoparticles to coat the surface of the bacteria in order to investigate its impacts on the rate of biodesulfurization of diesel. This work further developed a kinetic model using Michaelis-Menten equation coupled with mass transfer factor in order to investigate the kinetic parameters of biodesulfurization of diesel.

2. MATERIALS AND METHODS

A microorganism was isolated by enrichment culture. The isolated microorganism was purified and transported to the Chevron Biotechnology Centre MAUTECH, Yola where some cultural, morphological and characteristic tests were carried out for the purpose of identification and confirmation of its identity. Further confirmation of the identity was carried out by Polymerize Chain Reaction (PCR)

To obtain the Fe_3O_4 nanoparticles, 25 mL of 0.2 mM ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$) was mixed with 100 mL of 0.1 mM ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) solution in a flask. The testing begins at a temperature of 35 °C and after 90 minutes until the temperature reaches 80°C in refluxing condition under a nitrogen atmosphere while vigorously stirring the reaction mixture to prevent the development of particles larger than 25 nm. Then 1 g of glycine followed by 140 mL of NH_4OH (25% wt) was added to the reaction mixture under vigorous stirring until the pH was measured to be in the range of pH 10 – 13. The black precipitates were collected after cooling the reaction mixture to room temperature. Suspensions of the black precipitates were then washed several times with deionised water and were subsequently dried overnight at 50 °C. For complete coverage, the magnetite nanoparticles were synthesized by adding Polyethylene Glycol, PEG in two steps before synthesis (40 mL of solution containing 2 mg mL^{-1} PEG 1500MW) and after synthesis (60 mL of solution containing 2 mg mL^{-1} PEG 1500MW).

To coat the isolated microorganism with the synthesized magnetic nanoparticle, 15 mL of a magnetic suspension (15 g Fe_3O_4 nanoparticles per litre of saline water) was mixed with 100 mL of a cell suspension (25 g of cells/L of saline water). The microbial cells were coated by adsorbing the magnetic nanoparticles. The coated cells were concentrated on the side of the vessel containing the suspension and separated from the suspension medium with the aid of external magnet.

Biodesulfurization experiment was carried out by mixing 20 mL of diesel with 10 mL of sulfur-free phosphate buffer of pH 7 containing 0.5 mL of the cells suspension in sterile distilled water and 2%w/v glucose solution in a 250 mL flask. The flask was incubated on a Gallenkamp rotary shaker at 150 rpm for 5 hours in a temperature of 36 °C. The experiments were also conducted for 10, 15, 20, 25, 30, 35, 40, 45, and 50 hours in triplicates, the control experiment set up did not have the cell suspension. The experiments were done for both coated and uncoated cells of the isolate. The sulfur content was done with Gas Chromatograph Model 3800.

Model Development

The general mass balance equation of the substrate in the fuel over a batch reactor is given by

Input – Output – Reaction rate = Accumulation 1

But since the process is batch, input and output = 0

Therefore, Accumulation = – Reaction rate 2

There is no reaction in the fuel, so reaction rate is zero.

The material balance over the bacterium coated with nanoparticle, solid phase is given by

Accumulation = Rate of transfer of the substrate to the cell surface – Reaction rate 3

The rate of adsorption of DBT and BT into the reaction site (surface of the cells) from the bulk liquid is assumed to follow the Freundlich Isotherm. According to Muhammad et al., (2015), Freundlich isotherm is given by

$$Q = K(\mathrm{C}-\mathrm{C_s})^n \quad 4$$

Differentiating equation 4 gives

$$\frac{dQ}{dt} = nK\left(C - C_S\right)^{n-1}\frac{dC}{dt} \quad 5$$

$nK = K'$ Therefore equation 5 becomes

$$\frac{dQ}{dt} = nK'\left(C - C_S\right)^{n-1}\frac{dC}{dt} \quad 6$$

Where K' the Freundlich constant and the parameter n is the measure of heterogeneity of the surface of the adsorbent.

C is the concentration of DBT and BT in the liquid, C_s is the concentration of DBT and BT at the surface of the cell.

The external mass transfer is given by

$$r_{bf} = k_{La}(C - C_S) \quad 7$$

From equation 7, r_{bf} is the mass flux in $Kg/m^3.s$; K_L is the mass transfer co-efficient and a is the surface area to volume ratio in m^2/m^3

The rate of substrate disappearance in the 4S pathway according to Kareem (2010) is given by

$$-\frac{dC}{dt}=\frac{2kC}{K_M+C} \quad 8$$

On substituting equation 6, 7 and 8 into equation 3 gives

$$\frac{dC}{dt}=K'\left(C-C_S\right)^{n-1}\frac{dC}{dt}+k_La\left(C-C_S\right)-\frac{2kC}{K_M+C} \quad 9$$

On rearranging equation 9, we have

$$\frac{dC}{dt}=\frac{k_La\left(C-C_S\right)-\frac{2kC}{K_M+C}}{\left[1-K'\left(C-C_S\right)^{n-1}\right]} \quad 10$$

Equation 10 is the model equation for mass transfer influenced kinetics of biodesulfurization process based on 4S pathway and would be solved numerically by the 4th order Runge-Kutta method using Microsoft Excel.

The mass transfer coefficient k_La was obtained from slope of the plot of the LHS of equation 11 against time t

$$\ln\frac{C-C_S}{C-C_0}=k_Lat \quad 11$$

K' and n were obtained by linearizing the Freundlich isotherm,

$$q_e=K'C_e^{\frac{1}{n}} \quad 12$$

The linear form of equation 12 is

$$\ln q_e=\ln K'+\frac{1}{n}\ln C_e \quad 13$$

Where $q_e=\frac{m\left(C-C_S\right)}{V}$

The V is the volume of the solution; m is the mass of the magnetite while Cs is the equilibrium concentration. k and K_M were obtained from the plots of Lineweaver-Burk equation.

3. RESULTS AND DISCUSSION

Biochemical and morphological tests carried out on the isolated microorganism showed that the isolate has rod shape and raised elevation on nutrient agar with cream colour. It was observed to be Gram-negative due to their inability to retain the purple colour of the basic stain and, therefore, appeared pink under the light microscope. The isolate was also positive to the hydrogen sulfide test due to the present of black coloration of the lead acetate test paper. The result of the PCR confirmed that the isolate was *Pseudomonas stutzeri*.

The synthesized magnetite nanostructure was viewed by JEOL 1010 Telescopic Electron Microscope. Figure 1 showed the image, the particle size was predominantly 10.0 nm. Further characterization of the synthesized magnetite nanoparticles was obtained using XRD. This is to enable us understand the chemical composition of synthesized nanoparticles as well as the size of the nanoparticle. The output from XRD analysis yields a plot of intensity versus diffraction angle which can be used to determine the crystallographic planes that are being diffracted. The peaks of XRD patterns were analyzed and indexed using ICDD data base, comparing with magnetite standards (Lopez *et. al.*, 2010). As it can be seen in Figure 2, the numbers in parenthesis are reference standard pattern of Fe_3O_4 and give the Miller indices of pure Fe_3O_4 assigned to the observed peaks (Ansari *et al*., 2009).

The XRD pattern indicates the presence of predominantly Fe_3O_4 crystals. The discernible peaks which can be clearly identified in Figure 2 can be matched to, the (111), (220), (311), (400), (422), (511) and (440) planes of a cubic Fe_3O_4 unit cell, and it reflected by the well matching of the diffraction peaks with the magnetic pattern and it corresponds to that of magnetite structure. An estimation of the magnetite nanoparticles size has been performed from the Scherrer formula

$$D=\frac{K\lambda}{L\cos\Theta}$$

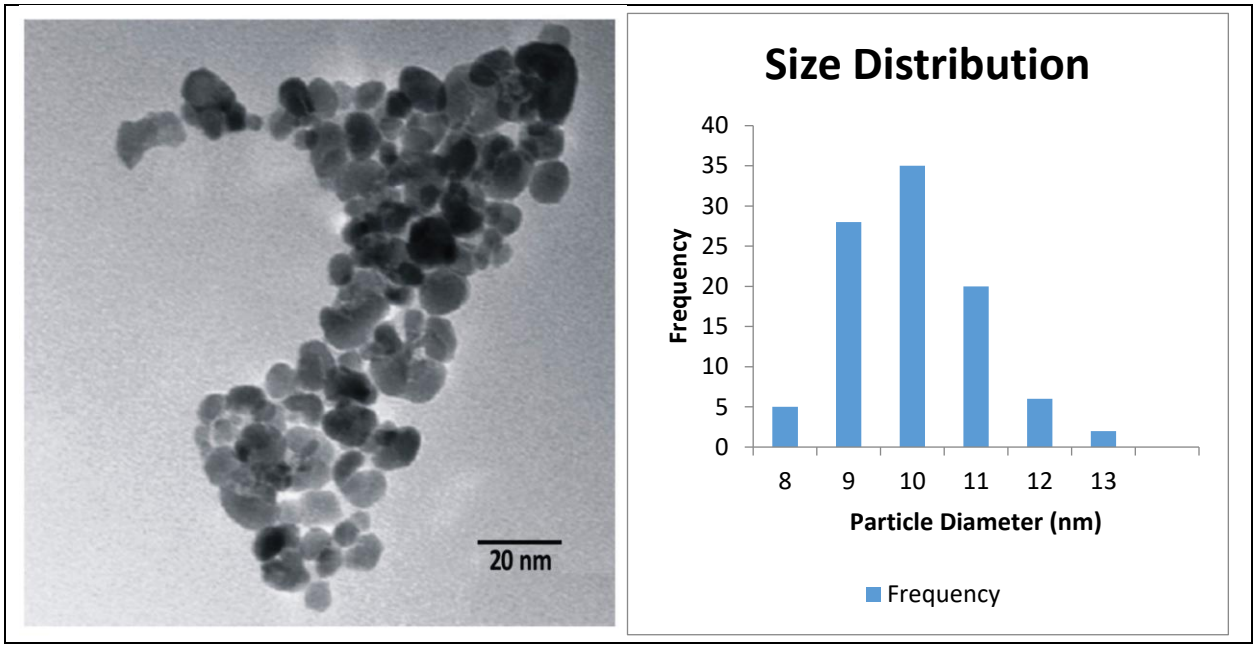

Figure 1 TEM Image and Particle Size Distribution of the Synthesized Magnetite

Where D is the crystallite mean size, λ is the X-ray wavelength (0.154 nm), L is the full width at half maximum (0.0142); θ is the corresponding Bragg angle; and K is the shape parameter, which is 0.89 for magnetite. Taking the highest intensity peak, namely the (311) plane, at 2θ = 35.7°, and the half maximum intensity width of the peak after accounting for instrument broadening, the calculated particle sizes was (10.15 ± 0.14) nm. The result is almost in agreement with the size as shown by TEM image which is 10.0 nm.

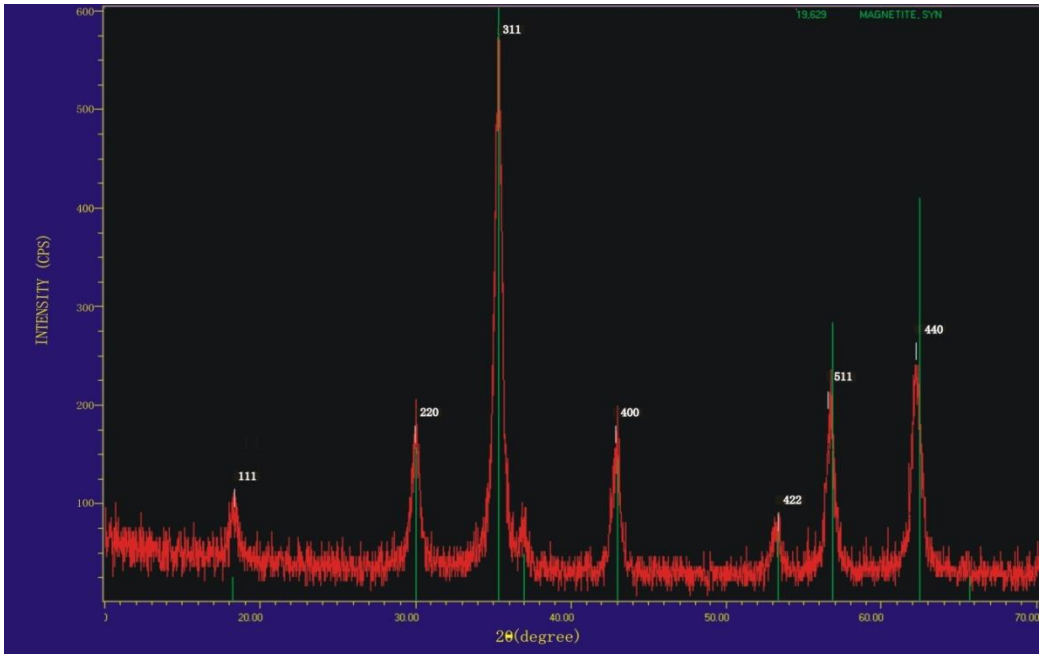

Figure 2: X-Ray Diffraction Patterns of the Synthesized Magnetite Nanoparticles

Analysis of the coated bacterium was done using Transmission electron micrographs. TEM image of the surfaces of cells coated with Fe_3O_4 nanoparticles is shown in Figure 3.

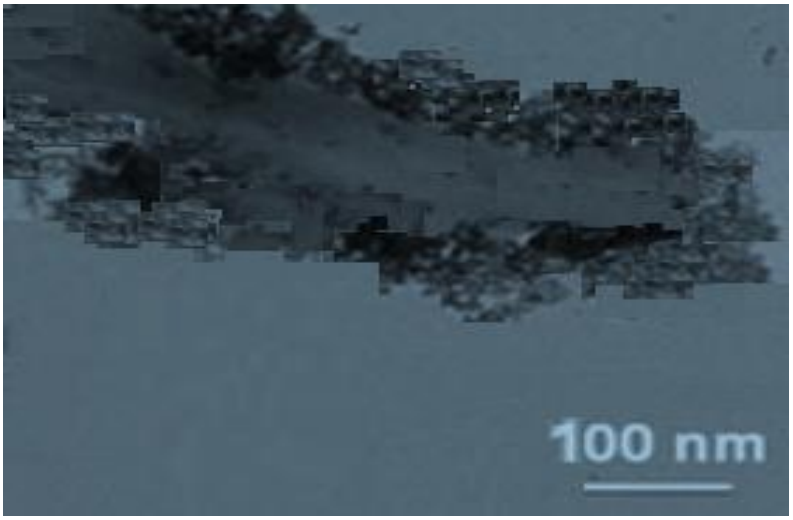

Figure 3: Magnetite-Coated Bacterium as Viewed by TEM

The Figure shows clearly that the magnetite nanoparticles were highly immobilized on the surface of the bacteria. This was due to magnetization effect of the nanoparticles (Ansari *et al.*, 2009) and the size of the nanoparticles (Bardania, 2013). A more uniform coating of the nanoparticles on the surface of bacterium cells can be attributed to the larger specific surface area and the higher surface energy of the nanoparticles synthesized in the presence of glycine, which leads to a lesser tendency for nanoparticles to aggregate, the consequence of this is that additional surface area that would enhance adsorption has been created.

Figure 4 shows the concentration – time profile for the biodesulfurization of DBT in diesel. The plot for coated bacterium showed that the substrate was desulfurized steadily from its initial concentration of 127.61 mg/L to 15.31 mg/L in 50 hours of desulfurization. This translates to about 88% desulfurization. The plot for uncoated bacterium showed that the substrate was desulfurized steadily from its initial concentration of 127.61 mg/L to 40.84 mg/L in 50 hours of desulfurization. This translates to over 68% desulfurization.

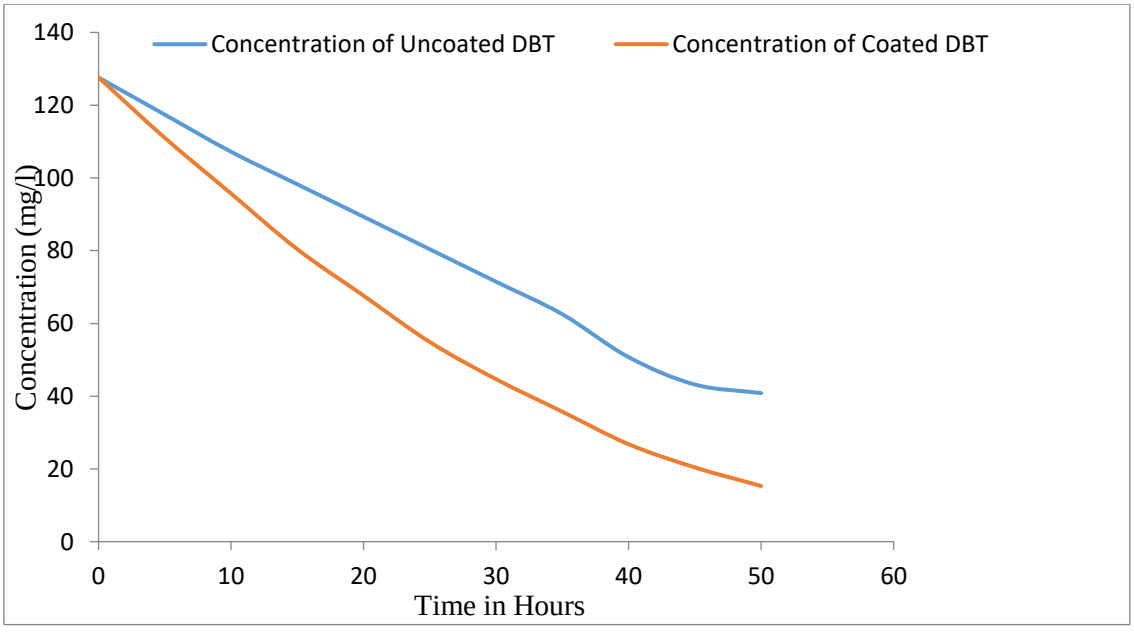

Figure 4: Plot of Experimental Data of DBT for Uncoated and Coated Bacterium

The augmented cellular permeability imparted by nanoparticle decoration of the cell surface, as employed in this study, had helped in improving the BDS kinetics (Karimi, 2016). In previous studies, Ansari *et al.* evaluated the coating of *R. erythropolis* IGTS8 by glycine-modified magnetite nanoparticles with a size range of 40–50 nm. It was shown that immobilization of magnetite nanoparticles on the surface of these bacteria

cells enhances their desulfurization activity by 56%, (Ansari *et al*., 2009). Although they determined the kinetic parameters using Optical waveguide lightmode spectroscopy (OWLS) which is an optical biosensor suitable for monitoring continuous adsorption processes. However, the results from this work have shown that coated bacterium (*Pseudomonas stutzeri*) increased biodesulfurization activity by 88% for DBT and 55% for BT. The increase in the rate of biodesulfulfurization of the coated bacterium is owing to the fact that biodesulfurization is extracellular, the microorganism secret enzymes which float at the surface of the nanoparticles thereby increasing the surface area of the reaction. The higher the surface area, the higher the rate of biodesulfurization. On the other hand, the increase may be attributed to the choice of the bacteria (Guobin *et al*., 2006) and the size of the magnetic nanoparticle as observed by Ansari, *et al*., (2009) and Bardania, (2013).

The kinetics of biodesulfurization of the uncoated bacteria followed the zero order kinetics while the coated one followed first order, this may be attributed to the likely increase adsorptive capacity created by the nanoparticle.

The measured kinetic parameters used in solving equation 8 are shown in Table 1, this equation was solved numerically using Runge-Kutta 4th order method with Microsoft Excel package.

The overall rate of substrate conversion is governed solely by the kinetics of the reaction. However, if mass transfer rate is lower than reaction rate, transport rate can be the step controlling the overall process rate and, moreover, the mass transfer rate may be influenced by the chemical rate of the bioprocess. Mass transfer limitations play an important role on the rate of reaction; the rate of conversion and product formation.

The kinetics data as presented in Table 1 shows that mass transfer is less in DBT when using coated bacterium. This therefore agrees with Bardania (2013) and Ansari (2009) that said mass transfer has little effect when magnetite nanoparticle is used to coat the bacteria strain capable of degrading sulfur. According to Felix (2009), the volumetric mass transfer coefficient is often used in order to compare the efficiency of bioreactors and as an important scale-up factor. In view of that, volumetric mass transfer coefficient was determined for the substrate (DBT) in order to design a bioreactor that can be used to take the technology to a market place.

Table 1: Parameters used to Solve Model Equation

	k_La (m^3/L)	K_{M1} (mg/L)	k_1 (mg/L.h)	K' (mg/L.h)	C_s(mg/L.h)	N
DBT Coated Bacterium	2.15	5.83	3.53	3.98	9.35	1.61
DBT Uncoated Bacterium	2.86	12.90	14.11	2.49	14.50	1.85

Figure 5 shows the experimental and simulated data plot of concentration of DBT versus time for uncoated bacterium. The level of agreement between the experimental data and the simulated data was determined using the Root Mean Square Error, RMSE, Table 2 gives the summary of the RMSE for each of the possible scenario. The lower the sum the better the level of agreement, as can be seen from the Table, the model equation coated system is lower, consequently, one may draw a conclusion that the model equation described the kinetics of the coated microorganism better. Figure 6 showed the experimental and simulated concentration – time profile of the biodesulfurization of DBT in diesel by *Pseudomonas stutzeri* coated with magnetite nanoparticle.

Table 2 Root Mean Square Error for the Model

	DBT
RMSE UNCOATED	2.13
RMSE COATED	0.38

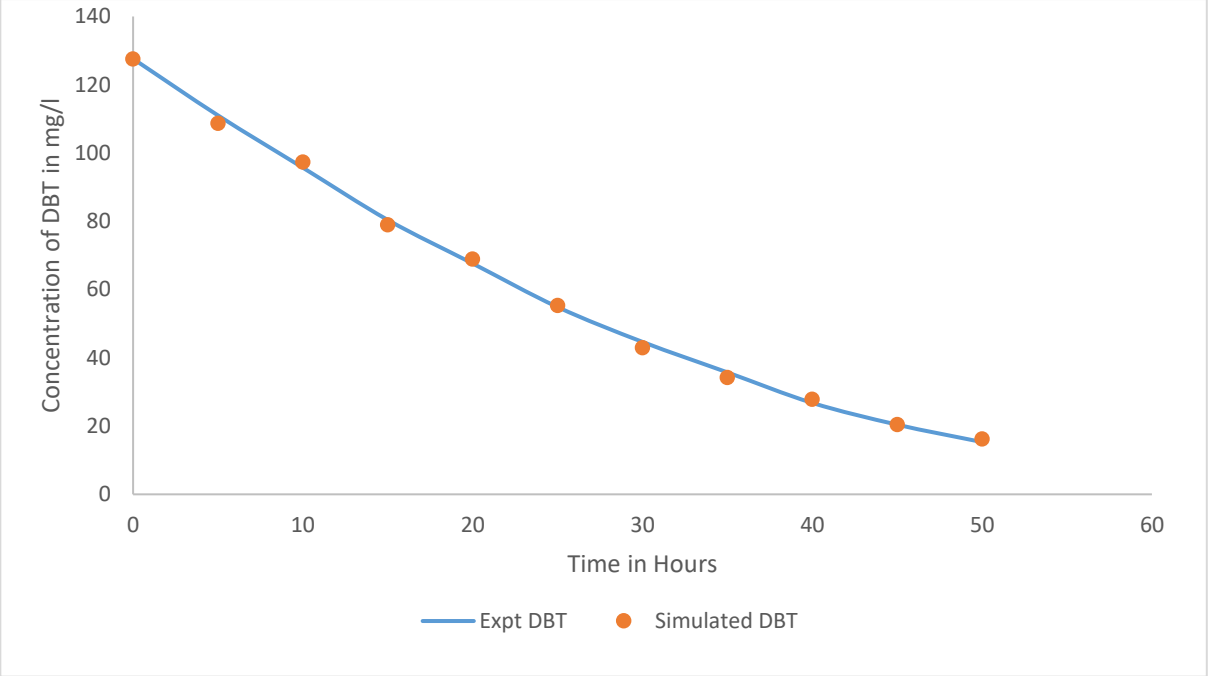

Figure 6 Experimental and Simulated Data Plot of Concentration of DBT versus Time using Coated Bacterium
It is worthy of mention that the biodesulfurization was carried out in diesel, a real petroleum feed.

4. CONCLUSION

In a nutshell, nanoparticles have the potential of enhancing microbial reaction. The coating of the isolated *Pseudomonas stutzeri* with the synthesized magnetic nanoparticle has shown great improvement in the biodesulfurization rate of diesel. The simulated data are also in good agreement with the experimental ones implying that all assumptions made in the development of the mathematical model are correct. Furthermore, the estimated and measured parameters would serve as useful tools for bioreactor design and analysis.

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