Modelling The Biodesulfurization Of Diesel

Semiu Adebayo Kareem*

Department of Chemical Engineering,

Modibbo Adama University of Technology,

Yola, Nigeria,

Alfred Akpoveta Susu

Department of Chemical and Polymer Engineering,

Lagos State University, Epe, Nigeria,

David Stan Aribike

Department of Chemical Engineering,

University of Lagos, Akoka, Yaba Nigeria,

Simon Cyril U. Nwachukwu

Department of Botany and

Microbiology,

University of Lagos, Akoka, Yaba Nigeria,

Abstract

The anaerobic biodesulfurization of diesel was carried out in the laboratory using an isolated bacterial strain, *Desulfobacterium indolicum.* Benzothiophene, dibenzothiophene, thiophene and 2, 5 – Dimethylthiophene were measured using a Gas Chromatograph with a Pulse Flow Photometric Detector to monitor the utilization of sulfur by the microorganism. The kinetic parameters of biodesulfurization like the maximum velocity rate constant, V_{max} and Michaelis Menten constant, K_M were determined by carrying out experiments in Erlenmeyer flasks using suitable medium and subsequent integration of Michaelis Menten equation. The partition coefficient was estimated through the efficacy of combining linear solvation energy relationships (LSERs) developed for pure systems through the application of linear solvent strength theory. A mathematical model showing the pattern of diesel biodesulfurization was developed. The developed model was solved numerically by the Finite Difference method. The model is able to describe all the experimental data with good statistical parameters, the simulated data were compared with the experimental findings to validate the model developed, the simulated results showed very good agreement with the experimental findings.

Keywords

Anaerobic Biodesulfurization, Linear Solvation Energy Relationships (LSERS), Simulated Data, Finite Difference Method, Pulse Flow Photometric Detector.

"1.   Introduction"

Biotechnology is becoming an attractive means of improving the efficiency of many industrial processes and resolving serious environmental challenges, the main reason for this is the extraordinary metabolic capabilities of microorganisms, microbial enzymes have been found to have capabilities of transforming a wide range of compounds. Biotransformation processes have several advantages over chemical transformation and these include; selectivity of microbial enzymes, energy efficiency, microbial enzymes are active under mild conditions and most importantly microbial enzymes are environment-friendly biocatalysts [1]. Biorefining is a possible alternative to some oil refining processes. The major potential applications of Biorefining are biodesulfurization, biodenitrogenation, biodemetallation and biotransformation of heavy crudes into lighter ones [2].

Sulfur has been described as the most undesirable element present in petroleum [3]; sulfur oxides released from fossil fuel combustion contribute to acid rain and air pollution [4]. The increasingly stringent limitations on SO_X emission are imposing an urgent requirement for refineries to reduce the sulfur content in the fuels they produce and thus its desulfurization is growing worldwide into a process critical to petroleum refinery profitability [5]. The conventional hydrodesulfurization is a higher pressure and high temperature catalytic process that converts organic sulfur to hydrogen sulphide by reacting crude oil fractions with hydrogen in the presence of an efficient inorganic catalyst. The reactivity of organosulfur compounds varies widely depending on their structure and the local environment of the sulfur atom. The conditions depend on the level of desulfurization required [6]. Hydrodesulfurization of diesel feedstock for instance for a low sulfur product requires a larger reactor volume, longer processing time, substantial hydrogen and energy input. Deep hydrodesulfurization results in deposition of carbonaceous coke on the catalysts, exposure of crude oil fractions to severe conditions which decrease fuel value of treated products. It requires new large capital investments and high operating costs. The H_2S generated by the process poisons the catalysts and shorten their life span [7].

Biodesulfurization is used as a more selective method for lowering the sulfur contents of petroleum products. Dibenzothiophene, DBT has been used as a model aromatic sulfur compound for the isolation and characterization of bacteria capable of biodesulfurizing organosulfur compounds found in a variety of fossil fuels [8]. Early work on biodesulfurization focused on organisms that degrade DBT. The pathways involved relied on oxidation and mineralization of the DBT carbon skeleton instead of sulfur removal and thus reduced the fuel value of the desulfurized product. Recent studies focused on organisms that use sulfur-selective oxidative and reductive pathways to remove sulfur from organic sulfur compounds and are capable of desulfurizing DBT and sterically hindered DBT compounds [9]. The 4S pathway is a specific desulfurization pathway in which DBT is desulfurized and converted to 2-hydroxybiphenyl, 2-HBP, through this pathway, the carbon skeleton of DBT is released intact and thus the calorific value of the fuel is not lost. Consequently, development of biocatalytic desulfurization for the selective removal of aromatic sulfur compound from petroleum products has focused on the 4S pathway [10], however, this practice bears little resemblance to the conditions the biocatalyst would encounter in commercial applications [11].

Kinetic equations, which describe the activity of an enzyme or a microorganism on a particular substrate, are crucial in understanding many phenomena in biotechnological processes. Quantitative experimental data is required for the design and optimization of biological transformation processes. A variety of mathematical models have been proposed to describe the dynamics of metabolism of compounds exposed to pure cultures of microorganisms or microbial populations of natural environment [12]. Characterization of the enzyme or microbe-substrate interactions involves estimation of several parameters in the kinetic models from experimental data. In order to describe the true behavior of the system, it is important to obtain accurate estimates of the kinetic parameters in these models [13]. Both derivative and integrated forms of equations derived for enzyme catalyzed reactions have been used to estimate kinetic parameters of microbiological processes. Estimates of kinetic parameters K and K_M have been calculated by fitting data to either integrated [14] or derivative [15] forms of Michaelis-Menten and Monod equation. Different approaches have been proposed for estimating the kinetic parameters, but progress curve analysis is the most popular because substrate depletion or product formation data from a single experiment are enough for parameter estimation. In this approach, substrate depletion or product formation-time course is used in the integrated form of the kinetic model for parameter estimation.

Modelling and simulation of internal loop air lift reactor was carried out using tank in series model under

unsteady conditions for biodesulfurization of hydrodesulfurized diesel oil by [16], the result showed that the internal loop air lift bioreactor can be used effectively to reduce organo sulfur compounds concentration from 500 ppm to almost nil in 120 hours. A mathematical model that describes the biodesulfurization of diesel in a trickle bed reactor was proposed by [3], their simulated values were able to explain the experimental observations satisfactorily. [17] Modelled the kinetics of the biodesulfurization bioprocess for the refining of pyrite ash by *Saccharomyces cerevisiae* and *Acetobacter aceti in* batch-type liquid- state bioreactors. The model provided predictions of ferric to ferrous iron rates and specific growth rates which can be used for the determination of oxidized and reduced ions. The ratios of dissolved sulfur to total sulfur have shown some promising results for *S. cerevisiae* to be used as a biodesulfurization and refining microorganism for pyrite ash and the other sulfide minerals. In this work, kinetic model for biodesulfurization of diesel was developed for sulfur specific reductive pathway in a batch reactor; the choice is informed due to its similarity to HDS. The implication is that, the modification to existing desulfurization facilities in refineries will be slight.

"2.   Methodology"

The anaerobic biodesulfurization of diesel was carried out in the laboratory using an isolated bacterial strain, *Desulfobacterium indolicum* and its data reported [18]. Mass balance on the substrates, the sulfur containing hydrocarbons in the fuel, diesel was done as previously described by [9]. The resulting differential equation was solved numerically using the Implicit Finite Difference Method. The choice was guided most importantly by convenience. Furthermore, it is accurate, consistent and stable. The kinetic parameters, the maximum rate constant, v_{max} and the Michaelis – Menten constant, K_M were obtained by direct integration of the Michaelis – Menten equation. The distribution coefficients of the substrates between the oil and the aqueous phases were estimated using a model developed by [19] for sulfur-containing organic substances in the fuel phase. The simulated results were compared with the experimental data of [18].

"3   Results and Discussion"

Scanty work has been reported on the biodesulfurization of real refinery feeds and some petroleum products [20] limiting the ability to assess the commercial potential of biodesulfurization. Microbial metabolism of organosulfur compounds is of interest in the petroleum industry not only for the biodesulfurization but also for their in-situ reduction of viscosity of the oil [9]. This work differs from others in this category as the anaerobic pathway is modelled; the advantage of the anaerobic pathway is that the absence of oxygen prevents the non-specific oxidation of hydrocarbons to colored, acidic, or gum-forming products. The anaerobic route is a potentially attractive biodesulfurization route to apply because of its sulfur specificity. From the pathway, it follows that the calorific value is maintained because C – C bonds are not altered. Furthermore, the reaction pattern is similar to HDS. However, growth under anaerobic conditions proceeds slowly, especially when organic compounds like thiophene and dibenzothiophene are involved in the conversion.

From a process point of view, the aerobic route has a major drawback. Sulfur is used as the assimilatory metabolism of aerobic bacteria. 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_2 availability is less optimal in emulsions with high biomass concentration. Furthermore, diluted sulfate is formed as the end product of the aerobic route that must also be removed, while the H_2S that is formed in anaerobic route can be treated with existing refinery desulfurization plants (e.g. Claus process). In the aerobic sulfur 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.

The quantitative parameters (measured, calculated and estimated) will represent a valuable pool of information that can be used for reactor design that will take the technology to the market place.

The mass balance on the sulfur containing organic compounds in diesel resulted in this model equation:

$$\frac{v_{max,i} C_i}{K_{M,i} + C_i} = -(1 + K_i)\frac{dC_i}{dt} \qquad 1$$

Where K_i is the Distribution coefficient of the sulfur containing organic compounds between diesel and water. The direct integration of the Michaelis – Menten equation yielded:

$$K_M \ln\left(\frac{C_0}{C}\right) + (C_0 - C) = v_{max} t \qquad 2$$

Further manipulation of equation 2 gives a more explicit one;

The model of [19] from which the distribution coefficients of the substrates between the oil and the aqueous phases were estimated is shown thus:

$$\log K_{i,fw} = \log\left[\frac{RT}{V_f P_i^0}\right] - \left(C_{aw} + r_{aw}R_2 + S_{aw}\Pi_2^H + a_{aw}\alpha_2^H + b_{aw}\beta_2^H + V_{aw}V_x\right) \qquad 4$$

where R is the universal gas constant, T is the temperature at which the coefficient is measured, V_f is the molar volume of diesel, P_i^0 is vapor pressures of the components of the Fuels parameters R_2 describes the excess molar refraction of solute i, Π_2^H describes the polarity/polarizability of solute i, α_2^H describes the hydrogen-bonding acidity of solute i, β_2^H describes the hydrogen bonding basicity of solute i, and Vx, describes the group-contributable molecular volume of solute i while c, r, s, a, b, and v are adjusted coefficients specific to the two-phase system, in this case air and water. The obtained values are shown in Table 2.

Equation 1 was solved numerically using the Finite Difference Method taking into account the multi-component nature of the substrates (sulfur containing organic compounds) in the diesel. The obtained concentration – time data was compared

$$\frac{C_0 - C}{\ln\frac{C_0}{C}} = -K_M + v_{max}\frac{t}{\ln\frac{C_0}{C}} \qquad 3$$

The bio-kinetic constants, K_M and v_{max} were estimated from equation 3 and are shown in Table 1.

Table 1: Kinetic Parameters Obtained From the Direct Integration of the Michaelis – Menten Equation (Equation 3)

Substances	v_{max}, (mg/L.hr)	K_{M}, (mg/L)
Benzothiophene	0.540	19.837
Dibenzothiophene	5.992	192.782

with the experimental data of [18] and is shown in Figure 1. The sets of data, the simulated and experimental were found to be statistically significant at 95% confidence level. The student's t-test statistical tool was used for the tests.

**Table2: The Distribution coefficients of Benzothiophene and Dibenzothiophene**

Substances	$K_{i,fw}$
Benzothiophene	2.312
Dibenzothiophene	2.038

The level of agreement between the simulated and the experimental data was determined using the sum of variances between them, in this method, the lower the sum the better the agreement. The result is shown in Table 3.

Table 3: Table Showing Level of Agreement between Simulated and Experimental data

T	Expt	Model	Variance
0	166.034	166.034	0.000
12	127.846	130.265	5.852
24	105.288	103.605	2.832
36	79.392	80.971	2.493
48	58.97	58.749	0.049
60	46.581	47.795	1.474
72	30.66	33.44	7.728
		Sum	**20.428**

The variances range from 0 to 7.728 and sum up to 20.428 which is the sum of variance for the sets of data. There is a good correlation among the points and one could infer that the mechanism proposed for the process is correct and that all assumptions made in developing the model are correct. Furthermore, one may conclude that all the estimated parameters are correct. It is worthy of mention here that the Michaelis-Menten equation was not transformed linearly in the course of parameter estimation, this was done in order to avoid the error associated with the dependent variable, thus making it not to be normally distributed and consequently leading to inaccurate parameter estimates [13].
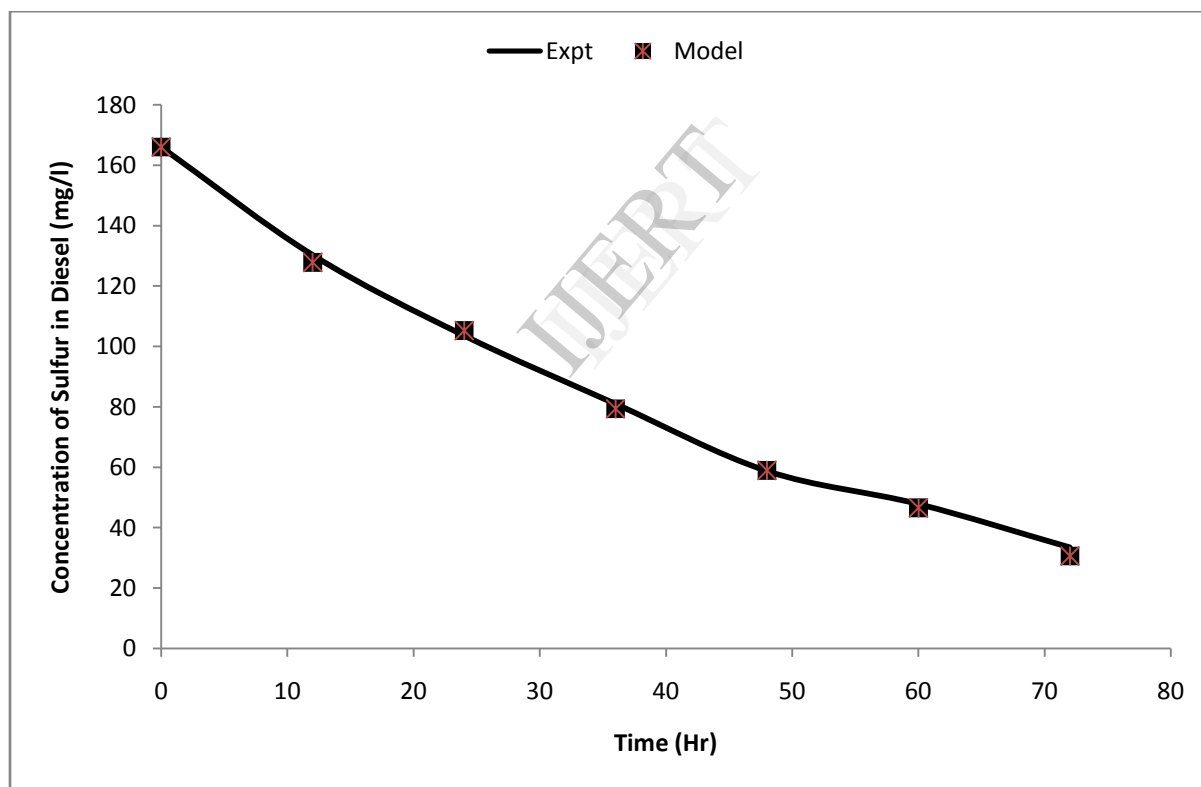
Figure 1: The Experimental and Simulated Concentration -Time Profile of Sulfur Containing Organic Compounds in Diesel

The developed model is one in which the kinetic was mass transfer driven. The substrates were assumed to be linearly adsorbed with the reaction taking place extracellularly, this assumption is line

with findings of [21] that the transport of substrates and products also contributed to desulfurization activity as demonstrated by the fact that cell-free

lysates of desulfurization cultures can exhibit a broader substrate range than the intact cell culture.

In conclusion, the need for more attention being paid to anaerobic biodesulfurization is important as it will take the process faster and cheaper than the aerobic pathway because of the minimal infrastructural change required to the existing ones in the refineries.

References

[1]. Mohebali, Ghasemali and Ball, Andrew S. Biocatalyst Desulfurization (BDS) of Petrodiesel Fuels. *Microbiology*, 154, 2008, pp. 2169 – 2183. DOI: 10.1099/mic.0.2008/017608-0

[2]. Le Borgne, R and Quintero, S. Biotechnological Processes for the Refining of Petroleum. *Fuel Process Technology,* 81, 2003, pp. 155 – 169.

[3]. Mukhopadhyaya, M., Chowdhury, R and Bhattacharya, P. Biodesulfurization of Hydrodesulfurized Diesel in a Trickle Bed Reactor – Experiments and Modelling. *Journal of Scientific and Industrial Research,* 65, 2006, pp. 432 – 436.

[4]. Gray, K. A., Mrachko, G. T and Squires, C. H. Biodesulfurization of Fossil Fuels. *Curr Opin Microbiol*, 6, 2003, pp. 229 – 235.

[5]. Yu, B., Xu, P., Shi, Q and Ma, C. Deep Desulfurization of Diesel and Crude Oils by a Newly Isolated Rhodococcus erytropolis strain. Appl. Environ. Microbiol., 72(1), 2006, 54 – 58. DOI:10.1128/AEM.72.1.54-58.

[6]. Gupta, N., Roychoudhury, P. K and Deb, J. K. Biotechnology of Desulfurization of Diesel: Prospects and Challenges. *Appl Microbiol Biotechnol,* 66, 2005, 356 – 366.

[7]. Egorova, M. Study of Aspects of Deep Hydrodesulfurization by Means of Model Reactions. Ph. D. Thesis, Swiss Federal Institute of Technology, Zurich, Switzerland. 2003.

[8]. Zang, S. H., Chen, H and Li, W. Kinetic Analysis of Biodesulfurization of Model Oil Containing Multiple Alkyl Dibenzothiophenes. *Appl Microbiol and Biotechnol,* 97(5), 2013, 2193 – 2200.

[9]. Kareem, S. A. Kinetics of Biodesulfurization of Diesel and Kerosene. A Ph.D Thesis, University of Lagos, Nigeria. 2010.

[10]. Torktaz, I., Etemadifar, Z and Derikvand, P. Comparative Modelling of Dszc, an Enzyme in Biodesulfurization, and Performing in Silico Point Mutation for Increasing Tendency to Oil. *Bioinformation,* 8(5), 2012, 246 – 250. DOI: 10.6026/97320630008246.

[11]. Wang, M., Li, W., Shi, Y., Wang, D and Feng H. Effect of Surfactant on Biodesulfurization by Corynebacterium Sp. 2D – 1 in the Presence of an Organic Phase. *J Zhejiang Univ. Science Suppl. II,* 2006, 371 – 375.

[12]. Minton A. P. The Influence of Macromolecular Crowding and Molecular Confinement on Biochemical Reactions in Physiological Media. J. Biol. Chem. 276 (14), 2001, 10577– 80.

[13]. Olsen S**.** Applications of Isothermal Titration Calorimetry to Measure Enzyme Kinetics and Activity in Complex Solutions. Thermochim. Acta 448, 2006, 12–18.

[14]. Goudar, C. T. and Delvin, J. F. Nonlinear Estimation of Microbial and Enzyme Kinetic Parameters from Progress Curve Data. *Wat. Environ. Res*. 73, 2001, 260-265.

[15]. Acuña-Argüelles, M. E., Olguin-Lora, P and Razo-Flores, E. Toxicity and Kinetic Parameters of the Aerobic Biodegradation of the Phenol and Alkylphenols by a Mixed Culture. *Biotechnol. Lett*. 25, 2003, 559 - 564.

[16]. Nandi, S. Biodesulfurization of Hydrodesulfurized Diesel in Airlift Reactor. *Journal of Scientific and Industrial Research,* 69, 2010, 543 – 547.

[17]. Ermurat, Y. Modeling the Kinetics of Pyrite Ash Biodesulfurization by *Saccharomyces Cerevisiae* and *Acetobacter Aceti* in Liquid State Bioreactors. Electronic journal of Biotechnology, 16 (2), 2013.

[18]. Kareem, S. A., Aribike, D. S., Nwachukwu, S. C. U and Latinwo, G. K. Microbial Desulfurization of Diesel by *Desulfobacterium indolicum. J Environ. Science & Engg*. 54(1), 2012, 98-103.

[19]. Arey, J. S and Gschwend, P. M. Estimating the Partition Coefficients for Fuel – Water Systems: Developing Linear Salvation Energy Relationships using Linear Solvent Strength Theory to Handle Mixtures. *Environ Sci Tech* 39, 2005, 2702 – 2710.

[20]. Fang, X. X., Zhang, Y. L., Luo, L. L., Xu, P., Chen, Y. L., Zhou, H. and Hai, L. Organic Sulfur Removal from Catalytic Diesel Oil by Hydrodesulfurization combined with Biodesulfurization. *Mod. Chem. Ind.* 26, 2006, 234–238.

[21]. Kilbane, J. J. Microbial Biocatalyst Development to Upgrade Fossil Fuels. *Curr. Opin. Biotechnol.* 17, 2006, 305 – 314.