Available online at www.sciencedirect.com

SCIENCE DIRECT®

Catalysis Today 106 (2005) 233–237

CATALYSIS TODAY

www.elsevier.com/locate/cattod

Bi-phasic bio-conversion of sulfur present in model organo-sulfur compounds and hydro-treated diesel

Sugata Guchhait [a], Dipa Biswas [b], Pinaki Bhattacharya [a], Ranjana Chowdhury [a,*]

[a] *Chemical Engineering Department, Jadavpur University, Kolkata 700032, India*
[b] *Department of Chemical Technology, University of Calcutta, Kolkata 700009, India*

Abstract

Bacterial strain of Rhodococcus sp. (JUBT1) isolated from petrol/diesel station has been used for the desulfurization of different model organo-sulfur compounds like DBT, substituted DBT, etc. which are difficult to remove in the conventional hydro-desulfurization of diesel fraction. The initial concentration of organo-sulfur compounds has been varied in the range of 100–1000 mg/dm^3. Under the present experimental range, the bacterial growth has been observed to follow Haldane-type kinetics characterizing the presence of substrate inhibition. The extent of inhibition by the substrate has been observed to increase with the number of substituents in the same range of initial concentration of different organo-sulfur compounds. The values of intrinsic kinetic parameters, like maximum desulfurization rate, $v_{\max}$, half saturation constant, K_S, inhibition constant, K_{Si} and the maximum substrate concentration, $C_{S_{\max}}$, corresponding to the maximum uninhibited rate of desulfurization, have been determined using each organo-sulfur compound having different number of substituents as limiting substrate. Relative changes in the values of the kinetic parameters have been correlated to the number of substitutions. Separate studies have also been conducted to determine the kinetics of bio-desulfurization of a hydro-treated diesel fraction. The concentration of sulfur in diesel was selected in the range of 100–500 mg/dm^3.

The effect of aqueous to non-aqueous ratio on the rate of specific desulfurization of hydro-treated diesel fraction in the range from 1:9 to 9:1 has also been studied in the present investigation. Mathematical models have been developed to predict the conversion of sulfur during batch-type bio-desulfurization of model compounds as well as diesel having known distribution of organo-sulfur compounds. The predictions of the model satisfactorily compare with the experimental results.

Keywords: Bi-phasic bio-desulfurization; Organo-sulfur compounds; Diesel; Growth kinetics

1. Introduction

Commercial crude oil fractions contain different types of sulfur compounds. Sulfur oxides are emitted to the atmosphere due to the combustion of these fractions, thus, causing serious environmental problems, such as acid rain. Desulfurization of petroleum fractions, particularly, diesel fuel is growing worldwide into a process critical to petroleum refinery profitability. Worldwide awareness led countries in the major developed regions to legislate almost sulfur-free highway diesel fuel for 2007. Near zero sulfur (NZS) [2,3] diesel fuel of 15 mg/dm^3 sulfur or less allows advanced post engine exhaust cleanup. Currently, diesel is treated by catalytic hydro-desulfurization (HDS). Although this process is energy intensive due to its requirement for high temperature and pressure, it is highly efficient in removing organic sulfur loads to 100 mg/dm^3 level, with the available range of catalysts (CoMo, NiMo, etc.) [1]. Further reduction of sulfur needs additional processing. Bio-desulfurization may serve as one of the efficient processes, supplementary to conventional hydro-desulfurization, to reduce the sulfur value of diesel to ultra low levels, e.g. 15 ppm. Although a few studies [1,4–6,8,9] have been reported on bio-desulfurization of diesel and pure organo-sulfur compounds, more systematic research studies should be conducted to explore the possibility of using this route efficiently.

---

* Corresponding author. Tel.: +91 33 24146378; fax: +91 33 24146378.
E-mail address: ftbe_bon@yahoo.com (R. Chowdhury).

0920-5861/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.cattod.2005.07.137

Nomenclature

C_S	substrate concentration, mg/dm^3
C_{S0}	initial substrate concentration, mg/dm^3
$C_{S_{max}}$	maximum substrate concentration, mg/dm^3
C_B	biomass concentration, mg/dm^3
v_s	desulfurization rate, mg/(dm^3 h)
v_{max}	maximum desulfurization rate, mg/(dm^3 h)
v_i	desulfurization rate of different model organo-sulfur compounds, mg/(dm^3 h)
v	desulfurization rate of lumped pseudo-organo-sulfur compound in diesel mg/(dm^3 h)
$Y_{x/s}$	yield coefficient = mass of biomass produced/mass of substrate consumed
K_S	half saturation constant, mg/dm^3
K_{Si}	inhibition constant, mg/dm^3
K'_s	modified half saturation constant for substrate-inhibited system, mg/dm^3
t	time, h
I.B.P.	initial boiling point
F.B.P.	final boiling point
Subscript	
i	any compound 'i'

Under the present investigation, a systematic growth kinetic study of a bacterial strain isolated from the soil of a local petrol station has been carried out using DBT, C1-DBT, C2-DBT, C3-DBT and diesel as limiting substrates. The concentration was varied in the range of 100–1000 mg/dm^3. In all the cases, both Monod- and Haldane-type kinetics have been attempted. Since the systems are characteristically substrate-inhibited ones, the threshold value of substrate concentration, above which the inhibitory effect becomes pronounced, has been identified for each component. The effect of aqueous to non-aqueous ratio on the rate of specific desulfurization of hydro-treated diesel fraction in the range from 1:9 to 9:1 has also been studied. A deterministic mathematical model has been developed to predict the concentration time history of biomass and substrates using suitable growth kinetics.

2. Materials and methods

2.1. Bacterial strain

Rhodococcus sp. (JUBT1) isolated from local petrol/diesel station soil were used.

2.2. Chemicals

DBT, C1-DBT, C2-DBT and C3-DBT (Sigma–Aldrich Fine Chemicals Limited), *N*-hexane, hexadecane (S.D. Fine Chemicals Pvt. Ltd.), KH_2PO_4 (GR Merck), Na_2HPO_4 (BDH E. Merck), $(NH_4)_2SO_4$ (Ranbaxy), $MgSO_4{\cdot}7H_2O$ (S.D. Fine-Chem Pvt. Ltd.), $CaCl_2{\cdot}6H_2O$ (E. Merck), $FeSO_4{\cdot}7H_2O$ (BDH E. Merck) were used for the present study.

2.3. Equipment

A B. Braun fermenter of 1.5 l working volume has been used.

2.4. Diesel

Diesel having the following specifications was used.

Compound	Diesel
I.B.P.	140 °C
F.B.P.	370 °C
Specific gravity	0.8216
Sulfur	500 ppm
Aromatic	27.16% (w/w)

2.5. Experimental media and growth condition

The sulfur-free medium (SFM) [7] contained KH_2PO_4 1.0 g, Na_2HPO_4 1.250 g, $(NH_4)_2SO_4$ 1.00 g, $MgSO_4{\cdot}7H_2O$ 0.500 g, $CaCl_2{\cdot}6H_2O$ 0.050 g, $FeSO_4{\cdot}7H_2O$ 0.005 g for 1 dm^3 having pH 6.8–7.2. DBT, C1-DBT, C2-DBT and C3-DBT solutions were prepared as 100 mg/dm^3 in hexadecane. Cells were cultivated in 150 ml flasks containing 20 ml SFM supplemented with 1 ml of different organo-sulfur compound solutions as the source of sulfur. Cell cultivation was carried out at 28 °C on a rotary shaker operated at 50 rpm.

2.6. Batch experiments

To determine the growth kinetics of the microorganisms with respect to different organo-sulfur compounds present in hydro-desulfurized diesel, batch experiments were conducted in Erlenmeyer flasks. For each compound, a set of experiments was performed for a period of 48 h varying the initial concentration in the range of 100–1000 mg/dm^3. For each initial substrate concentration, cell mass and the substrate concentration was determined at an interval of 4 h. Another experiment was carried out in a fermenter having a capacity of 1.5 l taking the ratio of diesel and aqueous medium in the range from 1:9 to 9:1 (v/v). The biomass concentration was determined using a dry weight method, while the sulfur concentration was determined using X-ray fluorescence spectroscopy (Oxford). A separate set of experiments was also conducted using diesel as the sole sulfur source.

3. Theoretical analysis

Taking the results of batch-type experiments of each model organo-sulfur compounds and diesel, microorganisms growth kinetics have been determined. High substrate concentration is the cause of substrate inhibition. Haldane-type kinetics, for substrate-inhibited growth, has been applied along with Monod-type kinetics. Monod- and Haldane-type kinetics are as follows:

Monod-type:

$$v = \frac{v_{\max} C_S}{K_S + C_S} \tag{1}$$

Haldane-type:

$$v = \frac{v_{\max} C_S}{K_s' + C_S + \frac{[C_S]^2}{K_S}} \tag{2}$$

In the kinetic analysis, intrinsic rates rather than effective rates have been used. Thus, heat and mass transfer limitations were eliminated [10]. Hence, the kinetic parameters may be described as intrinsic kinetic parameters. Their values, $v_{\max}$, K_S, K_s' and K_{Si} have been determined both graphically and by non-linear regression analysis. These are shown in Table 1.

For each organo-sulfur compound and for diesel, the value of maximum substrate concentration, $C_{S_{\max}}$, leading to the maximum substrate consumption rate has been determined using the following theoretical concept.

At $C_{S_{\max}}$,

$$\frac{d}{dC_S}(v_s) = 0$$

So, $C_{S_{\max}}$ will be

$$C_S = \sqrt{K_s' \times K_{Si}} = C_{S_{\max}} \tag{3}$$

The values of $C_{S_{\max}}$ have been given in Table 1. The values of K_s', $C_{S_{\max}}$ and K_{Si} have been correlated to the number of substituents in DBTs.

3.1. Time history of concentration

The following differential mass balance equations have been used to describe the time history of the concentration of different organo-sulfur compounds, also in the diesel.

Model compounds:

$$\frac{dC_{Si}}{dt} = -v_i C_B \frac{1}{Y_{x/Si}} \tag{4}$$

Diesel:

In the mathematical analysis, all the sulfur compounds in diesel have been lumped into a pseudo-compound. Hence, the rate of consumption of the pseudo-sulfur compound is given by,

$$\frac{dC_S}{dt} = -v C_B \frac{1}{Y_{x/s}} \tag{5}$$

The desulfurization rate, v_s, has been calculated using Monod- and Haldane-type kinetics during simulation using 4th-order Runga Kutta technique.

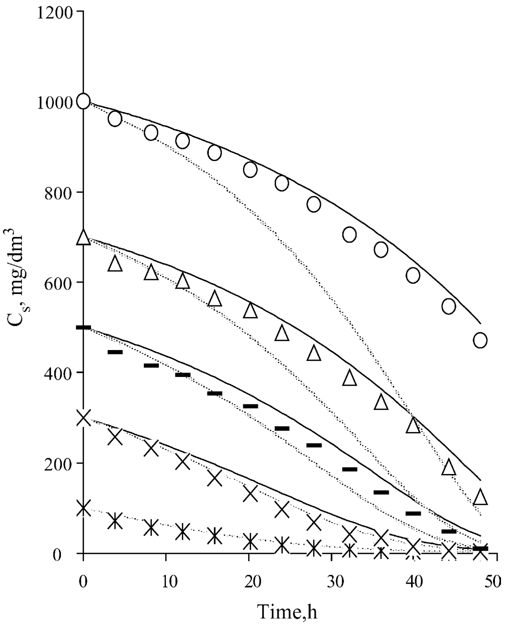

Fig. 1. Time history of concentration of C1-DBT with initial parameter as substrate concentration—simulated (lines: Monod (- - -); Haldane (—)) and experimental (points: (○) $C_{S0} = 1000$; (△) $C_{S0} = 700$; (–) $C_{S0} = 500$; (×) $C_{S0} = 300$; (⋇) $C_{S0} = 100$).

4. Results and discussion

Simulated values of concentration of C1-DBT using Eqs. (1), (2) and (4) have been plotted against reaction time in Fig. 1, with varying initial concentration (100–1000 mg/dm^3) as a parameter. The experimental values have been plotted in the same figure. Analysis of the figure reveals that the Monod-type kinetics is able to explain the transient behavior only for the initial substrate concentration of 100 mg/dm^3, above which Haldane-type kinetics is more suitable to predict the reality. Substrate inhibition is validated above 100 mg/dm^3 of initial substrate concentra-

Table 1
Kinetic parameters of different organo-sulfur compounds and of diesel

Compound	K_s or K_s' (mg/dm^3)	$C_{S_{\max}}$ (mg/dm^3)	K_{Si} (mg/dm^3)	$v_{\max} \times 100$ (mg/(dm^3 h))	$Y_{x/s}$
DBT	100	407.42	1659.92	645	0.40
C1-DBT	130	427.00	1402.69	686	0.40
C2-DBT	165	446.34	1207.40	819	0.45
C3-DBT	245	465.47	884.36	842	0.50
Diesel	205	432.23	950.00	710	0.52

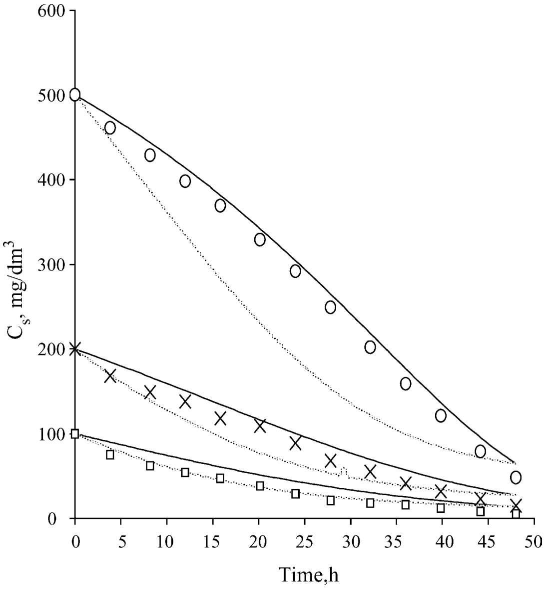

Fig. 2. Time history of concentration of organo-sulfur in diesel with initial concentration of sulfur as parameter—simulated (lines: Monod (- - -); Haldane (—)) and experimental (points: (○) $C_{S0} = 500$; (×) $C_{S0} = 200$; (□) $C_{S0} = 100$).

tion in each case. This is also true (curves not shown) for other compounds like DBT, C2-DBT and C3-DBT; with the increase in the number of substituents, the simulated conversion diminishes over the entire range of initial concentration. This also confirms to the experimental observations. This may be due to the steric hindrance caused by attached substituent groups. In Fig. 2, the concentration time history of sulfur compounds in diesel simulated using Eqs. (1), (2) and (5) have been plotted. In the same figure, the experimental values have been superimposed. It is observed that the theoretical plots are very close to the experimental ones. The validity of the Haldane model is established also in case of diesel.

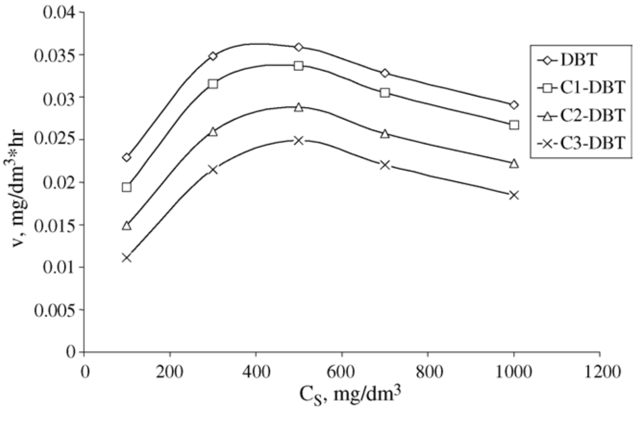

Fig. 3. Variation of maximum initial rate of desulfurization with concentration of substrate for different organo-sulfur compounds.

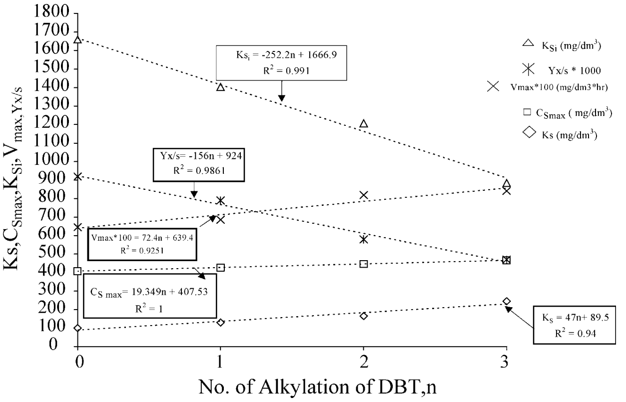

Fig. 4. Correlation of kinetic parameters (K_S, K_i, v_{max}, $C_{S_{max}}$, $Y_{x/s}$) with number of substituents, *n*.

From Fig. 3, it is evident that v reaches a maximum value at a substrate concentration, $C_{S_{max}}$, above which it decreases. The value of $C_{S_{max}}$ has been observed to increase with the number of substituents, *n*, in DBT. However, the value of v_{max} shows the reverse trend.

K_S, v_{max}, K_{Si} and $C_{S_{max}}$ have been plotted against the number of substituents in DBT in Fig. 4. It has been observed that while $C_{S_{max}}$, v_{max} and K_S have increased with the number of substituents, K_{Si} and $Y_{x/s}$ have decreased with it. Linear correlation of the number of substitution with each parameter has also been incorporated in the figure, so that knowing the composition of organo-sulfur compounds in the feed diesel, one can predict the ultimate achievable conversion.

The desulfurization rate of diesel has been plotted against the concentration of non-aqueous phase in the aqueous–non-aqueous mixture in Fig. 5. From the figure, it is apparent that the desulfurization rate increases gradually as the volumetric concentration of non-aqueous phase increases from 10 to 30%. When the volumetric concentration of non-aqueous phase increases above 30%, the rate of desulfurization gradually decreases. This may be due to the fact that as the ratio of non-aqueous to aqueous phase increases, the availability of sulfur compound increases leading to better growth of biomass causing higher rate of substrate consumption. However, as the proportion of non-aqueous

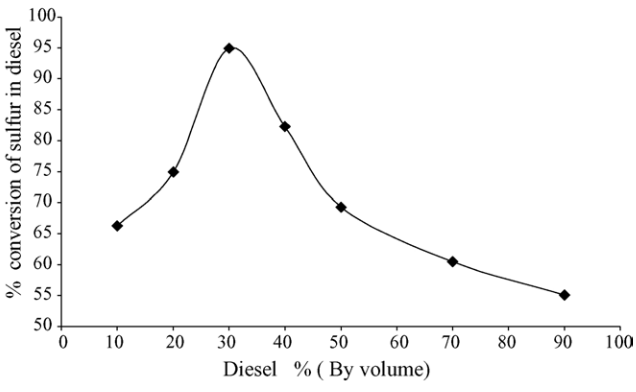

Fig. 5. Effect of diesel concentration in growth medium on sulfur conversion.

phase increases beyond 30%, the diffusion of oxygen gets highly retarded due to the formation of a thick non-aqueous layer above the aqueous one and hence the consumption rate of substrate gets retarded. This may be justified by the fact that although the fermenter is provided with an efficient sparger through which air is continuously bubbled, due to the lower diffusion rate of oxygen through the non-aqueous layer in comparison to aqueous one, the transfer rate of oxygen becomes slower when the volume of non-aqueous phase increases with respect to aqueous one.

5. Conclusion

Growth kinetics of microbial strains isolated from a local diesel/petrol station have been determined using model organo-sulfur compounds and diesel as limiting substrates. The initial concentration of organo-sulfur compounds has been varied in the range of 100–1000 mg/dm^3. The strains have been observed to follow substrate-inhibited growth following Haldane-type kinetics. Kinetic parameters are highly affected by the increase in the extent of substituents of DBT and linear correlations have been incorporated to explain the functionality of the parameters with number of substituents. A mathematical model consisting of differential material balance equations along with a Haldane kinetic model is capable of predicting the overall trend of desulfurization of organo-sulfur compounds as well as of diesel. The influence of diesel to aqueous phase ratio in the growth medium of microbial strain on the sulfur removal efficiency has also been studied.

Acknowledgement

Authors highly acknowledge the help rendered by Indian Oil Corporation Limited for sulfur estimation.

References

[1] H. Singh, M. Malhotra, N. George, S.K. Ghosh, Petrotech, India (2001).

[2] C.L.M. Marcelis, M.V. Leeuwen, H.G. Polderman, A.J.H. Janssen, G. Lettinga, Biochem. Eng. J. 16 (2003) 253–264.

[3] M.J. Grossman, M.K. Lee, R.C. Prince, V. Minak-Bernero, G.N. George, I.J. Pickering, Appl. Environ. Microbiol. 67 (4) (2001) 949–1952.

[4] T. Kaby, A. Ishihara, H. Tajima, Ind. Eng. Chem. 85 (1992) 334–338.

[5] R. Chowdhury, E. Pedernera, R. Reimert, Am. Inst. Chem. Eng. J. 48 (2002) 126–135.

[6] M.L. Shuler, F. Kargi, Bioprocess Engineering: Basic Concepts, Pearson Eduction Inc., New Jersey, USA, 2000.

[7] H.P. Schmauder, Methods in Biotechnology, M. Schweizer, Taylor & Francis, 1997.

[8] S. Abbad-Andaloussi, M. Warzywoda, F. Monot, Oil Gas Sci. Technol. 58 (2003) 505–513.

[9] K. Kohtaro, F. Toshiki, S. Rika, I. Yoshitaka, K. Kuniki, U. Shoji, Appl. Environ. Microbiol. 68 (8) (2002) 3867–3872.

[10] J.M. Smith, Chemical Engineering Kinetics, Mc-Graw Hill International Edition, Singapore, 1981.