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Bio-desulfurization of model organo-sulfur compounds and hydrotreated diesel—Experiments and modeling

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Abstract

The bacterial strain, namely, *Rhodococcus* sp. (JUBT1) isolated from petrol/diesel station, has been used for different model organo-sulfur compounds like, DBT, alkylated DBT, etc., which usually remain unchanged during the conventional hydro-desulfurization of the 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. Although the growth of the bacterial strain is of substrate-inhibited type for all model organo-sulfur compounds, used as limiting substrates, the extent of inhibition is, however, different. For the same values of initial concentrations the inhibition is more pronounced for the organo-sulfur compounds containing larger number of alkyl substitutions. The values of intrinsic exponential growth phase kinetic parameters like $\mu_{\max}$, maximum specific growth rate, K_S, half saturation constant, K_{Si}, inhibition constant, have been determined using each organo-sulfur compound of different number of alkylation as limiting substrates. Relative change in the value of kinetic parameters has been correlated to the number of substitution. A mathematical model has 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 have been compared with the experimental results satisfactorily.

Keywords: Bio-desulfurization; Organo-sulfur compounds; Hydrotreated diesel; Growth kinetics

1. Introduction

Desulfurization of diesel fuel is growing worldwide in a process which is 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 the most common sulfur specification is 500 mg/dm^3 (350 mg/dm^3 in Europe) [2]. Ultra low sulfur diesel (ULSD) fuel of less than 50 mg/dm^3 becomes the specification both in European union and Japan in 2005 [2,3]. European Commission further adopted a 50 mg/dm^3 sulfur specification for road diesel fuel beginning January, 2005 [2,3]. A USA EPA rule phases in 15 mg/dm^3 sulfur highway diesel fuel starting June, 2006 [13–15]. In Japan, 10 mg/dm^3 sulfur fuel in 2007 has already been announced [13–15]. Currently, diesel is treated thermo-chemically following hydro-desulfurization (HDS) method [7]. Although this process is energy intensive due to its requirement for high temperature and pressure it is highly efficient in removing organic sulfur load to 100 mg/dm^3 level. However, with the available range of catalysts (CoMo, NiMo, etc.) [1] this process is not suitable for the removal of polyaromatic sulfur compounds like alkylated dibenzothiophene, napthothiophenes, etc. [10] present in diesel to an ultra low level (10–50 mg/dm^3) required by the new US and European norms. From the reported review [1–10] it is clear that the conventional way of lowering the sulfur level from 100 mg/dm^3 to an ultra low level (10 mg/dm^3) would be to add a second stage to the existing hydro-treating unit. The second stage would require substantial modifica-

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Nomenclature

C_B	biomass concentration (mg/dm^3)
C_S	substrate concentration (mg/dm^3)
C_{Smax}	maximum substrate concentration (mg/dm^3)
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)
r_S	consumption rate of substrate (mg/dm^3 h)
t	time (h)
$Y_{x/S}$	yield coefficient = mass of biomass produced/mass of substrate consumed

Greek letters

μ	specific growth rate (h^{-1})
μ_{max}	maximum specific growth rate (h^{-1})

Subscript

i	any compound ‘i’

tion of the desulfurization process, primarily through using higher pressure, increasing hydrogen consumption, reducing space velocity and choice of catalysts. To reduce the apprehended cost of conventionally desulfurized diesel to an ultra low level, a few new technologies like sulfur adsorption, sulfur oxidation, bio-desulfurization, are under development to replace the second hydro-treating stage [1]. Among them, bio-desulfurization may be a potential and innovative alternative.

It is evident from the literature [1,3,9] that the majority of the organo-sulfur compounds remaining unconverted after the conventional hydro-desulfurization are mostly the alkylated dibenzothiophenes. Although a few works [1,4–6,8,10] 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.

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, C_1-DBT, C_2-DBT, C_3-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. 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, C_1-, C_2-, and C_3-DBT purchased from Sigma–Aldrich Fine Chemicals limited were used. *N*-hexane, hexadecane of liquid chromatography grade were used.

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), Silica gel (100–200 mesh) (Sisco Research Laboratory Pvt. Ltd.) were used for the present study.

2.3. Diesel

Diesel having the following specification was used: initial boiling point—140 °C; final boiling point—370 °C; specific gravity—0.8216; sulfur content—500 ppm [having C_1-DBT: 40% W, C_2-DBT: 30% W, C_3-DBT: 20% W and others: 10% W] aromatic content (%, w/w) −27.16.

2.4. Experimental media and growth condition

The sulfur-free medium (SFM) [11] was composed of 1. KH_2PO4—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 per 1 dm^3 having pH (6.8–7.2). DBT, C_1-, C_2-, C_3-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. It may be mentioned that the Erlenmeyer flasks containing aqueous growth medium were autoclaved for 15 min at 121 °C and 0.2 MPa. Other non-aqueous substrate solutions including diesel were exposed to UV radiation for 30 min for sterilization purpose. The culture medium was inoculated with the bacterial culture in a laminar-flow hood, using sterile pipette. Flasks were plugged with sterile cotton to avoid further contamination by organisms other than JUBT1. Cell cultivation was carried out at 28 °C on a rotary shaker operated at 150 rpm.

2.5. Batch experiments

To determine the growth kinetics of the microorganisms with respect to different organo-sulfur compounds present in hydro-de-sulfurised 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 were determined at an interval of 4 h. Biomass concentration was determined using a dry weight method, while the sulfur concentration were determined using X-ray fluorescence spectroscopy (Oxford) following ASTM-D4294-03 method. A separate set of experiments was also conducted using diesel as the sulfur source.

3. Theoretical analysis

Using the data derived from batch type experiments for each organo-sulfur compound and diesel, growth kinetics of the microorganisms have been determined. As high substrate concentration may cause inhibition, known as substrate inhibition, Haldane [12] type kinetics applicable for substrate-inhibited growth has been attempted along with the most simple Monod-type kinetics. The Monod and Haldane type kinetics are given as follows:

Monod type:

$$\mu = \frac{\mu_{\max} C_S}{K_S + C_S} \tag{1}$$

Haldane type:

$$\mu = \frac{\mu_{\max} C_S}{K'_S + C_S + \frac{[C_S]^2}{K_{Si}}} \tag{2}$$

The kinetic parameters, $\mu_{\max}$, K_S, K'_S and K_{Si} have been determined both graphically and by non-linear regression analysis. These have been given in Table 1.

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

$$\frac{d}{dC_S}(r_S) = 0, \qquad \text{or} \ \frac{d}{dC_S}\left(\frac{\mu_{\max} C_S}{K'_S + C_S + \frac{C_S^2}{K_{Si}}}\right) = 0,$$

$$\text{or} \ \frac{\mu_{\max}\left[\left(K'_S + C_S + \frac{C_S^2}{K_{Si}}\right) - C_S\left(1 + \frac{2C_S}{K_{Si}}\right)\right]}{\left(K'_S + C_S + \frac{C_S^2}{K_{Si}}\right)^2} = 0,$$

$$\text{or} \ K'_S + C_S + \frac{C_S^2}{K_{Si}} - C_S - \frac{2C_S^2}{K_{Si}} = 0,$$

$$\text{or} \ \frac{C_S^2}{K_{Si}} = K'_S, \qquad \text{or} \ C_S = \sqrt{K'_S K_{Si}} = C_{S\max} \tag{3}$$

The values of $C_{S\max}$ are given in Table 1. The values of K'_S, $C_{S\max}$ and K_{Si} have been correlated to the number of alkylation in DBTs.

3.1. Time history of concentration

To know the time history of concentration of different organo-sulfur compounds, the following differential mass balance equations have been used,

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

$$\frac{dC_B}{dt} = \mu C_B \tag{5}$$

The specific growth rate μ has been calculated using Monod type and Haldane type kinetics during simulation using 4th order Runga Kutta technique.

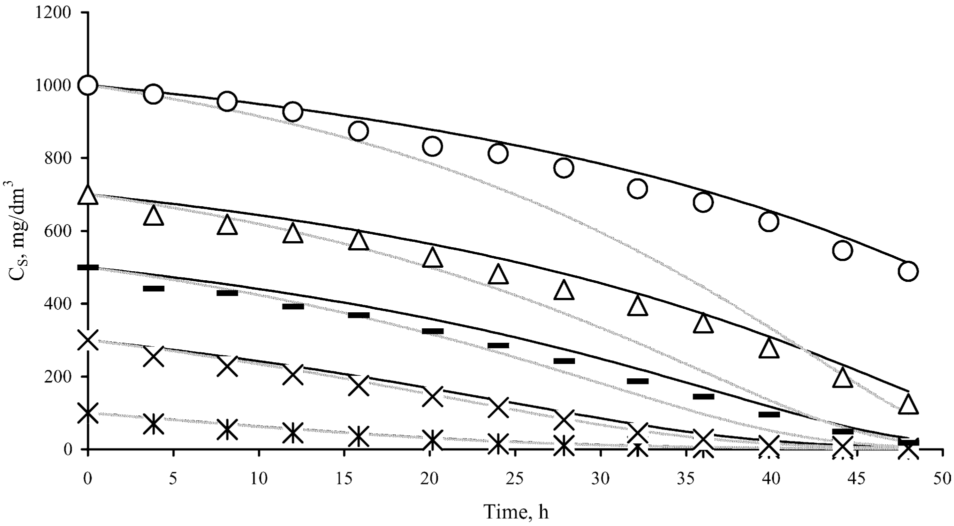

Fig. 1. Simulated (lines: Monod - - -; Haldane —) and experimental (points: (◯) $C_{S0} = 1000$; (△) $C_{S0} = 700$; (▬) $C_{S0} = 500$; (×) $C_{S0} = 300$; (*) $C_{S0} = 100$) time history of concentration of DBT with initial substrate concentration as parameter.

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

Compound	K_S or K'_S (mg/dm^3)	C_{Smax} (mg/dm^3)	K_{Si} (mg/dm^3)	μ_{max} ($\times 10^4$) (h^{-1})	$Y_{x/S}$ ($\times 10^3$)
DBT	100	407.42	1659.92	467	920
C_1-DBT	130	427.00	1402.69	457	790
C_2-DBT	165	446.34	1207.40	406	580
C_3-DBT	245	465.47	884.36	394	470
Diesel	205	432.23	950.00	402	720

4. Results and discussion

In Fig. 1, simulated values of concentration of DBT have been plotted against reaction time, varying initial concentration as a parameter. The experimental values have been plotted on the same figure. Analysis of the figure reveals that the Monod type kinetics are able to explain the transient behavior only for the initial substrate concentration of 100 mg/dm^3, above which Haldane type kinetics are more suitable to predict the reality. In Figs. 2–4 , the same plots have been made for C_1-, C_2-, and C_3-DBT respectively. In each case, substrate inhibition is validated above 100 mg/dm^3 of initial substrate concentration. In Fig. 5 values of specific growth rate for different organo-sulfur compounds have been plotted against their initial concentration. From the close observation of Fig. 5, it is evident that μ reaches a maximum value at a certain substrate concentration, C_{Smax}, above which it decreases. The value of C_{Smax} has been observed to increase with the number of substitution, n, in DBT. The value of μ_{max}, however, shows the reverse trend.

In Fig. 6, K_S, μ_{max}, K_{Si}, and C_{Smax} have been plotted against the number of alkylation in DBT. It has been observed

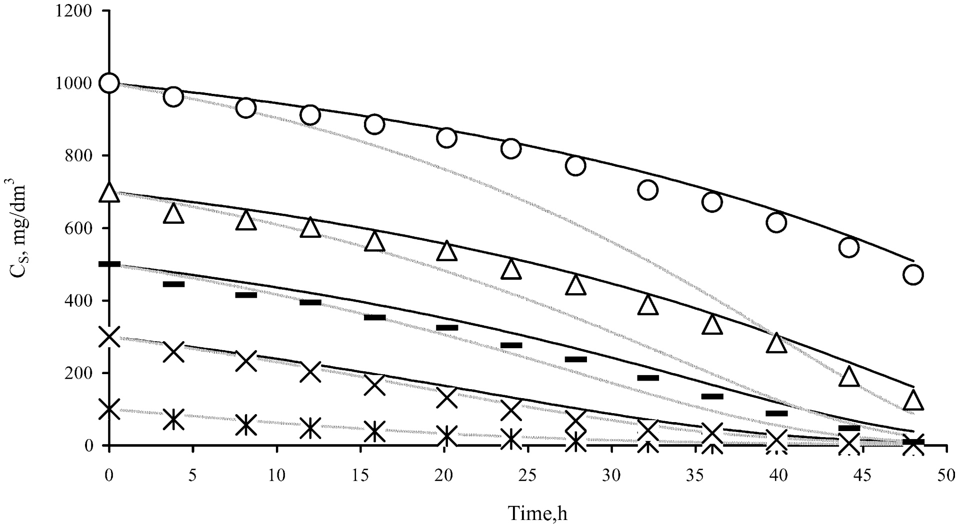

Fig. 2. Simulated (lines: Monod - - -; Haldane —) and experimental (points: (○) C_{S0} = 1000; (△) C_{S0} = 700; (▬) C_{S0} = 500; (×) C_{S0} = 300; (*) C_{S0} = 100) time history of concentration of C1-DBT with initial substrate concentration as parameter.

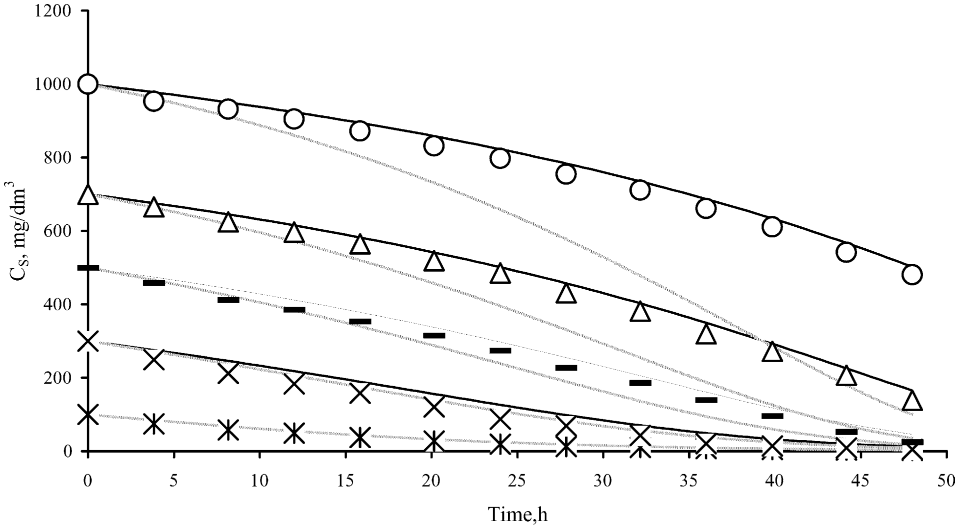

Fig. 3. Simulated (lines: Monod - - -; Haldane —) and experimental (points: (○) C_{S0} = 1000; (△) C_{S0} = 700; (▬) C_{S0} = 500; (×) C_{S0} = 300; (*) C_{S0} = 100) time history of concentration of C2-DBT with initial substrate concentration as parameter.

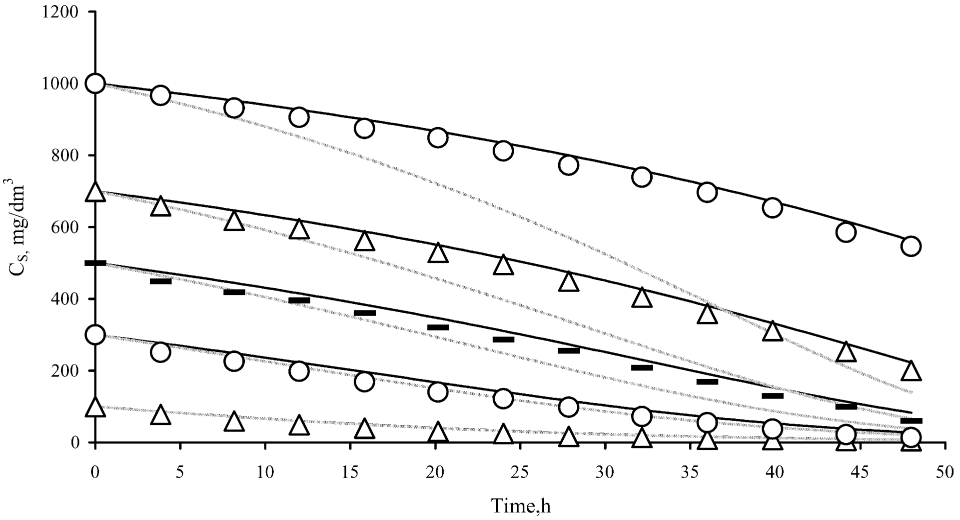

Fig. 4. Simulated (lines: Monod - - -; Haldane —) and experimental (points: (◯) C_{S0} = 1000; (Δ) C_{S0} = 700; (▬) C_{S0} = 500; (×) C_{S0} = 300; (*) C_{S0} = 100) time history of concentration of C3-DBT with initial substrate concentration as parameter.

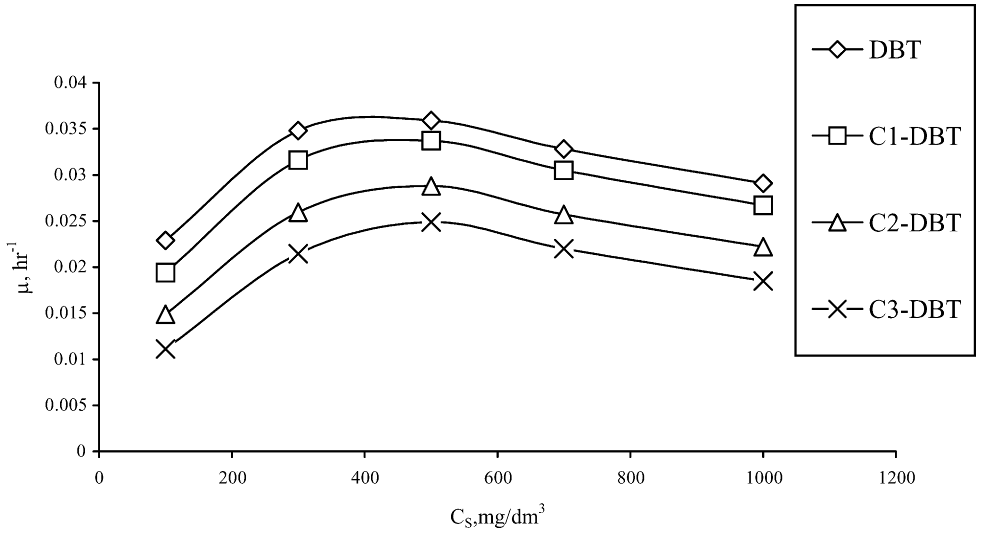

Fig. 5. Simulated (line) and experimental (point) variation of specific growth rate with concentration of substrate for different organo-sulfur compounds.

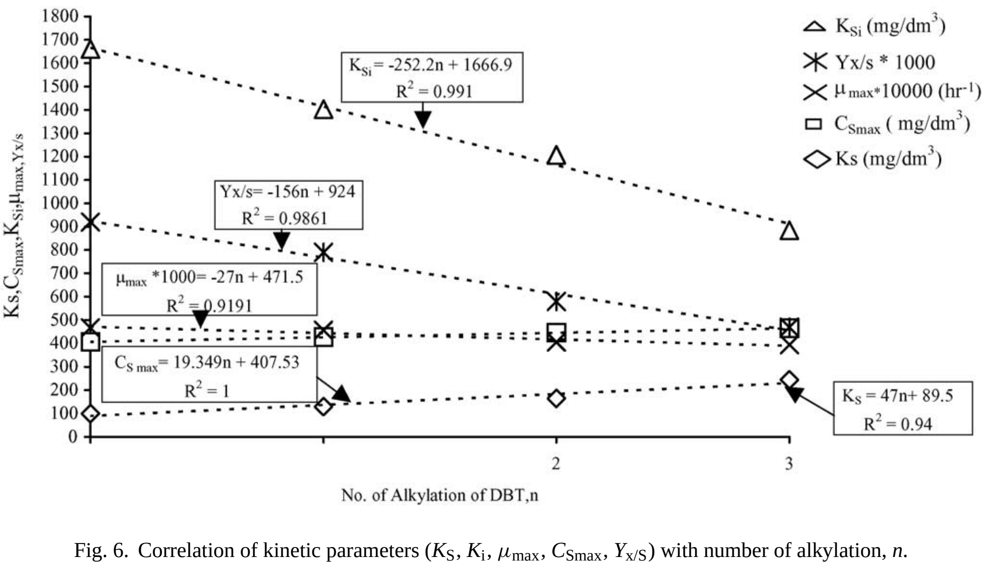

Fig. 6. Correlation of kinetic parameters (K_S, K_i, μ_{max}, C_{Smax}, $Y_{x/S}$) with number of alkylation, *n*.

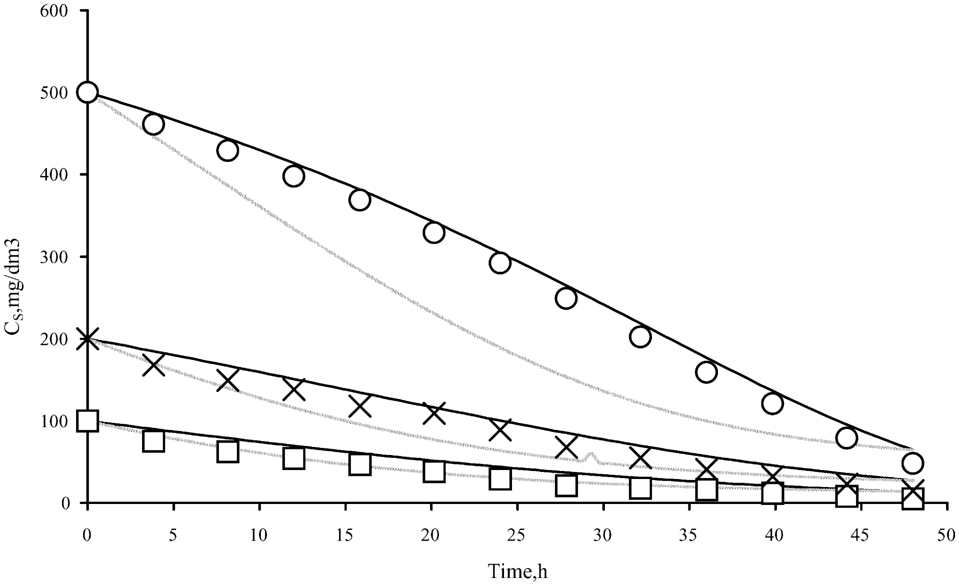

Fig. 7. Simulated (based on linear correlation - - -; diesel kinetics —) and experimental (points: (○) $C_{S0} = 500$; (×) $C_{S0} = 200$; (□) $C_{S0} = 100$) time history of concentration of organo-sulfur in diesel with initial concentration of sulfur as parameter.

that, while C_{Smax} and K_S have increased with the number of alkylation, K_{Si}, $\mu_{\max}$, $Y_{x/S}$ have decreased with it. Linear correlation of number of substitution with each parameter has also been incorporated in the figure, so that only by knowing the composition of organo-sulfur compounds in the feed diesel one can predict the ultimate achievable conversion. In Fig. 7, the concentration time history of sulfur compounds in diesel simulated using Eqs. (1) and (2) and the above linear correlations of Fig. 7, have been plotted. On the same figure another set of plots has been made using the theoretical results obtained using the differential mass balance equations and the kinetic parameter of diesel itself. On the same figure, the experimental values have been superimposed. It is observed that the theoretical plots are very close for initial organo-sulfur concentration up to 200 mg/dm^3 and are capable of explaining the experimental trend. At higher initial concentration of 500 mg/dm^3 only the simulated values based on the growth kinetics using diesel is capable of predicting the experimental trend. The validity of Haldane model is established also in case of diesel. Moreover, the applicability of the linear correlations of number of alkylation, *n*, with different kinetic parameters in the lower initial concentration range of diesel up to 200 mg/dm^3 is also confirmed. Although the microbial strain was identified following morphological and biochemical tests, further attempts will be made to characterize the culture using DNA sequencing, etc. Moreover, identification by r16RNA test is also awaited.

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 substrate. 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 alkylation of DBT and linear correlations have been incorporated to explain the functionality of the parameters with number of alkylation. A mathematical model using differential material balance equations along with the Haldane model and linear correlation of kinetic parameters and number of alkylation is capable of predicting the overall trend of desulfurization of organo-sulfur compounds as well as diesel.

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