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Determination of Growth Kinetic Parameters of a Desulfurizing Bacterium, *Gordonia Alkanivorans* RIPI90A

Zahra Azimzadeh Irani[a,b], Fatemeh Yazdian[a*], Ghasemali Mohebali[a], Majid Soheili[a], Mohammad Reza Mehrnia[b]

[a*] Iranian research Institute of petroleum Industry, P.O.Box:14665-1998, Tehran, Iran. yazdianf@ripi.ir
[b] School of Chemical Engineering, University Collecge of Engineering, University of Tehran, P.O.Box 11365-4563, Tehran, Iran.

Growing cells of a desulfurizing bacterium, *Gordonia alkanivorans* RIPI90A was used to study biodesulfurization of hydrotreated diesel in batch shake flasks. Sulfur concentration was varied from 0.012 g/L to 0.028 g/L. Moreover, specific growth rates of the culture were fitted to kinetic models of Haldane and Monod in order to determine the values of batch growth kinetic parameters like maximum specific growth rate (μ_{max}), half saturation coefficient (K_S) and substrate inhibition constant (K_{Si}). Concentration of total sulfur compounds in the diesel and hydrotreated diesel was varied in the range of 2-5 g/L and 0.007-0.028 g/L respectively. Under these ranges, the experimental results have shown acceptable agreement with Haldane kinetic for diesel and Monod kinetic for hyrotreated diesel.

1. Introduction

Combustion of fossil fuels with different types of sulfur compounds emits sulfur oxides that can react with moisture in the air and cause serious environmental problems like acid rain (Soleimani et al., 2007). Therefore more strict regulations order very low sulfur contents in oil fractions like diesel. Diesel is currently treated by Hydrodesulfurization (HDS) to comply with theses regulations. However, because of high capital and operating expenses of HDS process, Biodesulfurization (BDS) may use to remove the recalcitrant sulfur molecules under mild pressure and temperature to ultra low levels (Guchhait et al., 2005a, b).
The target of this work is the investigation of BDS process of hydrotreated diesel by growing cells of *Gordonia alkanivorans* RIPI90A and determination of batch growth kinetic parameters of this bacterial strain for diesel and hydrotreated diesel in biphasic media. This process was carried out using different initial total sulfur concentrations as limiting substrate in diesel and hydrotreated diesel. Haldane type kinetic is used to explain substrate inhibition growth of microbial culture because of high substrate concentration in diesel. In the case of hydrotreated diesel, Monod type kinetic was exploited for determination of the kinetic parameters.

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2. Materials and methods

2.1 Biocatalyst

The biocatalyst used was *Gordonia alkanivorans* RIPI90A. This strain was selected among 23 strains that were gathered on the basis of their growth rate and extent of desulfurization from all around Iran. The detailed description of the biocatalyst is reported by Mohebali et al. (2007). This aerobic Gram (+) strain was maintained as a suspension in nutrient broth containing 20% (v/v) glycerol, at -70 °C and also on glass beads under the same conditions.

2.2 Chemicals

All chemicals were of analytical grade. Normal hexadecane and dibenzothiphene (DBT) were purchased from Merck. N, N' dimethylformamide (DMF) were obtained from Riedel- de Haen.

2.3 Diesel

Diesel having the following specification was used: initial boiling point: 250 °C; final boiling point: 385 °C; specific gravity: 8370 kg/m^3; sulfur content of diesel: 8 g/L; sulfur content of hydro treated diesel: 0.03 g/L.

2.4 Micro-organism and culture conditions

The culture was initially grown in 250mL Erlenmeyer flask containing 100mL of Minimal salt (MS) medium having the composition (gram per liter of deionized water) KH_2PO_4 (6), Na_2HPO_4 (4), NH_4NO_3 (1.2), $MgCl_2.6H_2O$ (0.75), $MnCl_2.4H_2O$ (0.004) $CaCl_2.2H_2O$ (0.001), $FeCl_3$ (0.001), containing 2 g/L sodium benzoate as carbon source (MS-SB) medium. DMF was used as co-solvent in which DBT was dissolved and then added to the medium as sulfur source. The pH was adjusted prior to autoclaving to 7.08. All inoculated liquid media were incubated at 30 °C on a rotary shaker operated at 120rpm for 72h.

2.5 Batch growth and biodesulfurization study

Diesel and hydrotreated diesel with different initial sulfur concentrations were used. It is assumed that all the sulfur compounds in diesel and hydrotreated diesel were lumped into a pseudo-compound and then diluted at four dilution ratios using hexadecane to give final sulfur concentrations. Batch experiments were performed in 100ml Erlenmeyer flasks containing 15ml MS-SB supplemented with 5ml of diesel or hydrotreated diesel with different total sulfur concentrations and then incubated at 30 °C on a rotary shaker operated at 120 rpm. The reaction broths were always prepared with 10% (v/v) of initial biomass concentration. This was accomplished by transferring directly 100mL fresh culture in liquid medium broth to 1L MS-SB. A set of experiments was performed for a period of about 30h varying the initial concentration of sulfur substrate in the range of 2-5 g/L for diesel and 0.007-0.028 g/L for hydrotreated diesel. Following incubation at an interval of 4h and for each initial sulfur substrate, the samples were centrifuged (12000 rpm, 5 min). The organic phase was analyzed by UVfluorescence detection (vario TRACE) for quantification of total sulfur concentration while biomass concentration was determined using a dry weight method.

2.6 Software used

Regression analysis was performed with the data analysis tool pack of Microsoft Excel. The model equations were solved using nonlinear regression method by MATLAB 7.6.0.

3. Theoretical analysis

The values of biomass concentration derived from batch experiments for diesel and hydrotreated diesel were analyzed by applying Haldane and Monod type kinetic equations in order to determine the kinetic parameters.
Haldane type:

$$\mu = \frac{\mu_{max} C_S}{K_S + C_S + \frac{[C_S]^2}{K_{Si}}} \quad (1)$$

Monod type:

$$\mu = \frac{\mu_{max} C_S}{K_S + C_S} \quad (2)$$

Where μ is the specific growth rate (h^{-1}), C_S the substrate concentration (g/L), μ_{max} the maximum specific growth rate (h^{-1}), K_S the half saturation coefficient (g/L) and K_{Si} is the substrate inhibition constant (g/L). The values of kinetic parameters were determined by nonlinear regression analysis with MATLAB.

4. Results and discussion

4.1 Effect of initial sulfur concentration on biodesulfurization

Fig. 1 shows the time profile of biodesulfurization of hydrotreated diesel by the growing cells of desulfurizing bacterium, *Gordonia alkanivorans* RIPI90A. A different relation between desulfurization rate and initial sulfur concentrations was observed (data not shown). A maximum desulfurization rate could be achieved at 0.028 g/L of sulfur. This may be due to the fact that as the availability of sulfur compound increases, better growth of biomass causing higher rate of substrate consumption, indicating a strong influence of sulfur concentration on its desulfurization rate. The capability of *Gordonia alkanivorans* RIPI90A for desulfurization of DBT to hydroxybiphenyl (HBP) via 4s pathway (Mohebali et al., 2007) and inhibition effects of HBP production, may cause the desulfurization rate becomes constant after 30h.

4.2 Effect of initial sulfur concentration on the growth of the culture

The growth of bacterium was monitored periodically over about 30h, in order to obtain growth profile both for diesel and hydrotreated diesel. The evolutions of the biomass concentration with diesel in different initial sulfur substrate are shown in Fig.2. The amount of maximum growth decreases with increase of initial sulfur substrate concentration and maximum growth is obtained in 2 g/L, because the culture growth could be easily attributed to the substrate inhibition that is occurred in high substrate concentrations.

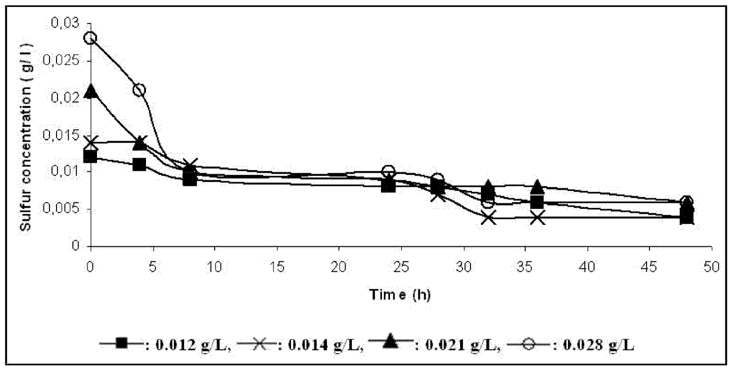

Figure 1: Time profile of biodesulfurization by Gordonia alkanivorans RIPI90A.

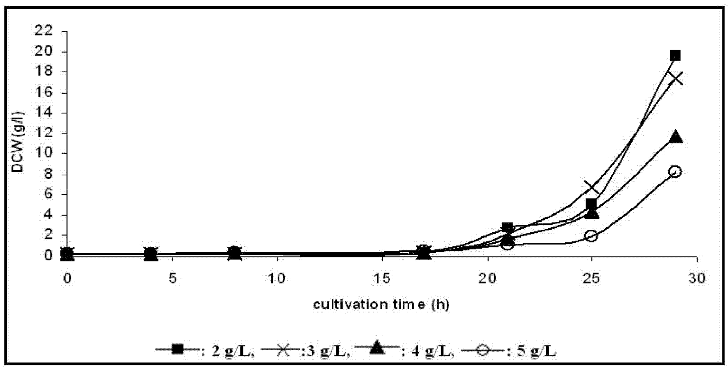

Figure 2: Biomass growth of Gordonia alkanivorans RIPI90A with diesel for different sulfur substrate concentration.

The evolutions of the biomass concentration with hydrotreated diesel in different initial sulfur substrate are shown in Fig.3. The amount of maximum growth increases with increase of initial sulfur substrate concentration and maximum growth is obtained in 0.028 g/L. This may be due to the increase of sulfur compounds availability that causes better growth of biomass with hydrotreated diesel in biphasic media.

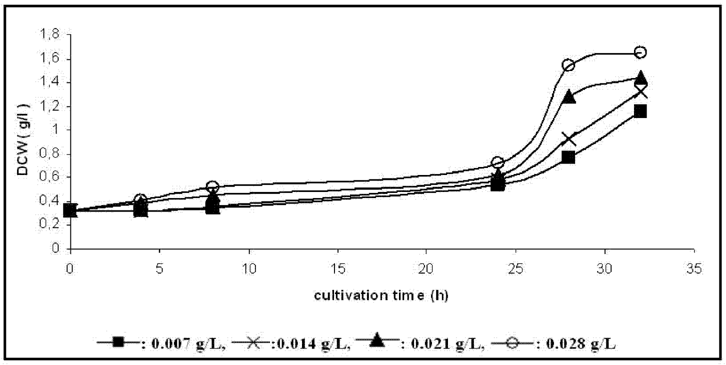

Figure 3: Biomass growth of Gordonia alkanivorans RIPI90A with hydrotreated diesel for different sulfur substrate concentration.

4.3 Modeling the kinetics of the culture growth

In order to determine growth kinetic parameters, specific growth rates (μ) of the culture at different sulfur concentration were calculated as per the following relationship:

$$\mu = \frac{1}{X}\frac{dX}{dt} \qquad (3)$$

Where X is biomass concentration (g/L) at time, t (h) and μ is the specific growth rate (h^{-1}) that is calculated from the slope of linear exponential growth curve versus time ($gL^{-1}\ h^{-1}$), dividing the average cell mass concentration (g/L) for each concentration of sulfur in the range of 2-5 g/L for diesel and 0.007-0.028 g/L for hydrotreated diesel. The model equations were solved using nonlinear regression method by MATLAB and were first applied directly on the experimental data on specific growth rate of the culture at different sulfur concentrations. The kinetic constants of growth of the culture obtained from these models along with Sum of squares due to error (SSE) and Coefficient of determination (R^2) between experimental and predicted rate values are shown in Table1.

Table 1: Specific growth rate kinetic parameters obtained for different models during biodesulfurization of diesel and hydrotreated diesel by Gordonia alkanivorans RIPI90A

Compound	Model	u_{max} (h^{-1})	K_s (g/L)	(g/L) K_{si}	R^2	SSE
Diesel	Haldane	0.459	35.5	1924	0.85	0.00022
Hydrotreated Diesel	Monod	0.095	0.02	-	0.99	$9.02*10^{-7}$

According to these values, Haldane model for diesel and Monod model for hydrotreated diesel are proved to be adequate fits as determined by their Sum of squares due to error and Coefficient of determination. This may be due to higher substrate concentration in diesel (2-5 g/L) that causes substrate inhibition behavior in the system.

5. Conclusions

Biodesulfurization of hydrotreated diesel was studied using growing cells of a desulfurizing bacterium; *Gordonia alkanivorans* RIPI90A in batch shake flasks. Experimental results indicate that desulfurization rate increases with the increase of the initial sulfur substrate. This result has been related to the increase of biomass growth that causes higher rate of substrate consumption. The desulfurization rate stops before the complete removal of sulfur compounds. Several authors have reported that the BDS process by growing cells may be deactivated by the HBP accumulation.

The growth of strain RIPI90A showed a direct response to the initial sulfur concentration and it decreases with the increase of the initial sulfur substrate in diesel. This result has been related to the initial substrate concentration which caused the inhibition growth of biocatalyst. On the other hand, the growth of strain in hydrotreated diesel increases with the increase of the initial sulfur substrate. As expected, higher initial sulfur concentrations had a positive influence over the biocatalyst growth. Growth kinetic parameters were determined by fitting the experimentally obtained values of the specific growth rates at different sulfur concentrations to suitable kinetic models. It has been observed that the growth of substrate follows Haldane model for diesel and Monod model for hydrotreated diesel.

In conclusion, this study revealed the potential of growing cells of RIPI90A in biodesulfurization of hydrotreated diesel and obtained growth kinetic parameters of this strain in biphasic media.

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