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Biodesulfurization of Natural Gas: Growth Kinetic Evaluation

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Abstract: The present study focused on evaluation of various kinetic models for hydrogen sulfur removal by means of active microorganisms. The microorganisms used for the removal of hydrogen sulfide were isolated from a local hot spring. The experiments were conducted with natural gas at initial pressures of 1 to 1.8atm. Several kinetic models such as; Andrew, Contois, Logistic, Monod, Moser, Tessier and Verhulst models in a batch culture were used to describe the microbial growth and substrate utilization. At low pressure (1atm), the bacterial behavior were substrate related and growth dependent; thus, Monod and Tessier models were unable to explain the microbial behavior. At gas pressure of 1.2atm, maximum cell dry weight of 3.136 and 1.724g.l^{-1} were obtained with Logistic and Verhulst models, respectively. The obtained regression values for Logistic model were reasonably acceptable for all initial gas pressures. As the gas pressure was increased to 1.8atm, the inhibition coefficient may be dominated in growth kinetic. Andrew's equation was also able to predict inhibition constant.

Key words: Hydrogen sulfide · Kinetic models · Substrate consumption rate · Microbial growth · Gas pressures

INTRODUCTION

Hydrogen sulfide is a flammable gas that has a characteristic malodour of rotten eggs. Upon inhalation, hydrogen sulfide reacts with enzymes in bloodstream and inhibits cellular respiration which is resulting in pulmonary paralysis, headache, dizziness, nausea, sudden collapse, staggering, drowsiness [1]. The existence of hydrogen sulfur in natural gas creates serious problems which comprise the disposal of hazardous waste materials, corrosion during transmission and distribution, reduced well production, decline gas combustion capacity, public nuisance, environmental pollution from emitted gas and high capital cost [2-7].

Traditional physico-chemical methods such as incineration, adsorption, absorption, thermal and chemical oxidation and alkanolamine processes have been used for the treatment of sour gas [8-10]. These conventional methods are energy intensive, high chemical and capital cost and also associated with pollutions [11]. Based on cost of equipment, reliable and less polluting operation, biological treatment exhibit to be the most economical and efficient alternative for the removal of hydrogen sulfide [12-16]. These processes operate at ambient temperature and atmospheric pressure; thus eliminate high costs for heat and pressure generation as required in a variety of chemical processes. The biological routes are easily applicable and minimize waste formation [7, 17-19]. Kinetic models can be used to gain a better understanding of the microbial growth and substrate consumption for process description governed by the microorganisms [20-24]. The objective of present work described here is to investigate several kinetic models for hydrogen sulfide removal by the isolated microorganisms from a hot spring. Experimental data was fitted by kinetic models and kinetic parameters were determined.

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MATERIALS AND METHODS

Microorganism and Growth Media: The sulfur oxidizing bacteria used in this work has been isolated from Ramsar hot spring (Ramsar, Iran). It was grown in an anaerobic serum bottle media. The media was incubated at 30°C and 180 rpm. The serum bottles containined 50ml liquid media; with media composition in grams per liter given as follow: 2.0 KH_2PO, 2.0 K_2HPO, 0.6 NH_4Cl, 0.4 $MgCl_2.6H_2O$, 8.0 $Na_2S_20_3.5H_2O$, 2.0 yeast extract, 2ml vitamin solution and 1ml trace element solution. The trace element solution consisted ($g.l^{-1}$) of 50 Na_2-EDTA, 11 $ZnSO_4.7H_2O$, 7.34 $CaCl_2.2H_2O$, 2.5 $MnCl_2.4H_2O$, 0.5 $CoCl_2.6H_2O$, 0.5 $(NH_4)_6Mo_7O_{24}.4H_2O$, 5.0 $FeSO_4.7H_2O$, 0.2 $CuSO_4.5H_2O$. The pH of trace element solution was adjusted to 6.0 using 1 M NaOH solution. The vitamin solution contained ($mg.l^{-1}$): 10 Thiamine-HCl.$2H_2O$, 20 Nicotinic acid, 20 Pyridoxine-HCl, 10 p-Aminobenzoic acid, 20 Riboflavin, 20 Ca-pantothenate, 1.0 Biotin, 1.0 Vitamin B_{12}. The pH of Vitamin solution was adjusted to 7.0. Distilled water was added to make 1-liter of broth solutions. The initial media pH was 6.5 and monitored by pH meter (HANA, 211, Romania). All chemicals used for the experiments were analytical graded and supplied by Merck (Darmstadt, Germany).

Analytical Methods: Batch experiments were carried out in sealed serum bottles with a volume of 125ml. The serum bottles contained liquid media which was prepared according to growth media composition discussed above. In each experiment, 50ml of fresh media transferred into the serum bottles under nitrogen gas. Gas impermeable rubber septum and aluminum crimp seals were used to seal the bottles for being used under various initial pressures. The bottles with liquid media were sterilized at 121°C for 15min. The sterilized serum bottles were inoculated with 3ml of seed culture. The inoculated culture was purged with a mixed gas from iron cylinder of compressed gas through a two-stage stainless steel regulator under variable initial pressures. The mixed gas comprises of the components of H_2S, CO_2, Ar and CH_4 gas. The experiments were conducted with various initial total pressures at 1 to 1.8atm with 0.2 intervals. The argon was selected as internal standard for gas analysis. The serum bottles were placed horizontally on an orbital shaker (Stuart, S1500 and UK) set at agitation rate of 180rpm and 30°C.

The gas and liquid samples were taken in a time interval of 4 h. The liquid samples were analyzed for optical density at a wavelength of 600nm using a spectrophotometer (Unico, 2100, USA). According to standard calibration curve, the cell dry weight concentration was also determined based on turbidity of the media by light absorbance as a function of cell dry weight. A gas-tight syringe (SGE, Australia) was used to take a 1ml of the gas sample for GC analysis. Gas chromatograph (Agilent, 7890A, USA), equipped with a thermal conductivity detector (TCD) was used for gas analysis. A packed column (HayeSep Q) with 80/100 mesh (Supelco, USA) was used to separate hydrogen sulfide, argon, methane and carbon dioxide. The initial oven temperature was 80°C. The oven temperature was programmed with a rate of $10°C.min^{-1}$ until reached 140°C and remained at that temperature for 1min. The injector and detector temperatures were 100 and 250°C, respectively. Helium gas was used as carrier gas at a flow rate of 25 $ml.min^{-1}$. Several kinetics models such as; Andrew, Contois Logistic, Monod, Moser, Tessier and Verhulst models were used to describe the behavior of microbial growth and substrate consumption by the active microorganisms for natural gas within pressure range of 1 to 1.8atm.

RESULTS AND DISCUSION

Hydrogen sulfide as an inorganic sulfur compound for cultivation of the isolated bacteria in batch media was used. One of the simple unstructured rate models for the batch culture defined as Malthus law is expressed as follows [25]:

$$\frac{dx}{dt} = \mu X \quad (1)$$

Where X is cell concentration of bacteria ($g.l^{-1}$), t is time (h) and μ is the specific growth rate (h^{-1}). This model predicts unlimited growth with respect to incubation time; while an inhibition term may provides limited growth which is dependent on cell concentration.

Logistic Kinetic Model: This model incorporated inhibition term; that means the model project inhibition coefficient which is proportional to cell density [25]. Also, the specific growth rate may be inhibited by high substrate concentration. In this case, the growth kinetics of microorganism is determined with respecting to logistic model. The specific growth rate for logistic model is defined by the following equation:

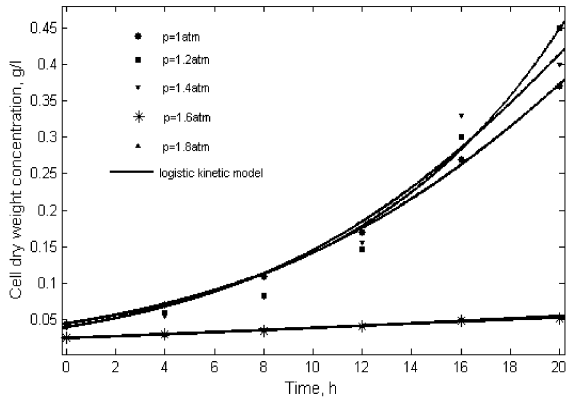

Fig. 1: Microbial cell concentration grown at various initial gas pressures obtained by logistic kinetic model

$$\mu = \mu_m \left(1 - \frac{x}{x_m}\right) \tag{2}$$

Where μ is the specific growth rate (h^{-1}), μ_m is the maximum specific growth rate (h^{-1}) and X_m is the maximum cell dry weight ($g.l^{-1}$). The logistic model leads to a lag phase, an exponential initial growth rate and a stationary growth concentration (X_m) which is described in the following equation [25]:

$$X = \frac{X_0 e^{\mu_m t}}{1 \frac{X_0}{X_m}(1 - e^{\mu_m t})} \tag{3}$$

Equation (3) gives the cell density with respect to time. Figure 1 shows the cell dry weight of mixed culture obtained in batch experiment with 5 initial gas pressure ranged 1 to 1.8atm.

A stepwise increase in gas pressure resulted in direct proportional increase in hydrogen sulfide concentration as gaseous substrate. At 1.6 and 1.8atm as the initial gas pressure did not show any influence on cell dry weight concentrations. As the initial gas pressure increased from 1 to 1.2atm, there was also an increase in cell dry weight, but as the gas pressure rose to 1.8atm, the cell concentration was decreased. The maximum cell dry weight was obtained with initial gas pressure of 1.2atm. In the batch bioreactor, the exponential growth rates were clearly observed with initial gas pressure in the pressure range of 1 to 1.4atm.

Monod Kinetic Model: Monod kinetic model is considered as one of the unstructured models which are dependent to substrate concentration as follows [25]:

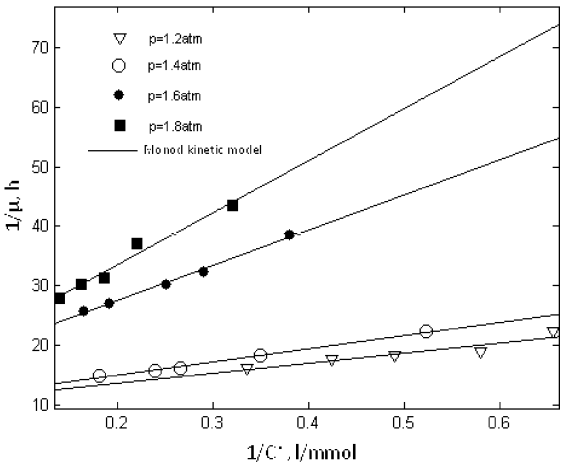

Fig. 2: Experimental data for microbial growth and substrate consumption at various gas pressures fitted to Monod model

$$\mu = \frac{\mu_m C^*_{H_2S}}{K_p + C^*_{H_2S}} \tag{4}$$

Where μ and μ_m are the specific growth rate and maximum specific growth rate for H_2S, respectively. The term $C^*_{H_2S}$ is represents hydrogen sulfide concentration in gas phase in equilibrium with liquid phase and K_p is Monod constant for H_2S. The value of $C^*_{H_2S}$ was calculated based on relationship between the partial pressure of hydrogen sulfide and gas solubility known as Henry' s law constant $(C^*_{H_2S} = P_{H_2S,gas}/H)$. The linearized form of Monod model is expressed by the following equation:

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_p}{\mu_m} \times \frac{1}{C^*_{H_2S}} \tag{5}$$

The illustrated plot of $(1/\mu)$ verse $(1/C^*_{H_2S})$, (Lineweaver-Burk plot), is shown in Figure 2.

The obtained kinetic parameters are shown in Table 1. Microbial growth and substrate consumption at low pressure such as 1atm did not follow Monod kinetic model. The regression value for the experimental data fitting to Monod model at 1.2atm was unsatisfactory (R^2=0.88). However, the regression analysis and kinetic parameters obtained at 1.4, 1.6 and 1.8atm were reasonably acceptable. Thus, Monod kinetic model is capable to describe the culture growth and substrate consumption behavior at 1.4, 1.6 and 1.8atm.

Contois Kinetic Model: Contois kinetic model is one of the unstructured models depends two terms such as substrate and cell concentrations. The following equations are the Non-linear and linear forms of Contois model [25]:

Table 1: Kinetic parameters at various pressures obtained by fitting the experimental data with different kinetic models

	Pressure, atm				
Kinetic models	1	1.2	1.4	1.6	1.8
Logestic					
$x_0(g,l^{-1})$	0.0450	0.0450	0.0400	0.0250	0.0250
$\mu_m(h^{-1})$	0.1250	0.1238	0.1408	0.0748	0.0813
$x_m(g,l^{-1})$	1.0670	3.1360	1.0220	0.0781	0.0733
$R^2(-)$	0.9984	0.9821	0.9637	0.9910	0.9865
Monod					
$\mu_{max}(h^{-1})$	-	0.0980	0.0949	0.0632	0.0621
$K_p(mmol,l^{-1})$	-	1.6540	2.0980	3.7440	5.4260
$R^2(-)$	-	0.8865	0.9921	0.9958	0.9718
Contois					
$\mu_{max}(h^{-1})$	0.0930	0.0612	0.0680	0.0466	0.0440
$K_p(mmol,g^{-1})$	3.7570	1.1531	2.3215	38.510	48.870
$R^2(-)$	0.9771	0.9243	0.9886	0.9829	0.9237
Moser					
$\mu_{max}(h^{-1})$	0.1870	0.0706	0.0714	0.0431	0.0448
$K_p(mmol^p,g^{-1})$	4.5210	1.1960	2.2100	4.5650	13.058
$R^2(-)$	0.9601	0.9104	0.9931	0.9957	0.9742
Tessier					
$\mu_{max}(h^{-1})$	-	0.0694	0.0716	0.0447	0.0465
$K_p(mmol^p,l^{-1})$	-	1.3250	1.9240	2.9570	4.9540
$R^2(-)$	-	0.9125	0.9968	0.9971	0.9630
Verhulst					
$\mu max(h^{-1})$	0.0915	0.0619	0.0683	0.0551	0.0475
$x_m(g,l^{-1})$	0.6580	1.7240	0.9350	0.1040	0.1380
$R^2(-)$	0.9031	0.9015	0.9770	0.9302	0.8560
Andrew					
K_p(atm)	-	3.9160	4.9200	7.0270	-
$\mu max(h^{-1})$	-	0.1763	0.1719	0.1017	-
$K_p(mmol^p,l^{-1})$	-	5.6930	8.3210	13.920	-
$R^2(-)$	-	0.9900	0.9900	0.9900	-

$$\mu = \frac{\mu_m C^*_{H_2S}}{K_p X + C^*_{H_2S}} \quad (6)$$

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_p}{\mu_m} \times \frac{X}{C^*_{H_2S}} \quad (7)$$

The linearized plot (1/μ) verse $(X/C^*_{H_2S})$ illustrated in Figure 3. The useful kinetic parameters (μ_m and K_p) were determined. Summary of the regression values and obtained kinetic parameters are reported in Table 1. The obtained data for all initial gas pressures fitted to Contois model were quite promising. The slope of illustrated plot, (K_p/μ_m) was highly dependent on gas pressures. Figure 3 shows data plotted based on Contois model; as the slope of the lines related to low gas pressure was insignificant; while the slope of the line was sharply increasing as the initial gas pressure increased. Therefore high K_p values were obtained at 1.6 and 1.8atm initial gas pressures.

Moser Kinetic Model: Moser kinetic model is strictly related to substrate concentration. Equations 8 and 9 represent the Non-linear and linear form of Moser model [25]:

$$\mu = \frac{\mu_m C^{*n}_{H_2S}}{K_p + C^{*2}_{H_2S}} \quad (8)$$

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_p}{\mu_m} \times \frac{X}{C^{*n}_{H_2S}} \quad (9)$$

Where μ and μ_m are the specific growth rate and maximum specific growth rate for H_2S, respectively. The term K_p is saturation constant for H_2S and n is constant as the exponent of substrate concentration. The illustrated plots of (1/μ) verse $(1/C^{*2}_{H_2S})$ were obtained by Matlab software is shown in Figure 4. The slope, (K_s/μ_{max}), the intercept, $(1/\mu_{max})$ and the exponent of substrate concentration for n = 2, the kinetic constants was determined by Moser model. The kinetic parameters are reported in Table 1.

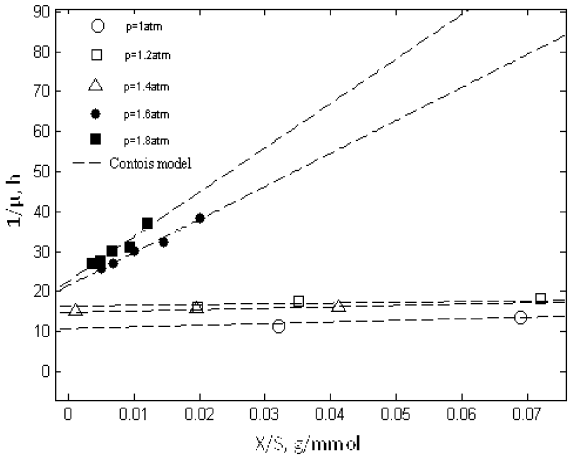

Fig. 3: Experimental data for microbial growth and substrate consumption at various initial gas pressures fitted to Contois model

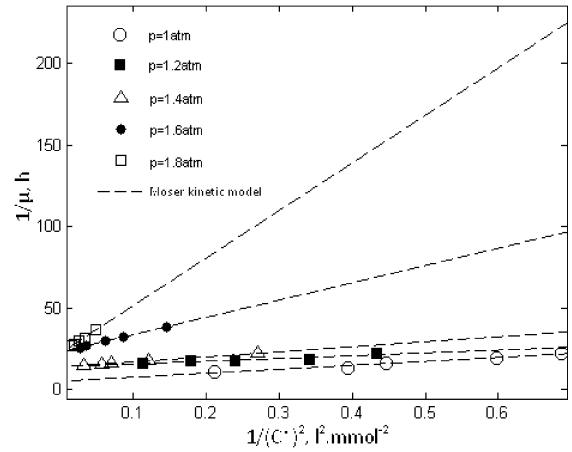

Fig. 4: Experimental data for microbial growth and substrate consumption at various initial gas pressures fitted to Moser model

Figure 4 shows the regression values for the fitting of experimental data achieved for cell growth and substrate consumption in Moser kinetic model. The obtained results were well fitted with the projected model. Here, the best regression analysis was also obtained at 1.4, 1.6 and 1.8atm. In fact, at low pressures of 1 and 1.2atm, the bacterial behavior did not follow Monod and Moser kinetic model; thus the unstructured models were substrate related and growth dependent.

Verhulst Kinetic Model: Verhulst kinetic model depends to cell concentration. This model has two kinetic constants of maximum specific growth rate (μ_{max}) and maximum cell concentration(X_m). Verhulst model is expressed by the following equations [25]:

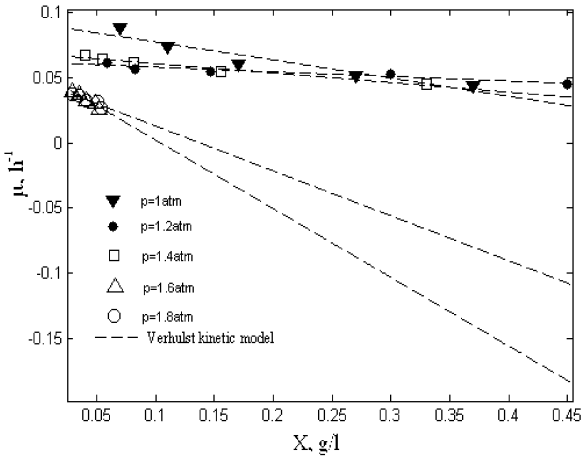

Fig. 5: Experimental data for microbial growth and substrate consumption at various initial gas pressures fitted to Verhulst model

$$\mu = \mu_m - \frac{\mu_m}{X_m} X \tag{10}$$

The plots of (μ) verse (X) for all initial gas pressures are depicted in Figure 5. The kinetic parameters determined by the slope of (μ_{max} / X_m) and intercept of (μ_{max}) are summarized in Table 1.

The obtained regression values for linear plot at pressures of 1 to 1.6atm were in acceptable range, but the regression value at pressure of 1.8atm was quite low (0.855). Since, Verhulst model is only depend to cell concentration; where the value of 0.855 indicates that the bacterial behavior at high initial gas pressure was related to both cell density and substrate concentration.

Tessier Kinetic Model: Tessier model is another unstructured model depends on substrate concentration. Linear and Non-linear form of this model is given by the following equations [25]:

$$\mu = \mu_m (1 - e^{\frac{-C^*_{H_2S}}{K_p}}) \tag{12}$$

$$\ln\left[1 - \frac{\mu}{\mu_m}\right] = -\frac{C^*_{H_2S}}{K_p} \tag{13}$$

Application of experimental data and Matlab software for the plot of (μ) verse ($C^*_{H_2S}$) was illustrated in Figure 6. The obtained kinetic constants are also summarized in Table 1.

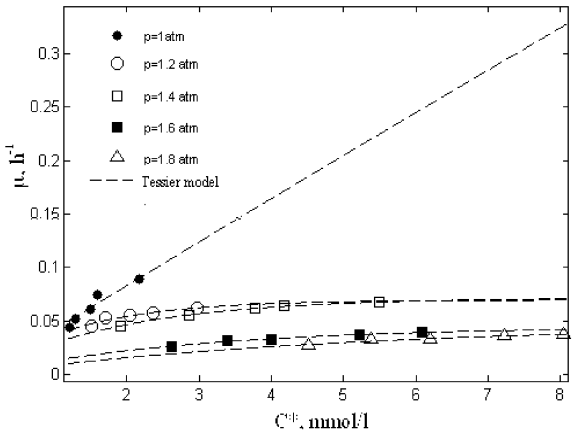

Fig. 6: Experimental data for microbial growth and substrate consumption at various initial gas pressures fitted to Tessier model

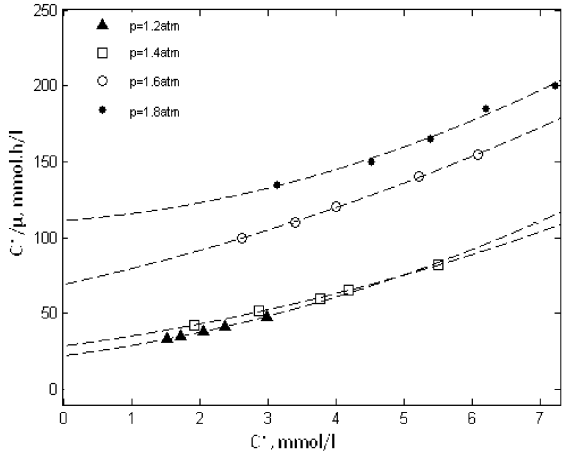

Fig. 7: Experimental data for microbial growth and substrate consumption at various initial pressures fitted to Andrew model

The regression analysis for the linear plot of Tessier model fitted with the cell concentration and substrate consumption rate by the microorganisms was accepted and quite satisfactory. The bacterial kinetic behavior at low gas pressure (1atm) did not follow Tessier model which was similar results obtained for Moser and Monod Kinetic models. These results lead to conclusion that at the low gas pressure of 1atm, the growth behavior did not fit to any unstructured models which are directly related to substrate concentration.

Andrew Kinetic Model: Andrew's model is proposed the following equation for the growth- dependent which incorporate substrate inhibition [26, 27].

$$\mu=\frac{\mu_m C^*_{H_2S}}{K_p+C^*_{H_2S}+(C^*_{H_2S})^2/K_i} \quad (14)$$

Where μ is the specific growth rate (h^{-1}), μ_m is the maximum specific growth rate for H_2S (h^{-1}), K_p is hydrogen sulfide concentration in gas phase in equilibrium with liquid phase ($mmol.l^{-1}$), $C^*_{H_2S}$ is Monod constant for H_2S ($mmol.l^{-1}$) and K_p is the inhibition constant ($mmol.l^{-1}$). Equation (14) was modified to a new expression as stated as follows:

$$\frac{C^*_{H_{2S}}}{\mu}=\frac{K_p}{\mu_m}+\frac{C^*_{H_2S}}{\mu_m}+\frac{\left(C^*_{H_2S}\right)^2}{\mu_m K_i} \quad (15)$$

Figure 7 shows the growth-dependent of H_2S by the microorganism with initial gas pressures of 1.2 to 1.6atm. The hydrogen sulfide flux has increased by augmentation of hydrogen sulfide concentration $(C^*_{H_2S})^2$ as easily used by the microorganisms in the culture media. The mixed culture exhibited more H_2S inhibition in a batch process under initial gas pressure of 1.6atm. This behavior may be due to the toxicity of hydrogen sulfide which has inhibited the activity of the microorganisms. The inhibition constants for the total pressure of 1.2 and 1.6atm were 5.69 and 13.92 ($mmol\ H_2S.l^{-1}$), respectively. The lowest value for inhibition coefficient was devoted to the lowest pressure 1.2atm. As the pressure of gas increased the inhibition coefficient was also increased. The kinetic parameters for rate models with inhibition and mass transfer coefficients are summarized in Table 1.

CONCLUSION

The removal of hydrogen sulfide from mixed gas was successfully carried out in a batch bioreactor using microorganisms isolated from hot spring. Experiments were conducted with various initial gas pressures with natural gas at 1 to 1.8atm, which comprise variable hydrogen sulfide concentration. The experimental data fitted to several kinetic models (Andrew, Contois, Logistic, Monod, Moser, Tessier and Verhulst models) were led to kinetic parameters under various initial gas pressures. It was observed that maximum cell dry weight of 3.136 and $1.724 g.l^{-1}$ were obtained with Logistic and Verhulst models, respectively. Logistic model described the microbial growth and substrate utilization better than the other projected models ($R^2 > 0.96$). Andrew's equation

also predicted the inhibition constant; the maximum specific growth rate (μ_{max}) for Andrew's model was 0.176h^{-1}. The low regression values (R^2 =0.88) for the experimental data fitting to Monod model at 1.2atm was unsatisfactory. It also concluded that the microorganism isolated from a hot spring was capable of oxidizing sulfur compound and significant amount of the hydrogen sulfide from natural gas was removed.

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Nomenclature

Ar	Argon
C^*	Equilibrium concentration of hydrogen sulfide (mmol.l^{-1})
CH_4	Methane
CO_2	Carbon dioxide
H	Henry' s law constant (atm.l.$mmol^{-1}$)
H_2S	Hydrogen sulfide
K_i	Inhibition constant (mmol.l^{-1})
K_p	Monod constant for H_2S (mmol.l^{-1})
n	Exponent of concentration (-)
$P_{H_2S,\,gas}$	Partial pressure of H_2S in the gas phase (atm)
t	Time (h)
TCD	Thermal conductivity detector
X	Cell dry weight concentration (g.l^{-1})
X_0	Initial cell dry weight (g.l^{-1})
X_m	Maximum cell dry weight (g.l^{-1})
μ	Specific growth rate (h^{-1})
μ_m	Maximum specific growth rate (h^{-1})

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