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Growth Kinetic Study in Biological Removal of Hydrogen Sulfide from Natural Gas in Batch Bioreactor

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ABSTRACT

The objective of the present study was to assess the Non-structured kinetic models of microorganisms used in biological desulfurization of hydrogen sulfide from gas stream in a batch bioreactor. The microorganisms used for the removal of hydrogen sulfide were originated from a hot spring. The experiments were conducted with mixed gas at operating temperatures of 25 to 45°C with a time interval of 5h. Different kinetic models such as Monod, Logistic, Tessier, Moser and Verhulst are considered and compared by means of statistical tools. Temperature of 35°C revealed the highest regression values by fitting the experimental data obtained from measurement of bacterial growth and substrate utilization with all used models. At this temperature, the achieved regression values for Monod, Logistic, Tessier, Moser and Verhulst models were 0.95, 0.98, 0.92, 0.93 and 0.91, respectively which were reasonably acceptable. The maximum specific growth rates were also determined to be 0.315, 0.240, 0.858, 0.33 and 0.143 h^{-1} for Monod, Logistic, Tessier, Moser and Verhulst model, respectively. In addition, the hydrogen sulfide removal was 72% at 35°C. The maximum cell dry weight was 0.733 $g.l^{-1}$ which achieved by Verhulst model. The inhibition coefficient was another parameter which was evaluated as a parameter affecting growth kinetics. As the gas temperature was increased to 45 °C, the inhibition coefficient may be dominated in growth kinetic. In overall, the microorganism isolated from a hot spring was capable of oxidizing sulfur compound and caused an acceptable amount of the hydrogen sulfide to be removed.

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1. INTRODUCTION

Removal of the H_2S from gases has attracted many attentions because of some reasons such as health, odor problems safety, and corrosion during transmission and distribution and also preventing pollution with sulfur dioxide upon combustion of the gases. Various technologies have been developed for removal of hydrogen sulfide from gas streams in the past, such as chemical absorption, adsorption, membrane separation, and adsorption/oxidation and biological methods[1]. Among these methods, the Claus process is the most common and widely used method. However, there are some major problems in this process [2, 3]: large energy consumption, low regenerative rate, the solvent loss and equipment corrosion. For adsorption methods, activated carbons [4-7] and many metal oxides have been used as sorbents for removing hydrogen sulfide by chemical adsorption from gas streams [8, 9]. On the other hand, biological processes operate at ambient temperature and atmospheric pressure; thus exhibit to be the most efficient and economic alternative for the removal of hydrogen sulfide [9-12].

In biological process, microorganism kinetics may significantly vary with the change in the culture conditions. Maximum growth rate and maximum cell dry weight must be determined as an input for process optimization, modeling and scale-up. Kinetic models can be used to achieve a better understanding of the microbial growth and substrate consumption for process description governed by the microorganisms [12-14].

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These mathematical equations represent the behavior of the microorganisms and are derived from experimental data. The final growth kinetic models are derived based on all of the factors that influence the growth of microorganism. Structural growth models due to expressing behavioral reactions of cells are more complex than the unstructured growth models. In the non-structural model, because of addressing the complex interactions of intracellular reactions, the amount of substrate utilization and production of extracellular metabolites will be studied [15].

The aim of present work was to use an isolated anaerobic organism for biodesulfurization of natural gas in a batch culture. Monod, Logistic, Tessier, Moser and Verhulst kinetic models for evaluation of the effect of temperature on hydrogen sulfide removal and microbial cell growth were also investigated. Experimental data was fitted by kinetic models and kinetic parameters were determined.

2. MATERIALS AND METHODS

2. 1. Materials and Media The sulfur oxidizing bacteria used in this study has been isolated from Ramsar hot spring (Iran). It was grown in an anaerobic serum bottle media incubated at 30℃ and 180 rpm. The serum bottles containined 50ml liquid media; with media composition in grams per liter given as follow: 2.0 KH_2PO_4, 2.0 K_2HPO_4, 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 included ($g.l^{-1}$) 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 $CuSO_4.5H_2O$. Distilled water was added to make 1-liter of broth solutions. The initial media pH was 6.8 and monitored by pH meter (HANA, 211, Romania). All chemicals used were analytical graded and provide by Merck (Darmstadt, Germany).

2. 2. Analytical Methods Batch experiments were done in sealed serum bottles with a volume of 125ml. The serum bottles contained 50ml of fresh media prepared under nitrogen gas. Gas impermeable rubber septum and aluminum crimp seals were applied to seal the bottles for being used under various operating temperatures. The bottles with liquid media were sterilized at 121℃ for 15min. The mixed gas includes of the components of H_2S, CO_2, Ar and CH_4 gas. The experiments were conducted with various operating temperatures on an orbital shaker (Stuart, S1500, and UK) set at agitation rate of 180rpm from 25 to 45 °C with a time interval of 5 °C.

The optical density of liquid samples were measured at a wavelength of 600nm using a spectrophotometer (Unico, 2100, USA). Gas chromatograph (Agilent, 7890A, USA) equipped with a thermal conductivity detector (TCD) was utilized for gas analysis. Helium gas was applied as carrier gas at a flow rate of 25 $ml.min^{-1}$. Monod, Logistic, Tessier, Moser and Verhulst models were used to describe the behavior of microbial growth and substrate consumption rate by the isolated microorganisms for natural gas within operating temperatures range of 25 to 45°C.

2. 3. Kinetic Models

2. 3. 1. Monod Kinetic Model Monod kinetic model is considered as one of the unstructured models which are dependent to substrate concentration as below [13]:

$$\mu = \frac{\mu_m C^*_{H_2S}}{K_S + C^*_{H_2S}} \quad (1)$$

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_S 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:

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_s}{\mu_m} \times \frac{1}{C^*_{H_2S}} \quad (2)$$

2. 3. 2. Logistic Kinetic Model This model included inhibition coefficient which is proportional to cell density. In addition, 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 described by the following equation:

$$\mu = \mu_m(1 - \frac{X}{X_m}) \quad (3)$$

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 defined in the following equation gives the cell density with respect to time [13]:

$$X = \frac{X_0 e^{\mu_m t}}{1 - \frac{X_0}{X_m}(1 - e^{\mu_m t})} \quad (4)$$

2. 3. 3. Tessier Kinetic Model The other model used for the description of growth kinetics and substrate utilization is Tessier model, which is based on an exponential function. The form of this model is given by Equation (5):

$$\mu = \mu_{max}(1 - e^{\frac{-S}{K_S}}) \quad (5)$$

2. 3. 4. Moser kinetic Model The mathematical formulation of the Moser model is given by:

$$\mu = \mu_{max} \frac{S^n}{K_S + S^n} \quad (6)$$

where, n is an adjustable parameter. The empirical equation proposed by Hermann Moser was a modified form of Monod equation. Moser upgraded the model of Monod with a parameter n (usually $n > 1$) to integrate effects of adoption of microorganisms to stationary processes by mutation. For n =1 the specific growth rate becomes equal to the Monod mode [16].

2. 3. 5. Verhulst Kinetic Model In some cases, Verhulst kinetic model has been used for the demonstration of growth characteristics of the cell population [17]. This model is shown in Equation (7) and contains two parameters; the maximum specific growth rate (μ) and maximum cell dry weight (X). It defines the relationship between cell growth and substrate utilization to describe the kinetic behaviors of a microbial cell population [18, 19].

$$\mu = \mu_{max}[1 - \frac{X}{X_m}] \quad (7)$$

2. 4. Substrate Consumption Rate The kinetics for biological hydrogen sulfide elimination reactions can be defined by Michaelis- Menten equation [20] stated as follows:

$$-\frac{dS}{dt} = \frac{\vartheta_m S}{K_m + S} \quad (8)$$

where, S is substrate concentration (g.l^{-1}), ϑ_m is the maximum reaction rate (g.h^{-1}.l^{-1}), t is the reaction time (h), and K_m is the saturation constant (h^{-1}). In this study, the substrate utilization by microorganism was followed the first-order reaction kinetic, while the kinetic parameter for the above reaction revealed that K_m was much greater than the substrate concentration. Equation (8) reduced to first order chemical reaction rate. The expression for substrate consumption with respect to time is written as first-order rate equation as follows:

$$-\frac{dS}{dt} = k_s . s \quad (9)$$

$$k_s = \frac{\vartheta_m}{K_m}$$

where, k_s is the first order rate constant (h^{-1}).

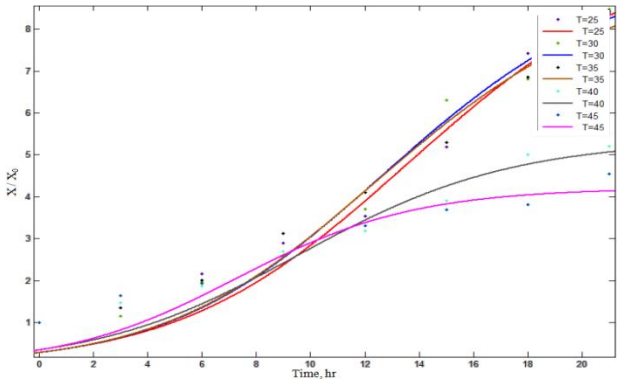

Figure 1. Experimental data for microbial growth and substrate consumption at various gas temperatures fitted to Monod model

3. RESULTS AND DISCUSSION

Figures 1 to 5 depict the experimental data for microbial growth and substrate consumption at various gas temperatures fitted to Monod, Logistic, Tessier, Moser and Verhulst model, respectively (After adding the mixed gas to serum bottle, pH was not adjusted). The achieved kinetic parameters are shown in Table 1.

3. 1. Fitting the Experimental Data with Monod Model The illustrated plot of $(1/\mu)$ verse $(1/C^*_{H_2S})$, (Lineweaver-Burk plot), is depicted in Figure 1. The achieved kinetic parameters are also shown in Table 1. The regression value for the experimental data fitting to Monod model at 45°C was unsatisfactory (R^2=0.87). However, the regression analysis and kinetic parameters attained at 25, 30, 35 and 40 °C were reasonably acceptable. Therefore, Monod kinetic model is able to describe the culture growth and substrate consumption behavior at 25, 30, 35 and 40°C.

3. 2. Fitting the Experimental Data with Logistic Model Figure 2 depicts the cell dry weight of mixed culture obtained in batch experiment with 5 operating temperatures from 25 to 45 °C. An increase in operating temperatures caused to direct proportional increase in hydrogen sulfide concentration as gaseous substrate. As the gas temperature increased from 25 to 35°C, there was also an increase in cell dry weight, but as the gas temperature rose to 40 and 45°C, the cell concentration was decreased. The maximum cell dry weight was obtained with gas temperature of 35°C. In the batch bioreactor, the exponential growth rates were obviously seen with gas temperature in the range of 25 to 35°C.

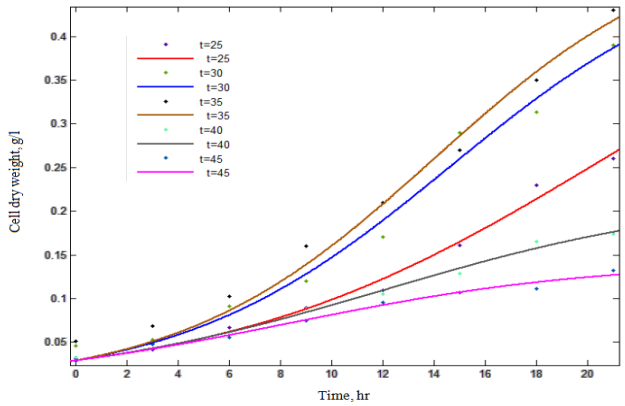

Figure 2. Microbial cell concentration grown at various operating temperatures obtained by Logistic kinetic model

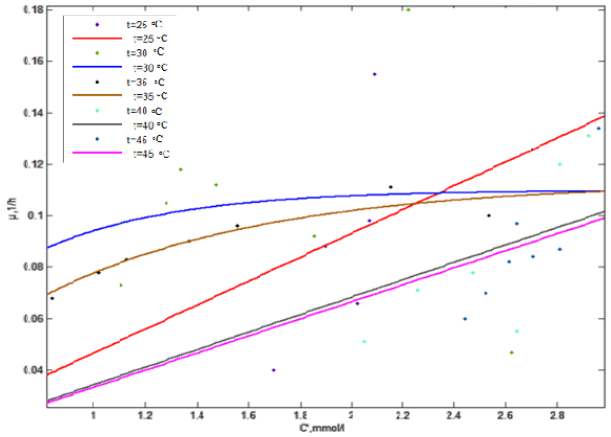

Figure 3. Experimental data for microbial growth and substrate consumption at various gas temperatures fitted to Tessier model

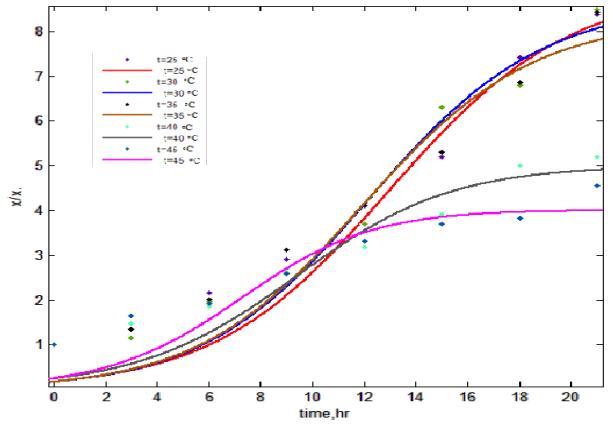

Figure 4. Experimental data for microbial growth and substrate consumption at various gas temperatures fitted to Moser model

3. 3. Fitting the Experimental Data with Tessier Model Figure 3 shows fitting the experimental data with Tessier model at various temperatures. The obtained kinetic parameters are presented in Table 1. It is shown that except the temperature of 35°C which was shown the appropriate compliance of regression equal to 0.92, at the other temperatures, there was no adequate correlation between experimental data and Tessier model. Applying Tessier model, the growth rate resulted to 0.858 h^{-1} which was the maximum value among the other studied kinetic models.

3. 4. Fitting the Experimental Data with Moser Model Assessment of the models parameter and curve fitting of cell growth with Moser model are shown in Figure 4. The results depicted that operating in all temperature have acceptable consistency but with an increase in temperature, compliance rate of experimental data were decreased. The maximum R-square and μ_{max} were 0.95 and 0.33 h^{-1}, respectively which was obtained at 30°C. It can be justified that the inhibition coefficient affected on the correspondence of data which caused to decrease compliance rate.

3. 5. Fitting the Experimental Data with Verhulst model The Verhulst model is still used often in environmental and industrial microbiology studies [21, 22]. Plotting the specific growth versus cell dry weight, we will able to determine the kinetic parameters. At low temperatures, experimental data did not follow the Verhalst model. However, with an increase in operating temperature, a suitable agreement was observed by fitting the experimental data with Verhalst model. In this kinetic model, the maximum cell dry weight of 0.733g.l^{-1} was obtained at 35 °C.

3. 6. Determination of the Hydrogen Sulfide Consumption Rate The amounts of Hydrogen sulfide in gas phase were determined according the procedure described at section 2.2. Figure 6 depicts the residual hydrogen sulfide in batch culture with respect to time under variable operating temperatures.

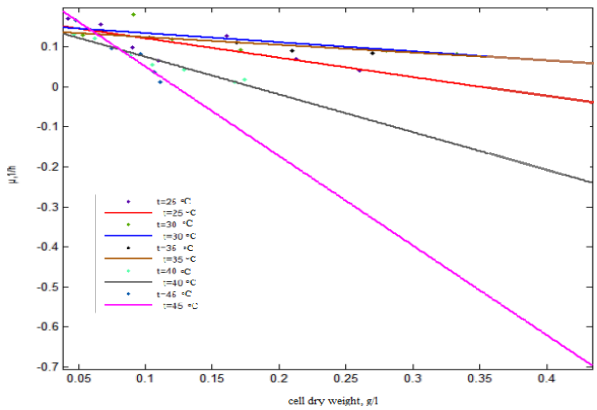

Figure 5. Experimental data for microbial growth and substrate consumption at various gas temperatures fitted to Verhulst model

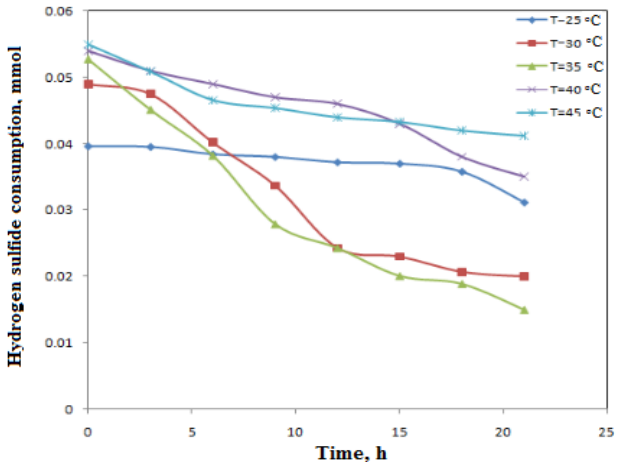

Figure 6. Residual Hydrogen sulfide in batch process

TABLE 1. Kinetic parameters at various temperatures obtained by fitting the experimental data with different kinetic models

Temperature(°C)	25	30	35	40	45
Logestic model:					
$\mu_m(h^{-1})$	0.138	0.191	0.204	0.156	0.165
$x_m(g.l^{-1})$	0.508	0.5	0.519	0.219	0.142
$R^2(-)$	0.99	0.98	0.98	0.99	0.98
Monod model:					
$\mu_{max}(h^{-1})$	0.29	0.313	0.315	0.298	0.353
$K_s(mmol.l^{-1})$	0.202	0.213	0.216	0.247	0.298
$R^2(-)$	0.96	0.97	0.95	0.92	0.87
Tessier mode:					
$\mu_{max}(h^{-1})$	-	-	0.858	-	-
$K_s(mmol.l^{-1})$	-	-	0.112	-	-
$R^2(-)$	-	-	0.92	-	-
Moser model:					
$\mu_{max}(h^{-1})$	0.312	0.33	0.33	0.31	0.36
$K_s(mmol.l^{-1})$	0.14	0.147	0.149	0.17	0.204
$R^2(-)$	0.92	0.95	0.93	0.87	0.81
Verhulst model:					
$\mu_{max}(h^{-1})$	-	-	0.143	0.168	0.274
$x_m(g.l^{-1})$	-	-	0.733	0.179	0.122
$R^2(-)$	-	-	0.91	0.96	0.944
Substrate consumption rate:					
$K_s(h^{-1})$	0.05	0.046	0.06	0.017	0.016
$R^2(-)$	0.88	0.94	0.98	0.93	0.89
Removal efficiency (%)	21.2	59	72	35.18	25.09

As the temperature increased from 25 to 45 °C, the removal of hydrogen sulfide was also increased while at high temperature of 40 to 45 °C, the removal efficiency was decreased.

4. CONCLUSION

The present paper focused on the hydrogen sulfide removal from mixed gas in a batch bioreactor using microorganisms isolated from a hot spring. Experiments were conducted with various initial temperatures. Five kinetic models such as Monod, Logistic, Tessier, Moser and Verhulst kinetics models was applied for the prediction of the bacterial growth rate and substrate consumption. By fitting the experimental data with used kinetic models, the kinetic parameters under various initial temperatures were achieved. It was observed that operation temperature had a significant influence on cell dry weights and H_2S uptake. The maximum and minimum K_s value was obtained 0.06 and 0.016 at temperatures of 35 and 45 °C, respectively. In addition, the maximum specific growth rate (μ_{max}) and maximum cell dry weight (X_m) was achieved as 0.858 h^{-1} and 0.733 $g.l^{-1}$ using Verhulst and Tessier kinetic model, respectively. In overall, the microorganism isolated from a hot spring was capable of oxidizing sulfur compound and caused an acceptable removal of the hydrogen sulfide in batch bioreactor.

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Hydrogen Sulfide

چکیده

هدف از مطالعه حاضر بررسی مدل های جنبشی غیر ساختاری از میکروارگانیسم ها در سولفورزدایی بیولوژیکی سولفید هیدروژن از جریان گاز در یک راکتور ناپیوسته می باشد. میکروارگانیسم مورد استفاده برای حذف سولفید هیدروژن از چشمه آب گرم سرچشمه گرفته است. آزمایش ها با گاز مخلوط در دماهای عملیاتی ۲۵ تا ٤٥ درجه سانتی گراد با فاصله زمانی ۵ ساعت انجام شد. مدل های جنبشی مختلف از جمله مونود، لجستیک، تیسیر، موزر و ورهالست در نظر گرفته شد و با استفاده از ابزارهای آماری مقایسه گردید. دما ۳۵ درجه سانتی گراد بالاترین مقدار رگرسیون را توسط برازش داده های تجربی بدست آمده از اندازه گیری رشد باکتری و مصرف سوبسترا با تمام مدل های مورد استفاده نشان داد. در این دما، مقادیر رگرسیون به دست آمده برای مدل های مونود، لجستیک، تیسیر، موزر و ورهالست به ترتیب برابر با ۰/۹۵، ۰/۹۸، ۰/۹۲، ۰/۹۳ و ۰/۹۱ بود که قابل قبول بودند. حداکثر نرخ رشد ویژه برابر با ۰/۳۱۵، ۰/۲٤، ۰/۸۵۸، ۰/۳۳ و ۰/۱٤۳ (عکس ساعت) به ترتیب برای مدل های مونود، لجستیک، تیسیر، موزر و ورهالست تعیین شد. علاوه بر این، حذف سولفید هیدروژن در ۳۵ درجه سانتی گراد ۷۲ درصد بود. حداکثر وزن خشک سلولی ۰/۷۳۳ گرم بر لیتر بود که توسط مدل ورهالست به دست آمد. ضریب بازدارندگی، پارامتر دیگری بود که به عنوان یک پارامتر موثر بر سینتیک رشد مورد بررسی قرار گرفت. هنگامی که درجه حرارت گاز به ٤٥ درجه سانتی گراد افزایش یافت، ضریب بازدارندگی ممکن بود در سینتیک رشد مهم شود. در مجموع، میکروارگانیسم های جدا شده از چشمه آب گرم قادر به اکسید کردن ترکیب سولفور بودند و باعث شدند مقدار قابل توجهی از سولفید هیدروژن حذف گردد.

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