

## Bioremediation of hydrogen sulfide from gas stream in continuous culture with cell recycle

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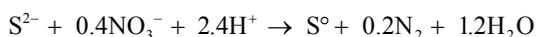
### Abstract

*Biodesulfurization of sour gas has been carried out in a continuous stirred tank bioreactor (3.5 L) equipped with absorption tank (1.5 L) and cell recycle unit (1 L). Pure culture of sulfur oxidized bacteria was isolated from native sulfur hot spring. Continuous biodesulfurization was conducted with cell decanter as cell separator. Cell concentration was increased from 1.35 to 1.96 g.L<sup>-1</sup> as cell recycle ratio was increased from 0 to 0.6. Maximum hydrogen sulfide transfer rate and volumetric mass transfer coefficient (k<sub>1a</sub>) was determined to be 0.0463 mol.m<sup>-3</sup>.h<sup>-1</sup> and 0.0202 h<sup>-1</sup> at 36°C and 250 rpm, respectively. Application of absorption tank improved sulfide removal rate under cell recycling anaerobic conditions approximately by 18%. The optimal dilution and gas flow rate were achieved at 0.0186 h<sup>-1</sup> and 2 mL.min<sup>-1</sup>. The removal of H<sub>2</sub>S at high concentration in the sour gas (5% v/v H<sub>2</sub>S, 5% v/v CO<sub>2</sub>, 10% v/v Ar, 80% v/v CH<sub>4</sub>) was determined to be 98%.*

**Keywords:** Biodesulfurization; Mass transfer coefficient; Cell recycle; Agitation rate; Hydrogen sulfide

### 1. Introduction

Natural gas plays an important role for the energy demands. Use of natural gas has increased to 35% over last decade and it is expected to increase to 53% by 2020 [1]. Natural gas contains some impurities such as hydrogen sulfide (H<sub>2</sub>S) known as sour gas. H<sub>2</sub>S is a toxic, flammable and corrosive gas which must be reduced to acceptable levels (less than 10 ppmv) before use [1]. On the other hand, H<sub>2</sub>S in gas stream can cause serious problems such as corrosion and environmental pollutions after combustion, reducing efficiency of fluid handling equipment and value of the products [2, 3]. Various methods were introduced to gas sweetening process. Because of economical and environmental issues of conventional gas sweetening methods, biodesulfurization (BDS) of sour gas is a breakthrough in field of natural gas processing. BDS is cost effective method that can remove H<sub>2</sub>S using sulfur oxidizing bacteria. These microorganisms can biologically mediate elimination reaction of H<sub>2</sub>S. In this biochemical reaction H<sub>2</sub>S is utilized as electron donor; while oxygen and nitrate act as electron acceptor in aerobic and anaerobic conditions, respectively [4, 5]. Partial oxidation of hydrogen sulfide under denitrification conditions leads to formation of sulfur and nitrogen. The major reaction is summarized as follows [6]:



The main drawbacks of the aerobic biological reactions are the occurrence of side reaction and disposal of the foul air containing H<sub>2</sub>S. In addition, in aerobic processes air is

mixed with the treated gas and this leads to a dilution of gas concentration. These difficulties may be resolved by the use of anaerobic treatment instead of an aerobic process. Thus, for the production of pipeline grade gas, anaerobic biofiltration is more feasible technology than the commonly used aerobic biodesulfurization [7]. Generally, anoxic and anaerobic operations have such drawbacks: biological nitrogen and phosphorus removal is not possible; much more sensitive to the adverse effect of low temperature on reaction rate; the process is slow with low cell propagation; have potential for production of odors and corrosive gases.

Investigation on BDS processes have been conducted by many researchers using various techniques [1, 2, 4, 8]. The aim of this work was to evaluate the functional properties of the native isolated bacteria in anaerobic continuous BDS operation. It is customary to use mixed culture for waste water treatment or for treatment of complex nature of contaminations. But, in other biological processes and specially for eliminating one particular component, pure culture may be more efficient than the mixed culture at controlled conditions. Since the removal of hydrogen sulfide is cell growth associated, then it is required to achieve high cell density and efficient mass transfer in the bioreactor. For this purpose, the bioreactor was equipped with gas absorption and cell recycling unit. The effect of agitation speed in absorption system on overall H<sub>2</sub>S removal efficiency was investigated. In order to obtain optimal condition, continuous BDS was conducted with various recycle ratio, dilution rate and gas flow rate.

## 2. Materials and Methods

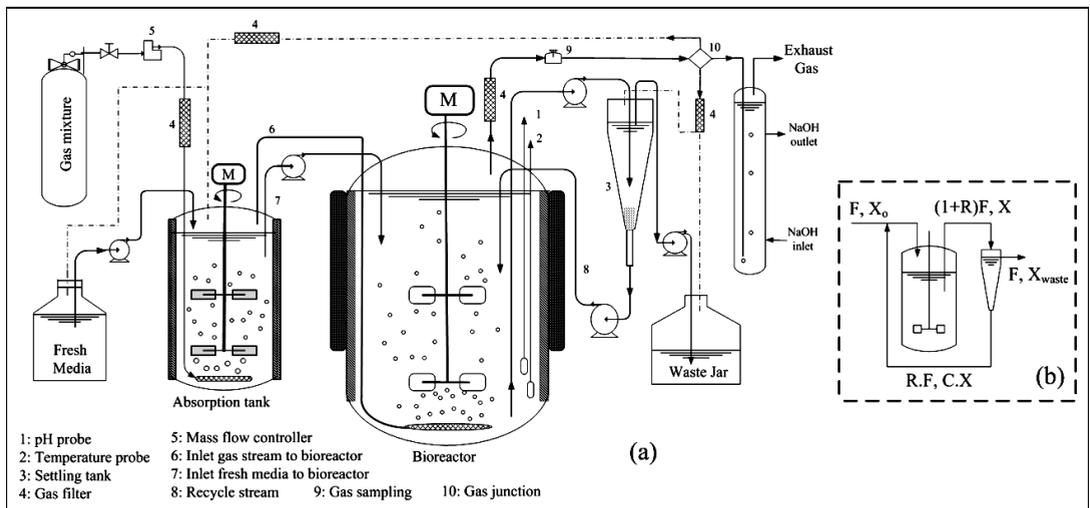
### 2.1. Bacteria and Growth Media

Based on survey, some bacteria residing in hot spring can oxidize or reduce sulfur compounds [9]. In this research, an isolated pure culture was identified from the sulfur hot spring located in the hill side of Alborz mountain (Ramsar, Iran). It was anaerobically grown in a rich media at 36°C in an incubator shaker (Stuart, S1500 and UK) with agitation rate of 180 rpm for 72 h. On the basis of previous work [10], the growth medium consists of: 0.6 g of NH<sub>4</sub>Cl, 0.2 g of MgCl<sub>2</sub>·6H<sub>2</sub>O, 1.2 g of KH<sub>2</sub>PO<sub>4</sub>, 1.2 g of K<sub>2</sub>HPO<sub>4</sub>, 0.3 g of NaNO<sub>3</sub>, 1 g of yeast extract, 7 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>·5H<sub>2</sub>O and 1 L deionized water. Also, 2 mL of vitamins solution and 1 mL of trace metals were prepared based on previous published data which is added to the synthetic medium [10]. All the chemicals used were analytical graded and supplied by Merck (Darmstadt, Germany).

### 2.2. Continuous Culture Operation

A defined medium was used to prepare inocula and cultivate the cells in a bioreactor. Initially, the bioreactor (Infors, Switzerland) with a working volume of 3.5 L was anaerobically operated under batch condition for an incubation period of 72 h at a fixed temperature of 36°C and controlled pH at 6.5. pH was adjusted using 0.1 molar of HCl and NaOH (Merck) solutions. The prepared mixed gas was comprised of H<sub>2</sub>S, CO<sub>2</sub>, Ar and CH<sub>4</sub> composite of 5, 5, 10 and 80% v/v, respectively. The high concentration of sour gas composition was prepared based on worst case of natural reservoirs in Iranian gas field. Iranian gas reservoirs contain sour gas may contained various composition and concentration of components. Based on National Iranian Gas Company (NIGC) surveys, natural gas contains 3 ppm (Tabnak, Persian Gulf and Sarajeh, Qom, Iran) to 50000 ppm (Khangiran, Khorasan and Salakh, Qeshm, Iran) hydrogen sulfide [11, 12]. In addition, it has been reported that in Marjan complex which is a large offshore oil field located in Persian Gulf; the reservoir is shared between Saudi Arabia and Iran, the H<sub>2</sub>S concentration varies from 10 to 40000 ppm [13]. The gas flow rate was controlled at 2 mL·min<sup>-1</sup> using a gas flow controller (dwyer, USA). The agitation rate was adjusted to 250 rpm after initial inoculation. A continuous mode of operation was started on third day at a liquid flow rate of 0.5 mL·min<sup>-1</sup> (0.008 h<sup>-1</sup>).

Cell concentration in bioreactor was increased by recycling cell biomass from the effluent stream. For this purpose a cell separator presented in Figure 1b (1 L settling separation funnel) was used. The concentrated cells were continuously recycled back to the continuous stirred tank bioreactor (CSTBR) with recycle ratio in the range of 0.1 to 1 (stream line 8 in Figure 1a). Mass transfer rate of  $H_2S$  was considered and volumetric mass transfer coefficients were calculated for various agitation rates in bioreactor. Absorption tank was employed to enhance mass transfer of hydrogen sulfide through BDS process. For this purpose, influent sour gas was firstly passed through an absorption tank with capacity of 1.5 L while fresh sterilized media was pumped into the adsorption tank then saturated media with  $H_2S$  transferred to the bioreactor (stream line 7 in Figure 1a). Absolutely, there was no organism present in the absorption tank. The saturated medium with sour gas experimented with various dilution rate of 0.008, 0.0133, 0.0186, 0.026, 0.035 and  $0.045\text{ h}^{-1}$ . In addition, from the absorption tank, the unabsorbed sour gas in the gas phase was sparged to the bioreactor under positive pressure influent gas pressure of 1.25 atm (stream line 6 in Figure 1a). The effect of various sour gas flow rates in the range of 2 to  $8\text{ mL}\cdot\text{min}^{-1}$  were monitored during the course of continuous operation. For safe operation, the unutilized sour gas and vent gas is absorbed by NaOH gas trap at the end of pipeline. In order to prepare anaerobic condition, the exhausted treated mixed gas was led to the system linings before the trap. That was preventing penetration of ambient atmosphere. In addition, use of inert gas such as nitrogen may interfere in gas analysis by gas chromatography (GC) instead of argon as internal standard. The schematic representation of the experimental set up is shown in Figure 1a.



**Figure 1.** (a) Schematic representation of the experimental set up of CSTBR; (b) Mass flow rates of bioreactor equipped with cell decanter

### 2.3. Determination of Mass Transfer Coefficient:

Mass transfer coefficient ( $k_L a$ ) was calculated by technique which has been reviewed in literature [14]. In steady state condition, based on mass balance, the difference of  $H_2S$  flow between inlet and outlet must be equal to the rate of  $H_2S$  transferred from gas phase to liquid phase.

$$N_A = \frac{1}{V_L} \left[ (v_g C_{H_2S, G})_i - (v_g C_{H_2S, G})_o \right] = \frac{1}{V_L R} \left[ \left( \frac{v_g P_{H_2S} S_G}{T} \right)_i - \left( \frac{v_g P_{H_2S} S_G}{T} \right)_o \right] \quad (1)$$

where  $V_L$  is the volume of liquid in the fermenter,  $v_g$  is the volumetric gas flow rate,  $C_{H_2S,G}$  is the gas-phase concentration of  $H_2S$ , and subscripts i and o refer to inlet and outlet gas streams, respectively. Also, the  $H_2S$  transfer rate through the liquid phase is:

$$N_A = k_L a (C_{H_2S,L}^* - C_{H_2S,L}) \quad (2)$$

Combination of Equations (1) and (2) resulted:

$$k_L a (C_{H_2S,L}^* - C_{H_2S,L}) = \frac{v_g P_T}{V_L R T} [(y_{H_2S,G})_i - (y_{H_2S,G})_o] \quad (3)$$

where  $y_{H_2S,G}$  and  $C_{H_2S,L}^*$  is the mole fraction of  $H_2S$  in the gas phase and equilibrium concentration of  $H_2S$  in liquid phase. Rearranging of Equation (3) gives an expression for  $C_{H_2S,L}^*$ :

$$C_{H_2S,L}^* = C_{H_2S,L} - \frac{v_g P_T}{k_L a V_L R T} [(y_{H_2S,G})_i - (y_{H_2S,G})_o] \quad (4)$$

$k_L a$  and  $C_{H_2S,L}^*$  were obtained by plot of  $C_{H_2S,L}^*$  versus  $[(y_{H_2S,G})_i - (y_{H_2S,G})_o]$  [15-17]. Furthermore, mass transfer rate of  $H_2S$  was calculated from Equation (2).

#### 2.4. Analytical Method:

The cell population was determined by optical density of the media using spectrophotometer (Unico, 2100, USA) at wavelength of 600nm. Gas chromatograph (Agilent, 7890A, USA) equipped with a thermal conductivity detector (TCD) was used for gas analysis based on previous studies [10]. The dissolved sulfide concentration was determined using a spectrophotometric method. The presence of dissolved sulfide in cultures is proved rapidly by its colloidal precipitation as  $CuS$  in a copper sulfate reagent. The copper reagent consisted of  $HCl$  ( $50 \text{ mmol.L}^{-1}$ ) and  $CuSO_4$  ( $5 \text{ mmol.L}^{-1}$ ). Aliquots of cultures added (1:40, v/v) to the acidic  $CuSO_4$  solution which remained stable for 20-40 seconds. During this short period, the absorbance of the end product was detected at wavelength of 480 nm.  $HCl$  ( $50 \text{ mmol.L}^{-1}$ ) added to the culture aliquot which served as blank [18]. All gas and liquid samples were taken in every 12 h.

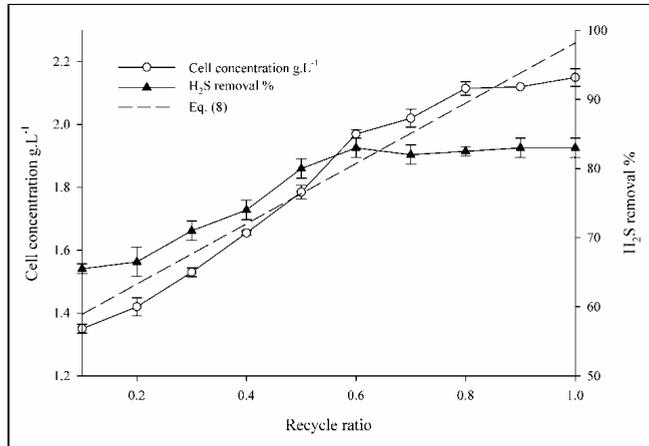
### 3. Result and Discussion

#### 3.1. Chemostat Operation with Cell Recycle:

The most economical way to provide cells in the inlet stream is to recycle the part of the outlet stream back to the inlet. Unlike the plug flow bioreactor, the continuous stirred tank bioreactor requires the cell separator in order to recycle. In this study, 1 L settler funnel as a cell separator was applied. Continuous experiments were commenced whereas a portion of the effluent culture was continuously recycled back to the bioreactor. Recycle ratio was considered in the range of 0.1 to 1 and the optimum value was obtained. Recycle ratio was defined as follows:

$$\text{Recycle ratio (R)} = \frac{\text{recycle flow rate}}{\text{feed flow rate}} \quad (5)$$

During the optimization of the recycle ratio, agitation speed, dilution and gas flow rates were kept constant at 250 rpm,  $0.0186 \text{ h}^{-1}$  and  $2 \text{ mL}\cdot\text{min}^{-1}$ , respectively.



**Figure 2.** H<sub>2</sub>S removal efficiency and cell concentration, in continuous operation with various recycle ratio: 0.1 – 1. At constant value of gas flow rate:  $2 \text{ mL}\cdot\text{min}^{-1}$ ; dilution rate:  $0.0186 \text{ h}^{-1}$ ; bioreactor agitation speed 250 rpm

Figure 2 illustrates the effect of recycle ratio on cell concentration and H<sub>2</sub>S removal efficiency. The cell concentration was gradually increased with recycle ratio (max. R= 0.8). At recycle ratio higher than 0.8; there was no significant improvement in cell density; that was probably due to inadequate time to precipitate. Capability of separator funnel was defined by a coefficient C for the concentrated cell stream. This parameter was determined by developing cell balance over the bioreactor and simultaneous cell decanter (see Figure 1b). At steady state condition with sterilized influent stream ( $X_0 = 0$ ), the resulted balance equation is stated as follows:

$$F \cdot X_0 + R \cdot F \cdot C \cdot X - (1 + R) \cdot F \cdot X + V \cdot \mu \cdot X = V \frac{dX}{dt} \Rightarrow \mu = (1 + R(1 - C))D \quad (6)$$

where, D is dilution rate which is defined by  $\frac{F}{V}$ . The specific growth rate can be explained by Malthus equation which is expressed as follows:

$$\mu = \mu_{max} \left( 1 - \frac{X}{X_{max}} \right) \quad (7)$$

Substituting Equation 6 into Equation 7 and rearrangement has resulted in the following equation for the cell concentration:

$$X = X_{max} \left[ 1 - \frac{D}{\mu_{max}} (1 + R(1 - C)) \right] \quad (8)$$

The value of C was obtained from the slope of plotting data for X versus R; which is illustrated in Figure 2. The value of C is determined to be 2.2 when D, R and  $\mu_{max}$  were  $0.0186 \text{ h}^{-1}$ , 0.6 and  $0.04 \text{ h}^{-1}$ , respectively. Cell concentration in the effluent stream of the cell decanter ( $X_{waste}$ ) can be obtained stated as follows:

$$(1 + R).X = R.C.X + X_{waste} \rightarrow X_{waste} = 0.53 \text{ g.L}^{-1} \quad (9)$$

Maximum H<sub>2</sub>S removal efficiency was gained 83% at R=0.6. Figure 2. shows that increasing of R from 0.6 to 0.8 did not exert significant effect on H<sub>2</sub>S% removal efficiency. This phenomenon may be due to accumulation of dead microorganism in bioreactor by high amount of recycle ratio. Therefore further experiments were conducted with R=0.6.

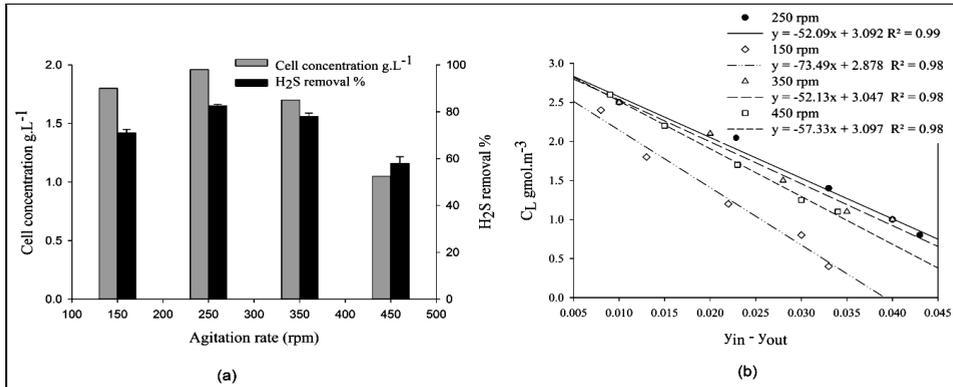
### 3.2. Optimum Agitation Rate

An important parameter for BDS process is agitation rate. This design parameter has major effect on the transfer of H<sub>2</sub>S bubble to the microorganisms and growth of them. Figure 3a depicts the effect of agitation rate on cell concentration and H<sub>2</sub>S removal efficiency. Four agitation rates were selected, ranging from 150 to 450 rpm. This set of experiments was accomplished by dilution rate and gas flow rate of 0.0186 h<sup>-1</sup> and 2 mL.min<sup>-1</sup>, respectively. Based on the obtained data, the suitable agitation rate determined to be 250 rpm. It is clear that transfer rate of H<sub>2</sub>S was increased by increasing in agitation rate. However, it was found out that increasing agitation rate from 250 to 350 and then 450 rpm caused to decrease cell concentration. That was due to high shear forces exerted by high agitation rate even agitation makes well suspension and uniformity of the media. Besides high agitation related to high energy consumption; but excessive shear due to vigorous mixing lead to loss of viability and cell disruption [19]. As it can be observed in Figure 3a, with increasing agitation rate from 250 to 350 rpm, in spite of declining cell density (decreased 13.2%), the removal efficiency of H<sub>2</sub>S was decreased to 6%. That was most probably due to enhancement of mass transfer rate resulted by high agitation. The amount of transferred H<sub>2</sub>S can be expressed in terms of volumetric mass transfer coefficient (k<sub>L</sub>a). The values of k<sub>L</sub>a, C<sub>H<sub>2</sub>S,L</sub><sup>\*</sup> and the maximum transfer rate of H<sub>2</sub>S were calculated from the slope and intercept of Figure 3b; the obtained data are summarized in Table 1. The maximum molar transfer rate of H<sub>2</sub>S was obtained by Equation 2 when [(y<sub>H<sub>2</sub>S,G</sub>)<sub>i</sub> - (y<sub>H<sub>2</sub>S,G</sub>)<sub>s</sub>] was at the highest value. According to the obtained data, by rising agitation rate from 150 to 250 rpm, k<sub>L</sub>a improved 40%. This was due to the fact that H<sub>2</sub>S bubbles were dispersed in small sizes at high mixing rate and therefore, surface area was enhanced [19-21]. However, increasing agitation rate to 450 rpm, k<sub>L</sub>a was slightly decreased. These data well agreed with the illustrated data in Figure 3a. In fact, the illustrated transfer rate is proportional to cell growth. At constant C<sub>H<sub>2</sub>S,L</sub><sup>\*</sup>, k<sub>L</sub>a was decreased when cell disruption occurred due to vigorous mixing [16, 22]. In fact physical absorption may assist the mass transfer of the process while the biological process is taking the advantage of physical absorption. This means limited solubility of H<sub>2</sub>S exists due to absorption process while presence of biocatalyst enhanced the hydrogen sulfide removal. In order to enhance mass transfer process for the justification and adjustment of operation process; it was decided to install absorption tank (see Figure 1a).

**Table 1.** The calculated value of k<sub>L</sub>a, C<sub>H<sub>2</sub>S,L</sub><sup>\*</sup> and the maximum transfer rate of H<sub>2</sub>S (N<sub>H<sub>2</sub>S</sub>)

Agitation rate (rpm)	k <sub>L</sub> a (h <sup>-1</sup> )	N <sub>H<sub>2</sub>S</sub> (mol.m <sup>-3</sup> .h <sup>-1</sup> )	C <sub>H<sub>2</sub>S,L</sub> <sup>*</sup> (mol.m <sup>-3</sup> )
150	0.0143	0.0354	2.878
250	0.0202	0.0463	3.092
350	0.0200	0.0410	3.047
450	0.0183	0.0390	3.098

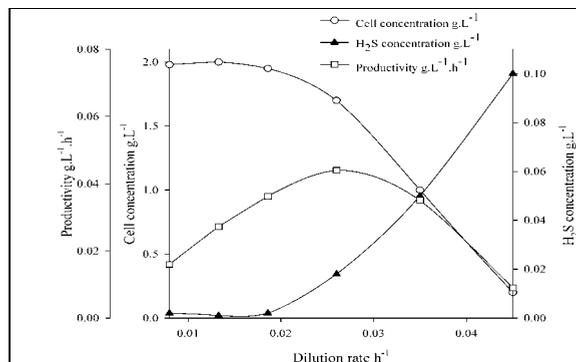
$$C_{H_2S,L}^* (\text{average}) = 3.028 \text{ mol.m}^{-3}$$



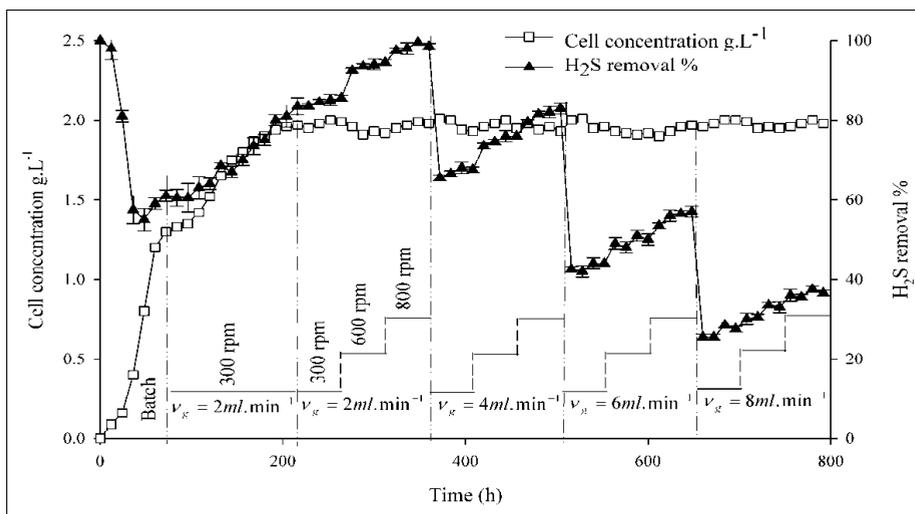
**Figure 3.** (a) H<sub>2</sub>S removal efficiency and cell concentration, in continuous operation with various bioreactor agitation rate; (b) Effect of bioreactor agitation rates on mass transfer coefficient ( $k_L$ ).

### 3. 3. BDS Efficiency

H<sub>2</sub>S removal efficiency and cell concentration were investigated in bioreactor with optimum cell recycle ratio and primary gas absorption pretreatment. Absorption tank was operated with agitation rate of 800 rpm; while, agitation rate in bioreactor was kept constant at 250 rpm. To find out the critical dilution rate, absorption tank was fed at the top with a fresh media with various dilution rates (0.008, 0.0133, 0.0186, 0.0266, 0.035 and 0.045 h<sup>-1</sup>) and at the bottom with gas flow rate of 2 mL.min<sup>-1</sup>. Use of absorption tank assisted to achieve H<sub>2</sub>S concentration in liquid phase near to saturation point which is 0.11 g.L<sup>-1</sup>. Saturated fresh media along with the excess amount of gas were fed into the bottom and top of the bioreactor, respectively (see stream 6 and 7 in Figure 1a). Figure 4. shows variations of cell productivity, cell and H<sub>2</sub>S concentration respect to dilution rate. Variations of Hydrogen sulfide concentration were monitored in liquid phase owing to evaluate the effectiveness of biological process without effect of physical absorption of H<sub>2</sub>S on removal efficiency. The maximum cell productivity in continuous dilution rate of 0.026h<sup>-1</sup> was 0.044 g.L<sup>-1</sup>.h<sup>-1</sup>. The main purpose of this work was to gain maximum H<sub>2</sub>S removal from the gas stream. Thus, the optimum dilution rate was chosen at 0.0186 h<sup>-1</sup> as a consequence of maximum H<sub>2</sub>S removal. By increasing dilution rate above the 0.0186 h<sup>-1</sup>, cell concentration was declined due to decreasing cell retention time. Reduction of cell concentration was continued till dilution rate at 0.035 h<sup>-1</sup> as after this dilution rate; the wash out was inevitable.



**Figure 4.** Effect of various dilution rates on cell productivity, cell and H<sub>2</sub>S concentration; at constant value of bioreactor agitation speed: 250 rpm, gas flow rate: 2 mL.min<sup>-1</sup>; absorption tank agitation speed: 800 rpm; recycle ratio: 0.6



**Figure 5.** H<sub>2</sub>S removal efficiency and cell concentration, in continuous operation with various gas flow rate (2, 4, 6 and 8 mL.min<sup>-1</sup>) and absorption tank agitation speed (300, 600 and 800 rpm). At constant value of dilution rate: 0.0186 h<sup>-1</sup>; bioreactor agitation speed: 250 rpm; recycle ratio: 0.6

A prolonged run of the BDS using the four gas flow rate (2, 4, 6, 8 mL.min<sup>-1</sup>) was conducted to ensure that it could be operated continuously at steady state. The bioreactor was successfully operated in continuous mode of operation without any serious problem for the duration of 27 days. Figure 5. shows the continuous operation by employing absorption tank at the following conditions; three agitation rates 300, 600 and 800 rpm, dilution rate of 0.0186 h<sup>-1</sup>. The absorption tank led to achieve high H<sub>2</sub>S transfer rate while high agitation rate was implemented in absence of cell. Cell concentration reached to constant amount of 1.96 g.L<sup>-1</sup> after period of 8 days and did not exert significant alteration respect to gas flow rate variations. High H<sub>2</sub>S conversion was achieved in low gas flow rate. However, H<sub>2</sub>S removal efficiency was declined to 36 % for gas flow rate of 8 mL.min<sup>-1</sup>; that was due to decrease in gas retention time. The obtained data showed that by increase in agitation in absorption tank from 300 to 800 rpm, the H<sub>2</sub>S removal efficiency was enhanced from 82 to 98%; while cell concentration and productivity was consistently kept at highest values. Results indicated that 98% of H<sub>2</sub>S of inlet sour gas with flow rate of 2 mL.min<sup>-1</sup> was removed while the cell concentration in the bioreactor was 1.96 g.L<sup>-1</sup>. Oxidations of sulfide under anaerobic condition lead to formation of sulfur or sulfate. Sulfur and sulfate are produced by partial and complete oxidation of H<sub>2</sub>S respectively. For gas flow rate of 2mL.min<sup>-1</sup> and dilution rate 0.0186 h<sup>-1</sup>, the defined measurement of sulfur showed concentration of 7.8 mg.L<sup>-1</sup>. The N:S ratio is an important and influential parameter in anaerobic/anoxic biological treatment; while for the biodesulfurization the removal of H<sub>2</sub>S and utilization of sulfur compounds were targeted by the active biocatalyst. Present work focused on biodesulfurizations which lead to elimination of hydrogen sulfide.

#### 4. Conclusion

The BDS process of sour gas by native isolated bacteria was successfully carried out in a CSTBR. Cell concentration was increased 55% by cell recycling with recycle ratio of 0.6. Maximum mass transfer rate and  $k_L a$  were calculated 0.0202 h<sup>-1</sup> and 0.0463 mol.m<sup>-3</sup>.h<sup>-1</sup> with 250 rpm agitation speed in bioreactor. The effects of various medium and gas flow rates

were monitored during the course of continuous operation. In order to gain high removal efficiency, the absorption tank with agitation rate of 300, 600 and 800 rpm was employed. Refining efficiency of sour gas was gained 98% with dilution and gas flow rate of 0.0186 h<sup>-1</sup> and 2 mL.min<sup>-1</sup>, respectively.

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## References

1. J. AMIRFAKHRI, M. VOSSOUGH, M. SOLTANIEH, Assessment of desulfurization of natural gas by chemoautotrophic bacteria in an anaerobic baffled reactor (abr). *Chem. Eng. Proc.*, 45(3), 232, 237. (2006).
2. A. BALL, B. RASEKH, A. KAYTASH, G. MOHEBALI, Biodesulfurization potential of a newly isolated bacterium, *gordonia alkanivorans* RIPI90A. *Enzyme Microb. Technol.*, 40(578), 58, 66 (2007).
3. M. RAMIREZ, M. FERNANDEZ, C. GRANADA, S. LE BORGNE, J.M. GOMEZ, D. CANTERO, Biofiltration of reduced sulphur compounds and community analysis of sulphur-oxidizing bacteria. *Bioresour. Technol.*, 102(5), 4047, 4053. (2011).
4. Z.A. IRANI, M.R. MEHRNIA, F. YAZDIAN, M. SOHEILY, G. MOHEBALI, B. RASEKH, Analysis of petroleum biodesulfurization in an airlift bioreactor using response surface methodology. *Bioresour. Technol.*, 102(22), 10585, 10591. (2011).
5. M. RASHTCHI, G. MOHEBALI, M. AKBARNEJAD, J. TOWFIGHI, B. RASEKH, A. KEYTASH, Analysis of biodesulfurization of model oil system by the bacterium, strain RIPI-22. *Biochem. Eng. J.*, 29(3), 169, 173. (2006).
6. M. FERNANDEZ, M. RAMIREZ, J.M. GOMEZ, and D. CANTERO, Biogas biodesulfurization in an anoxic biotrickling filter packed with open-pore polyurethane foam. *J. Hazard. Mater.*, 264, 529, 535, (2014).
7. K. TANG, V. BASKARAN, and M. NEMAT, Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries. *Biochem. Eng. J.*, 44(1), 73, 94, (2009).
8. A. DEHKORDI, M. SOBATI, M. NAZEM, An experimental investigation on the oxidative desulfurization of kerosene feedstock. *Energy Sources Part A*, 35(3), 226, 234, (2013).
9. J. M. MARTINKO, M. MADIGAN, Brock biology of microorganisms, Englewood Cliffs, NJ: Prentice Hall, 2005.
10. M. KHAVARPOUR, G. NAJAFPOUR, A. GHOREYSHI, M. JAHANSHAHI, B. BAMBAL, Biodesulfurization of natural gas: Growth kinetic evaluation. *Middle East J Sci Res*, 7, 22, 29, (2011).
11. A. RABBANI, H<sub>2</sub>S and sour oil and gas fields, AmirKabir University of Technology, 2011.
12. M.P. CHENAR, H. SAVOJI, M. SOLTANIEH, T. MATSUURA, S. TABE, Removal of hydrogen sulfide from methane using commercial polyphenylene oxide and cardo-type polyimide hollow fiber membranes. *Korean J. Chem. Eng.*, 28(3), 902, 913, (2011).
13. M.I. AL-EID, S.L. KOKAL, W.J. CARRIGAN, J.M. AL-DUBAISI, H.I. HALPERN, J.I. AL-JURAIID, Investigation of H<sub>2</sub>S migration in the marjan complex. *SPE Reservoir Eval. Eng.*, 4(06), 509, 515, (2001).
14. K. VAN'T RIET, Review of measuring methods and results in nonviscous gas-liquid mass transfer in stirred vessels. *Ind. Eng. Chem. Process Des. Dev.*, 18(3), 357, 364, (1979).
15. J.E. BAILEY, D.F. OLLIS, *Biochemical Engineering Fundamentals*. McGraw Hill International Editions, Singapore, 1986, pp. 393-480.
16. P.M. DORAN, *Bioprocess engineering principles*, Academic Press, 1995.
17. R. DUTTA, *Fundamentals of Biochemical Engineering*, Springer-Verlag Berlin Heidelberg, 2008, pp. 153-248.
18. R. CORD-RUWISCH, A quick method for the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J. Microbiol. Methods*, 4(1), 33, 36, (1985).
19. K.S.K. ISMAIL, G. NAJAFPOUR, H. YOUNESI, A.R. MOHAMED, A.H. KAMARUDDIN, Biological hydrogen production from co: Bioreactor performance. *Biochem. Eng. J.*, 39(3), 468, 477, (2008).
20. G. NAJAFPOUR, *Biochemical engineering and biotechnology*, Elsevier, 2006.
21. G. NAJAFPOUR, H. YOUNESI, A. MOHAMED, Continuous hydrogen production via fermentation of synthesis gas. *Pet. Coal*, 45(3/4), 154, 158, (2003).
22. H. HEYDARZADEH, G. NAJAFPOUR, A. GHOREISHI, H. YOUNESI. Elimination of hydrogen sulfide from sour gas in cstr bioreactor using native isolated strain of sulfur oxidizing bacteria. *Pak. J. Biotechnol.* 11(2) 67-78 (2014)