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ELIMINATION OF HYDROGEN SULFIDE FROM SOUR GAS IN CSTR BIOREACTOR USING NATIVE ISOLATED STRAIN OF SULFUR OXIDIZING BACTERIA

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ABSTRACT

Biodesulfurization of sour gas has been carried out in a continuous stirred tank bioreactor with culture volume of 3.5 L. Pure culture of sulfur oxidized bacteria was isolated from Ramsar (Iran) hot spring. Bioreactor performance was evaluated in terms of elimination capacity (EC, mg/L.h) and removal efficiency (RE, %). The highest RE% of H_2S at high concentration of sour gas was determined to be 99%. The effect of agitation rate on the removal efficiency was evaluated at four different gas flow rates ranged in 0.5 to 2mL/min. The optimum gas flow rate was achieved 0.5mL/min. In addition, the achieved optimal dilution rate and cell productivity were 0.01 1/h and 0.018 g/L/h, respectively. Furthermore, the limitation of dilution rates in the bioreactor and wash-out phenomena were considered. The biodegradation kinetic values obtained from Michaelis Menten model. The proposed model was satisfactorily fitted with the experimental data with three agitation rates in the bioreactor operation for the purpose of biodesulfurization of natural gas.

Keywords: Biodesulfurization; Elimination Capacity (EC); Hydrogen sulfide; Michaelis –Menten Model; Sulfur Oxidizing Bacteria.

INTRODUCTION

Odor pollution is a major environmental problem affecting human health due to utilization of natural resources. Odor effluents are mainly caused by sulfur compounds, such as hydrogen sulfide (H_2S), dimethyl sulfide, dimethyl disulfide and methyl mercaptan [1-4]. Malodorous hydrogen sulfide emissions are considered a public health concern due to their potential hazardous effects on human health. In addition to its unpleasant odor, H_2S is widely known for its extremely high toxicity, being common pollutant emitted from natural and synthetic hydrocarbon gas streams. H_2S removal, often called gas sweetening, is necessary to avoid corrosion of combustion engines and SO_x generation in the flue gases. Physicochemical processes typically perform to gas sweetening at high temperature and pressure. Whereas, microbiological sulfide oxidation processes are easily operated at ambient temperature and atmospheric pressure [1-7]. In case of

high volume metric gas flow rate, gas sweetening may be more efficient via physiochemical methods. On the other hand, biological methods should be considered as the most economical process operates at ambient conditions; that is more convenient and environmental friendly process. The biological process does not require chemical solvents or catalysts. Biological desulfurization can be carried out by various groups of microorganisms in aerobic and anaerobic conditions [8-12]. Different types of bioprocesses have been reviewed as methods to purify sour gas, including biofiltration, biotrickling and membrane bioreactors while using various advanced techniques [2, 13-18] .

Ramirez *et al.,* [19] have used two stage biotrickling filters for simultaneous treatment of hydrogen sulphide, methyl mercaptan, dimethyl sulphide and dimethyl disulphide. The first biofilter was inoculated with *Acidithiobacillus thiooxidans* and the second one with *Thiobacillus thioparus*. They have found that in the two-stage biofiltration, the removal efficiency did not appreciably decrease in the H_2S concentration [19]. Generally, aerobic processes biogas is mixed with the excess air and that leads to dilution of the biogas concentration. In addition, disposal of the excess air containing H_2S is the main drawbacks of the aerobic bioprocess. These difficulties may be resolved by the use of anaerobic treatment instead of an aerobic process. The advantage of anaerobic processes is sulfur oxidizing bacteria are able to reduce H_2S via cell metabolic pathway [20]. Amirfakhri *et al.,* [2] have evaluated an anaerobic baffled reactor (ABR) which was inoculated by activated sludge from municipal sewage treatment for the biodesulfurization of natural gas. They have nearly converted 61% of sulfide to elemental sulfur and sulfate [2].

The aim of present work was to investigate the act of native isolated bacteria in anaerobic continuous biodesulfurization. It is required to achieve optimal medium composition and gas flow rate for improving the biodesulfurization efficiency. For this purpose, the effect of agitation rate on removal efficiency and elimination capacity was investigated and the mass of cell population and productivity were monitored. For identification of the bioreactor limitation on operating condition, wash out phenomena was defined as dilution rates varied. In order to consider biodegradation kinetics, Michaelis–Menten model was investigated and the values of related kinetic parameters were obtained based on experimental data.

MATERIALS AND METHODS

Bacteria and Medium Preparation: ***B***acteria residing in sulfur spring have great potential to degrade and reduce sulfur compounds [21]. In this research, the mixed culture was sampled from the hot spring located in Ramsar (north of Iran). For the growth of mixed culture, the chemical compositions were listed in Table-1. The compositions of stock solutions of vitamins

and trace metals are summarized in Table-1. In addition, from the stock solutions of vitamins and trace metals (2 and 1 mL, respectively) were added to the prepared synthetic medium. All the chemicals used were analytical graded and supplied by Merck (Darmstadt, Germany).

Table -1: Chemical composition of culture

Nutrient composition	Concentration (g/L)	Composition of vitamins stock solution	Concentrat ion (mg/L)	Composition of trace metals	Concentrat ion (g/L)
NH_4Cl	0.6	ThiamineHCl.$2H_2O$	10	Na_2-EDTA	50
$MgCl_2.6H_2O$	0.2	Nicotinic acid	20	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.5
KH_2PO_4	1.2	Pyridoxine-HCl	20	$FeSO_4$. 7 H_2O	5
K_2HPO_4	1.2	p-Aminobenzoic acid	10	$MnSO_4$. 5 H_2O	2.5
$NaNO_3$	0.3	Riboflavin	20	$ZnSO_4$. 7 H_2O	11
$Na_2S_2O_3.5H_2O$	7	Ca-pantothenate	20	$CoCl_2.6H_2O$	0.5
Yeast extract	1	Biotin	1	$CaCl_2.2H_2O$	7.34
Vitamins stock solution	2 mL	Vitamin B12	1	$CuSO_4$	0.2
Trace metals	1 mL				

The isolated organism was stained and recognized as gram negative rod shape known as bacillus sp.; it was grown in a 125 mL degassed serum bottle under anaerobic condition at atmospheric pressure. The serum bottle was contained 50mL of liquid media and the remaining volume was allocated for the removal of hydrogen sulfide considered in sour gas [22]. The prepared mixed gas of the components of H_2S, CO_2, Ar and CH_4 comprise of 5, 5, 10 and 80 v/v %, respectively. The high concentration of sour gas composition was prepared based on sourest case of natural gas reservoirs in Iranian gas field. The sterilized media in serum bottles were inoculated with fresh isolated culture. To provide an anaerobic condition and acclimatize the isolated bacteria to H_2S, the mixed gas was purged into an inoculated culture. The incubation was performed in an orbital shaker (Stuart, S1500 and UK) with agitation rate of 180 rpm at 36 °C for 72 h.

Continuous Culture Operation: Initially, the bioreactor (Infors, Switzer land) with working volume of 3.5 L and all the reagent bottles were autoclaved in 121˚C and 20 minute. The acclimated seed culture was prepared and harvested at a mid exponential phase. The inoculum of 250mL seed culture was anaerobically transferred to the bioreactor containing 3250 mL medium. The sour gas was continuously fed into the bioreactor. Gas flow rate was controlled using a gas flow controller (dwyer, USA). The agitation rate was adjusted to 150 rpm after initial inoculation. Bioreactor was operated under batch condition with defined medium compo-

sition for duration of 72 h at a fixed temperature of 36˚C and controlled pH at 6.5. The pH was adjusted using 0.1 molar of NaOH and HCl (Merck) solutions. A continuous mode of operation was started on third day with liquid flow rate of 0.3mL/min (dilution rate of 0.0051/h). The effect of various gas flow rates in the range of 0.5 to 2 mL/min were monitored during the course of continuous operation. For the safe operation at the end of gas line, the unutilized excess sour gas and vent gases were absorbed by NaOH solution gas trap. Also, gas analysis was carried out on the exhausted gas from the bioreactor which is shown in gas sampling section (item 6 in Figure 1). Based on the aim of this work, analyses were focused on biodesulfurization process which leads us to discuss only on elimination of hydrogen sulfide concen-tration. The schematic representation of the experimental set up is shown in Figure 1.

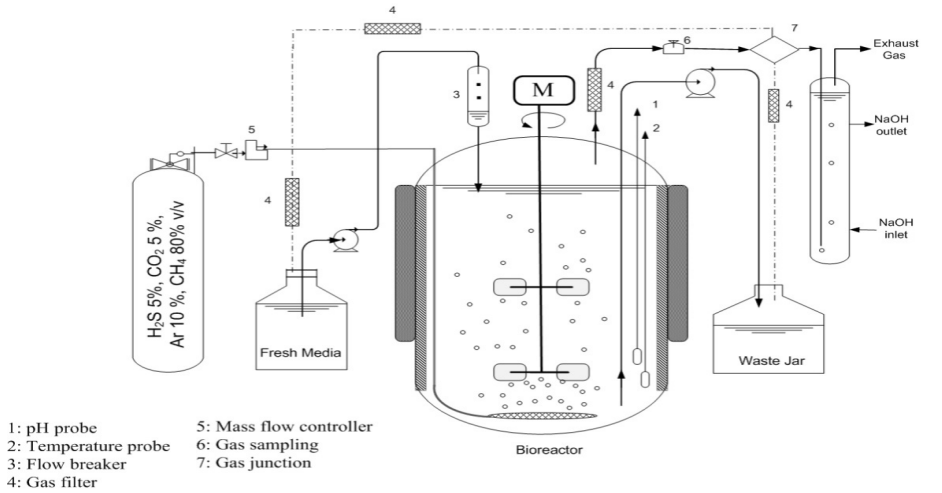


Figure 1: Schematic of the experimental set up of continuous operation

Analytical Method: The cell population was determined by optical density of the media using spectrophotometer (Unico, 2100, USA) at wavelength of 600nm (OD 600nm). 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 analyze hydrogen sulfide, argon, methane and carbon dioxide. The initial oven temperature was 80°C. The temperature was programmed with a step rate of 10°C/min until reached to 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 30 mL/min [22]. 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 mmol/L) and $CuSO_4$ (5 mmol/L). Aliquots of cultures added (1:40, v/v) to the acidic $CuSO_4$ solution which remained stable for 20-40 seconds. During this time, the absorbance of the end product was measured at 480nm wavelength. HCl (50 mmol/L) added by the culture aliquot served as blank [23]. All gas and liquid samples were taken in every 12 h. The gas analyses of samples were repeated twice and the calculated mean values were recorded. All collected data were replicated and average values were reported with standard deviation of in the neighborhood of less than 5%.

RESULT AND DISCUSSION

Continuous bioreactor operation

The bioreactor was initially operated under batch conditions at 36˚C. After achieving an appropriate cell population, bioreactor was fed from the top with a fresh media with desired dilution rates; while sour gas was sparged from the bottom of the bioreactor vessel with a fixed gas flow rate. The bioreactor was successfully operated in continuous mode of operation without any serious problem for the duration of 27 days.

Optimum Dilution Rate: The optimum dilution rate (the ratio of the liquid flow rate to the bioreactor volume) was obtained with respect to RE %, cell density and productivity. During experimental runs for optimization of the liquid flow rate, the agitation and gas flow rates were kept constant at 250 rpm and 0.5 mL/min. The pH of the bioreactor was controlled during the entire operation otherwise noted. Figure 2 shows variations of cell productivity, cell and H_2S concentrations in the continuous bioreactor operation at various fresh medium flow rates for defined optimum condition. Continuous operation was started at the liquid flow rate of 0.3 mL/min (0.0051/h) and then increased to 1.4mL/min (0.0241/h). Cell concentration slightly decreased to 1.22g/L while increasing in dilution rate up to 0.0151/h. The maximum cell productivity in continuous dilution rate of 0.015 1/h was 0.018 g/L/h, which is consistent with cell concentration variation. Hydrogen sulfide concentrations were monitored in liquid phase owing to evaluate the effectiveness of biological process without the effect of physical absorption of H_2S on removal efficiency.

However, the solubility of hydrogen sulfide in medium was measured to be nearly 100 mg/L; as shown in Figure 2. H_2S concentration in liquid phase, similar to cell concentration, was slightly declined with the rise of dilution rate up to 0.015 1/h. In view of the fact that H_2S is toxic and severely corrosive compound, its low concentration could make serious problems. In addition, the main purpose of this work was to gain maximum H_2S removal from the gas stream. Thus, the optimum dilution rate was chosen at 0.011/h as a conse-

quence of maximum H_2S removal. At the highest dilution rate of 0.024 1/h (1.4 mL/min) the cell population and productivity decreased, which was an indication of undesired wash-out phenomenon at this dilution rate. Thus, the limitation was found that bioreactor was unable to operate at any dilution rates beyond the value of 0.02 1/h. That was due to wash-out phenomena as the cell propagation and productivities were rated low in the culture broth.

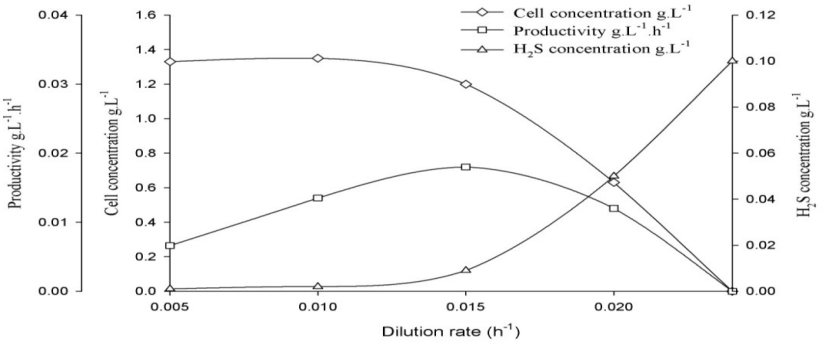


Figure -2: Effect of various dilution rates on cell productivity, cell and H_2S concentration

Biodesulfurization Efficiencies: Bioreactor performance was evaluated in terms of the elimination capacity (EC, mg/L.h) and the removal efficiency (RE, %); the related terms are defined in Table 2.

Table- 2: Definition of equations used in this paper

Parameter	Definition	Nomenclature
Elimination capacity (EC; mg/L.h) Removal efficiency (RE; %)	$EC = \frac{Q}{V}(C_{in} - C_{out})$ $\mathrm{RE} = \frac{C_{in} - C_{out}}{C_{in}} \times 100$	Q: gas flow rate (L/h) V: working volume (L) C_{in}: inlet concentration (mg/L) C_{out}: outlet concentration (mg/L)
Michaelis–Menten model	$EC = \frac{EC_{\max} \times C_{out}}{K_s + C_{out}}$	EC_{max}: maximal elimination capacity (mg/L.h) K_s: saturation constant (mg/L)

Figure 3 demonstrates 27 days of successful operation of the continuous bioreactor at operating conditions at various gas flow rates (0.5 to 2 mL/ min) and three agitation rates (150, 250 and 350 rpm), for defining an optimum condition for the biodesulfurization. The stepwise increase in gas flow rates are shown with dashed line. Continuous biodesulfurizartion was operated at the described conditions; data are illustrated in Figure 3 at dilution rate 0.01 1/h. Cell concentration has reached to constant

value of 1.2 g/L after 3 days of initial batch operation. Based on this figure, agitation rate was increased from 150 to 350 rpm in each gas flow rate step.

Figure 4 demonstrate the performance of continuous operation for duration of 648 hours, while in the course of H_2S removal experimental studies were conducted with known and exact variables (operating conditions: various gas flow rates (0.5 to 2 mL/min) and three agitation rates (150, 250 and 350 rpm) in each gas flow rate)). Changes of experimental condition caused fluctuation of actual obtained data; therefore, the real data are illustrated. Figure 4, shows cell concentration was highly dependent on agitation rates and did not exert significant alteration with respect to gas flow rate variations. Rise of agitation rates from 150 to 250 rpm make well suspension which improved mass transfer and cell propagation. However, it was found out that excessive increase in agitation rate from 250 to 350 caused to decrease cell concentration. That was due to excessive shear forces cause by vigorous mixing which make cell disruption. In addition, Figure 4 depicts that high H_2S conversion was achieved in low gas flow rate. However, H_2S removal efficiency was declined to 51 % for gas flow rate of 2 mL/min; that was due to decrease in gas retention time. Results indicate that a 99% of H_2S of inlet sour gas with flow rate of 0.5 mL/min was removed while the cell concentration in the bioreactor was maintained at 1.35 g/L.

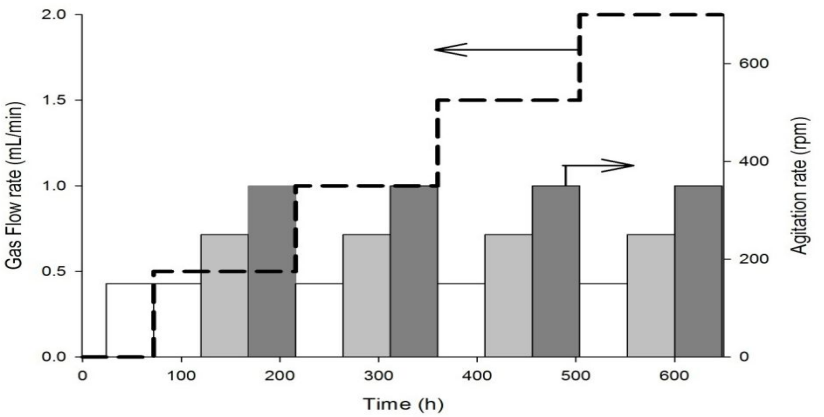


Figure 3: The continuous bioreactor operation protocols at various gas flow rates (0.5 to 2 mL/min) and three agitation rates (150, 250 and 350 rpm)

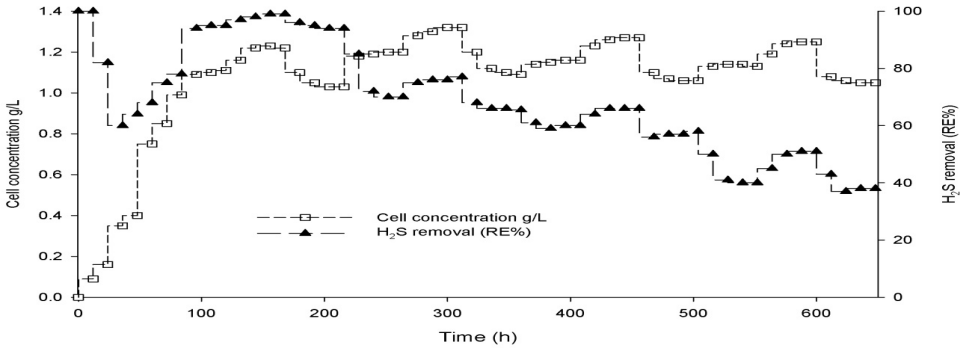


Figure- 4: H_2S removal efficiency and cell concentration, in continuous operation based on operation condition. dilution rate was kept constant at 0.01 h^{-1}

Biodegradation Kinetics: Elimination capacity is an important parameter when it is required to design long period operation based on results of the short period of experiments. In a continuous biodesulfurization, the elimination capacity follows a behavior that can be adequately described by a Michaelis–Menten model [24, 25]. This model makes correlation between removal efficiency and average concentration of component in the bioreactor using two constants EC_{max} and K_s. The EC_{max} is maximum elimination capacity and K_s is defined as saturation constant. In mixed bioreactor, there are no concentration variations within the reactor. This means that the concentration in the reactor is the same as that in the outlet stream. Therefore, in Michaelis-Menten model the average concentration of H_2S in bioreactor was replaced by its concentration in outlet liquid stream. The values for EC_{max} and K_s can be calculated by means of regressions from experimental measurements according to the following linearized equation derived from Michaelis-Menten model [18]:

$$\frac{1}{EC} = \frac{K_s}{EC_{\max}} \times \frac{1}{C_{H_2S\,out}} + \frac{1}{EC_{\max}} \qquad (1)$$

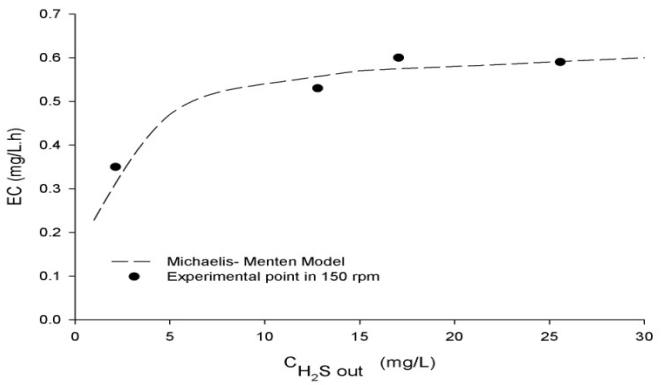
EC_{max} and K_s were obtained from intercept and slope of linearized equation. The biodegradation kinetics values obtained from the model and experimental data were summarized in Table 3. The constants were calculated at agitation rate of 150 to 350 rpm. Based on literature [18], the value of Michaelis–Menten constant at fixed condition is unchanged; while changing the substrate concentration and nutriaents compositions, the EC_{max} and K_s may vary. Therefore, the obtained values of K_s were consistently altered by increasing agitation rates from 150 to 350 rpm due to mass transfer operation. The EC_{max} increases with increasing in agitation rate and then

decreased. It can be attributed to occurrence of cell disruption at 350 rpm.

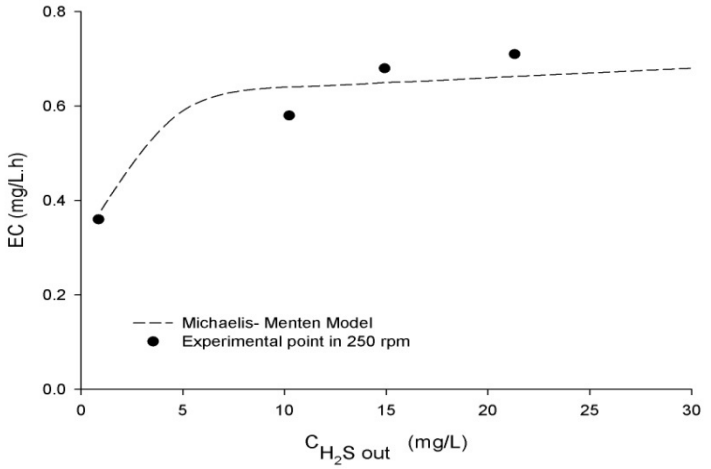
Figure 5 presents EC value versus C_{out} based on the projected model and experimental data for different agitation rates of 150, 250 and 350 rpm. As can be observed from this figure, the elimination capacity estimated based on the Michaelis–Menten model was fairly fitted with the experimental data.

Table -3: Biodegradation kinetics values determined from the Michaelis––Menten model

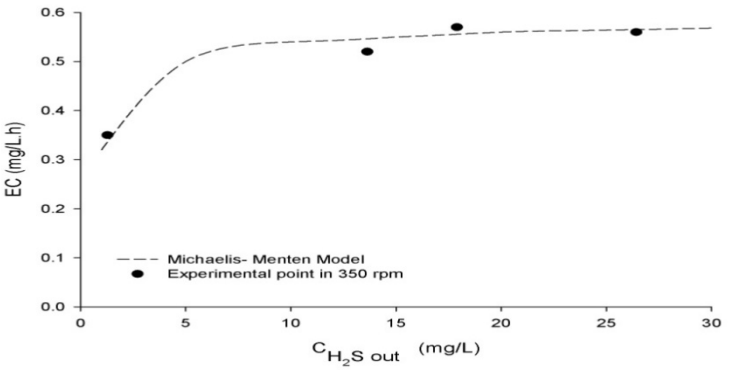
Agitation rate	EC_{max} (mg/L.h)	K_s (mg/L)
150 rpm	0.645	1.12
250 rpm	0.694	0.81
350 rpm	0.612	0.80



(a)



(b)



(c)

Figure 5: Elimination capacity as a function of H_2S concentration at different agitation rates: (a) 150 rpm, (b) 250 rpm, (c) 350 rpm. Experimental: points and models (Michaelis–Menten): dash line

Figure 6 presents Linewearver-Burk plot of the identified model explained by equation (1). Among these data, at agitation rate of 150 rpm resulted prefect fitting with R^2 of 0.98. From the slope and intercepts of lines, one can define these values for EC_{max} and K_S; the high value of EC_{max} may enhance the removal efficiency and also K_S as the ability of biological operation for elimination of the component. It dependents on inlet concentration, type of bioreactor and its geometrical dimension; while low values of K_S means high elimination capacity of the biofiltration.

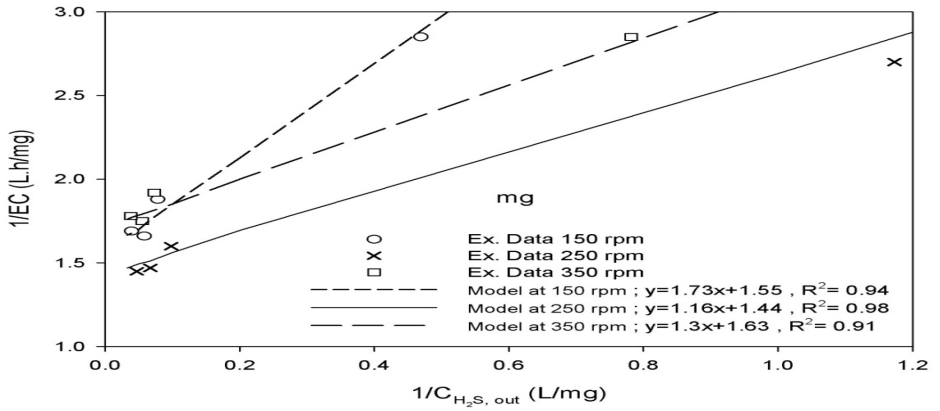


Figure -6: Linweaver-Burk plot of equation (1) and experimental data with Michaelis-Menten at agitation rates of 150, 250 and 350 rpm.

CONCLUSION

The Biodesulfurization process of sour gas by native isolated sulfur oxidizing bacteria was successfully carried out in a continuous stirred tank bioreactor. The effects of various medium (0.3 to 1.4 mL/min) and gas flow rates (0.5 to 2mL/min) were monitored during the course of continuous operation. The elimination capacity (EC, mg/L/h) and the removal efficiency (RE, %) were considered at agitation rate of 150, 250 and 350 rpm. Removal efficiency of sour gas was gained 99% with dilution and gas flow rate of 0.01 1/h and 0.5mL/min, respectively. Wash out phenomena was considered for the bioreactor. The Michaelis–Menten model, biodegradetion kinetics, was satisfactorily fitted with the experimental data.

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