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Anaerobic Oxidation of Hydrogen Sulfide in Batch Bioreactor Using Micro Organism Isolated from Ramsar Hot Spring

[1]*Ali Alemian*, [1]*Ghasem D. Najafpour*, [2]*Maryam* K*havarpour*,
[1]*Ali-Asghar Ghoreyshi and* [1]*Hamid Heydarzadeh* Darzi

[1]Biotechnology Lab. Faculty of Chemical Engineering,
Noushirvani University of Technology, Babol, Iran
[2]Department of Chemical Engineering, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

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Abstract: Removal of hydrogen sulfide from mixed gas was successfully carried out in a batch reactor. The isolated microorganism from Ramsar hot spring was cultured and adapted in an environment with hydrogen sulfide. At 35°C, maximum cell dry weight was 0.43 g/l. For the hydrogen sulfide removal, effect of temperature in the range of 25 to 45°C was investigated. The experimental results showed that the removal efficiency significantly increased with an increase in temperature from 25 to 35°C, then; a slightly decrease in media temperature at 45°C appeared. Maximum and minimum removal efficiencies of hydrogen sulfide were 72 and 25%, respectively. At 35°C based on first order biochemical kinetic; the rate of substrate utilization was 0.06 h^{-1}.

Key words: Hydrogen sulfide; Removal efficiency; Substrate utilization; Ramsar hot spring; Sulfur oxidizing bacteria

INTRODUCTION

Presence of hydrogen sulfide in natural gas is considered as one of the most noxious gases. Removal of the hydrogen sulfide from sour natural gas is required for gas sweetening, odors removal, corrosion during transmission, distribution and prevent environmental pollution with sulfur dioxide and hydrogen sulfur gases [1, 2]. The emission of odor gas especially hydrogen sulfide is quite essential for many industrial activities such as petro-chemical refineries, food processing, dye production, fuel treatment, wastewater treatment, natural gas processing and tanneries manufactures [3, 4]. Conventional technologies such as physical and chemical processes, including chemical oxidation, activated carbon adsorption, ozone oxidation and incineration have been used to remove hydrogen sulfide from gaseous stream [5, 6]. These traditional methods are energy intensive and required chemical and high capital costs [7]. Thus, extensive research led to biological alternatives for a variety of biological desulphurization processes for natural gas sweetening [8, 9]. Naturally, biological process operates at ambient temperature and atmospheric pressure may eliminate high costs for heat and pressure generation in a variety of chemical processes [10-14]. Hydrogen sulfide is converted into elemental sulfur via biological pathway [15]. The reduced sulfur compound such as hydrogen sulfide serve as electron donors for anaerobic phototrophic bacteria or provide growth energy for the colorless sulfur bacteria [16]. Various microorganism are capable of H_2S oxidation, including *Thiobacillus*, *Xanthomonas*, *Pseudomonas* and etc. [17, 18].

The purpose of present investigation was to evaluate hydrogen sulfide removal efficiency via oxidation of hydrogen sulfide by the isolated microorganism from Ramsar hot spring in a batch bioreactor under anaerobic condition. The effect of media temperature and concentration of hydrogen sulfide on growth of the isolated microorganism for the removal of hydrogen sulfide were investigated.

Corresponding Author: Dr. Ghasem D. Najafpour, Faculty of Chemical Engineering,
Noushirvani University of Technology, Babol, Iran. E-mail: najafpour8@yahoo.com.

MATERIALS AND METHODS

Microorganism and Growth Media: The mixed culture used for the removal of hydrogen sulfide was isolated from Ramsar hot spring (Ramsar, Iran). For the growth of mixed culture, a synthetic medium of 0.4 g/l NH_4Cl, 0.2 g/l $MgCl_2.6H_2O$, 0.2 g/l KH_2PO_4, 0.2 g/l K_2HPO_4, 0.2 g/l yeast extract and 8 g/l $Na_2S_2O_3.5H_2O$ was used. Also, 2 ml vitamin solution and 1ml trace element solution were added to the growth medium. The vitamin solution contained (mg/l): Thiamine-HCl.$2H_2O$, 10; Nicotinic acid, 20; Pyridoxine-HCl, 20; p-Aminobenzoic acid, 10; Riboflavin, 20; Ca-pantothenate, 20; Biotin, 1.0; Vitamin B12, 1.0. The pH of Vitamin solution was adjusted to 7.0. The trace element solution consisted of (g/l) Na_2-EDTA, 50; $ZnSO_4.7H_2O$, 11; $CaCl_2.2H_2O$, 7.34; $MnCl_2.4H_2O$, 2.5; $COCl_2.6H_2O$, 0.5; $(NH_4)6MO_7O_{24}.4H_2O$, 0.5; $FeSO_4.7H_2O$, 5.0; $CuSO_4.5H_2O$, 0.2. The pH of trace element solution was adjusted to 6.0 using 1 M NaOH solution. Media and equipment were autoclaved and sterilized at 121°C at 1.2 atm for 15 min. But, the vitamin and trace metal solutions filtered using a 0.45 µm filter. All the chemicals used for the experiments were analytical graded and supplied by Merck (Darmstadt, Germany).

Batch Experiments: Batch culture of the isolated organism was grown in a 125 ml de-gassed serum bottle under anaerobic condition at atmospheric pressure. To ensure anaerobic condition, the media in the serum bottle was prepared under nitrogen gas. The serum bottle was contained 50 ml of liquid media, the remaining volume was considered for the removal of hydrogen sulfide from the mixed gas. The serum bottle was sealed with rubber septum and aluminum crimp.

The sterilized serum bottles were inoculated with 3ml of seed culture. The inoculated culture was purged with a mixed gas from the gas tank 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 with the volume percentages of 5, 5, 10 and 80%, respectively.

Analytical Method: The serum bottles were placed horizontally on an orbital shaker (Stuart, S1500 and UK) with agitation rate of 180 rpm. The removal efficiency of hydrogen sulfide was investigated in a temperature range of 25 to 45°C with an increment of 5°C. The gas and liquid samples were taken in every 3 hours. The liquid samples were analyzed for cell optical density at a wavelength of 600 nm using a spectrophotometer (Unico, 2100, USA). According to the standard calibration curve, the cell dry weight concentration was also determined based on optical density of the media by light absorbance as a function of cell dry weight. 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 rate of 10°C. min^{-1} 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 45 $ml.min^{-1}$.

RESULTS AND DISCUSSION

Cell Growth Measurement: Initially, batch experiments were carried out using hydrogen sulfide as an inorganic sulfur compound for cultivation of the isolated bacteria. Fig. 1 shows the cell dry weight of bacteria at various temperatures. According to the obtained data, the maximum cell dry weight of 0.43 $g.l^{-1}$ was achieved at 35°C.

Degradation of Hydrogen Sulfide: Utilization of hydrogen sulfide was determined at five different temperatures for incubation period of 21 hours. According to Fig. 2a, maximum reduction of hydrogen sulfide in the gas phase

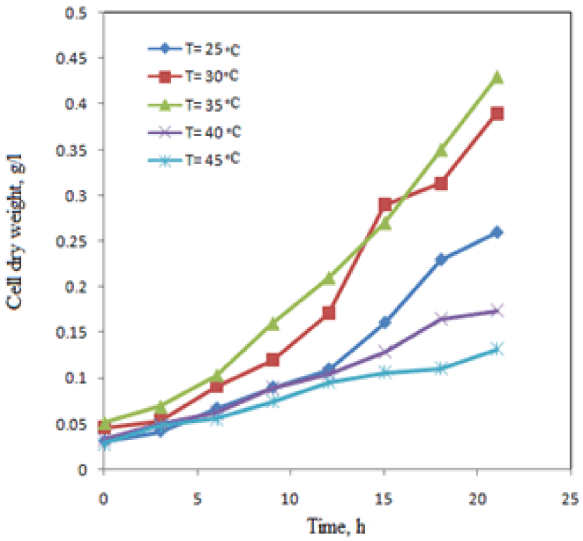

Fig. 1: Cell dry weight in an anaerobic condition with respect to incubation time

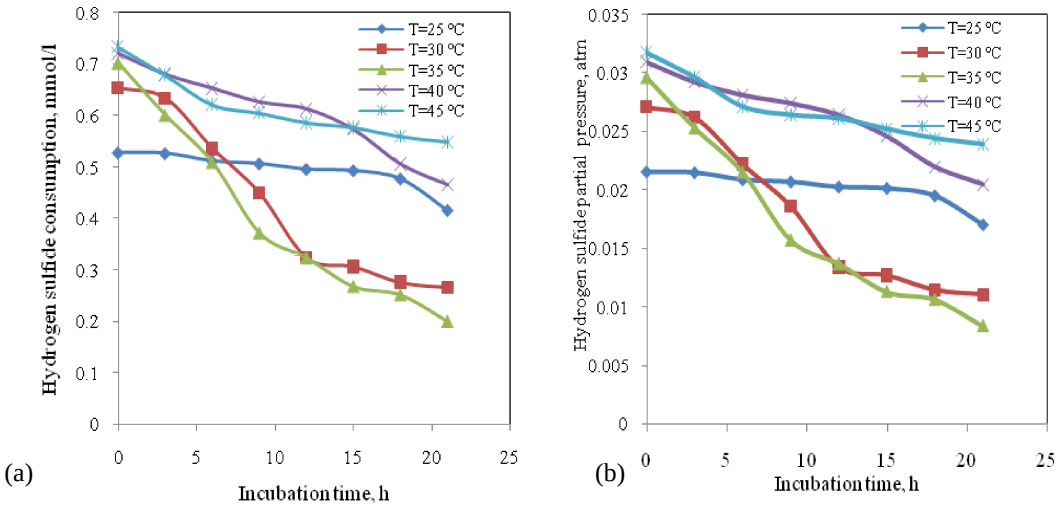

Fig. 2: a) Hydrogen sulfide concentration and b) Partial pressure of hydrogen sulfide, in an anaerobic condition with respect to time

Table 1: Removal of hydrogen sulfide and yield of biomass production at various temperatures

Temperature (°C)	Removal efficiency (%)	$Y_{x/s}$ (g cell/g substrate)
25	21.2	0.0079
30	59	0.0156
35	72	0.0132
40	35.18	0.0102
45	25.09	0.0101

was occurred at 35°C. The hydrogen sulfide concentration was reduced from 0.71 to 0.2 mmol. l^{-1}. Fig. 2b also shows reduction of hydrogen sulfide partial pressure with respect to incubation time at various temperatures. Maximum cell concentration and partial pressure reduction were obtained at the same temperature (35 °C). This behavior might be due to high ability of bacteria to uptake hydrogen sulfide.

However, the calculation for the removal of hydrogen sulfide from gas phase was based on ideal gas law. On the other hand, the yield of biomass production was calculated by the following relation. The obtained results are summarized in Table 1.

$$Y_{x/s} = -\frac{\Delta x}{\Delta s} \quad (1)$$

Kinetic of Substrate Consumption: Elimination of hydrogen sulfide via biological route is defined by Michaelis-Menten rate equation; stated as follows [19]:

Table 2: First order rate constant obtained at various temperatures

	T (°C)				
Kinetic parameters	25	30	35	40	45
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

$$-\frac{ds}{dt} = \frac{J_m S}{K_m + S} \quad (2)$$

Where, S is substrate concentration (g/l), J_m is the maximum specific growth rate ($g.h^{-1}.l^{-1}$), t is the reaction time (h) and K_m is the saturation constant (g/l). If the substrate utilization by microorganism follow the first-order reaction kinetic, then the kinetic parameter for the above reaction showed that K_m is much greater than the substrate concentration. Equation (2) simplified to first order chemical reaction rate. The expression for substrate consumption with respect to time is written as first-order rate equation stated as follows:

$$-\frac{ds}{dt} = k_s.S \quad (3)$$

Where, k_s is the first order rate constant (h^{-1}) which is defined as $k_s = \frac{J_m}{K_m}$. As shown in Fig. 3, by plotting the linear form of Equation (3) with respect to time, the first order rate constant was obtained in a temperature range of 25-45°C.

Table 2 summarized the values for first order kinetic constants at 5 different temperatures. The maximum rate constant at 35°C was 0.06 h^{-1}.

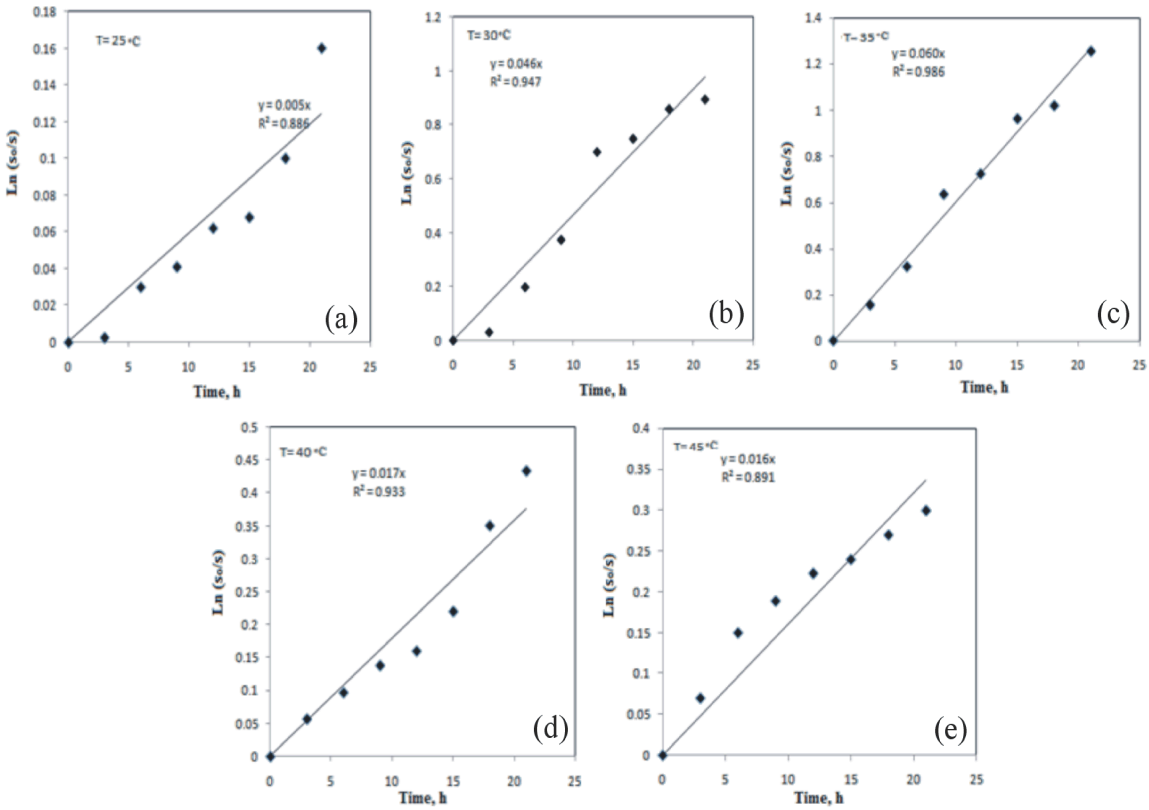

Fig. 3: Substrate consumption rate with respect to various temperatures; a) 25, b) 30, c) 35, d) 40, e) 45°C

At this temperature maximum consumption of hydrogen sulfide was observed. The values obtained for linear regressions based on the first order biochemical kinetic were in the acceptable range.

CONCLUSION

The removal of hydrogen sulfide from mixed gases was carried out in a batch bioreactor using the isolated microorganism from Ramsar hot spring. Experiments were conducted at 5 different temperatures. Maximum removal of hydrogen sulfide and cell growth occurred at 35°C. In batch culture, maximum hydrogen removal was 72%. In addition, at 35°C maximum rate constant was 0.06 h^{-1}. Also, it concluded that the isolated microorganism from Ramsar hot spring was able to reduce hydrogen sulfide from the mixed gases.

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