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Study on H_2S Removal in the Bio-Trickling Filter *via* *Denitrification thiobacillus* Under Anaerobic Conditions

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Denitrification thiobacillus has been used to inoculate a bio-trickling filter packed with volcanic rocks to remove H_2S from mixed gas under anaerobic conditions. A batch experiment was carried out to check the transformed form of element S under *Denitrification thiobacillus* under anaerobic conditions. The degradation mechanism of H_2S was predicted. Results showed that the removal efficiency of 93 % was obtained at pH of 7, the inlet gas empty bed residence time (EBRT) of 30 s, the inlet H_2S concentration of 500 ppm and the flow rate of the nutrient solution circulating counter-currently of 25 L h^{-1}. It was inferred that almost all the H_2S was transferred by biodegradation but not chemical absorption. It was found that H_2S was transferred into element S^0, SO_4^{2-}, $S_2O_3^{2-}$ and SO_3^{2-} *etc*. Calculated results showed that the total amount of element S after H_2S was degraded was equal to the amount before H_2S was degraded. When H_2S concentration was far less than NO_3^- concentration, H_2S was almost completely converted into SO_4^{2-}. On the contrary, when H_2S concentration was far more than NO_3^- concentration, the excess H_2S was transferred into S^0 by the reaction with SO_4^{2-} under *Denitrification thiobacillus*. When S^{2-}/NO_3^- was 1.25/2, nearly half of the H_2S was transferred into $S_2O_3^{2-}$ and SO_3^{2-}, 1/4 of the H_2S was transferred into SO_4^{2-} and element S^0 production was very little.

Keywords: Biodegradation, Anaerobic, Balance, Sulfur, H_2S, *Denitrification thiobacillus*.

INTRODUCTION

Biogas is a typical energy-rich gas containing various low concentration contaminants, such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), *etc.*[1], which is bad for human health and environmental protection because of the toxic and odorous character. H_2S and CH_3SH are the most common reduced sulfur compounds in biogas (the content of which is up to 95 % by mass). H_2S content in biogas ranges from 0.1 to 2 % (1000-20000 ppm)[2-4]. Siloxane can form silicon dioxide deposits on the surface of equipment up to several millimeters thick, which could lead to a decrease in the efficiency of the equipment and an increase in maintenance costs when biogas combusted[2]. The factors mentioned above restrict the usage of energy gas, so these contaminants must be filtrated before usage and reduced to acceptable levels prior to release. The removal of the contaminants from biogas effectively has been investigated by researchers all over the world[3,4].

Due to high chemical requirements, energy and disposal costs, the chemical contaminants removal processes are expensive. As a result, biological treatment methods for removal of pollutants desirable as an alternative to chemical treatment. A biological impurities removal process has the potential to overcome the disadvantages of chemical processes. In the case of H_2S and CH_3SH in biogas, anaerobic methods provide the inherent advantage of maintaining the anaerobic nature of the gas and avoid any potential safety problems.

Under anaerobic condition, both phototrophic and chemotrophic bacteria are suitable candidate microorganisms for H_2S bioxidation.

Studies on microbial ecology associated with phototrophic bacteria have shown that a species of green sulfur bacteria (GSB) *Cholorobiumlimicola* are the most suitable for sulfide removal[5]. Green sulfur bacteria are capable of oxidizing sulfide to elemental sulfur, requires only light, CO_2 and inorganic nutrients for growth and are strictly anaerobic. Green sulfur bacteria are non-motile and product elemental sulfur extracellularly. Phototrophic bio-trickling filter is an interesting concept for cost-effective H_2S removal from biogas due to their ability to operate for long periods of time without requiring a biomass separation and operate under variable loadings. This feature makes green sulfur bacteria suitable for elemental sulfur recovery from sulfide-containing gas. The overall photochemical reaction

by which green sulfur bacteria oxidizes S^{2-} to S^0 while reducing CO_2 to carbohydrates is[6]:

$$2nH_2S + nCO_2 \rightarrow 2nS^0 + n(CH_2O) + nH_2O$$

When light intensity and sulfide flow rate were adjusted to a point, all of the sulfide introduced to the bio-trickling filter was oxidized to elemental sulfur without the formation of sulfate. Under sulfide overloading conditions, light energy was not sufficient and sulfide accumulated in the reactor. When the reactor was in a sulfide under loading condition, the surplus light caused the formation of sulfate as shown[7]:

$$nH_2S + 2nCO_2 + 2nH_2O \rightarrow nSO_4^{2-} + 2nH^+ + 2n(CH_2O)$$

A number of chemotrophs are suitable for the biodegradation of H_2S. These bacteria grow and produce new cell material by using inorganic carbon (CO_2) as a carbon source and chemical energy from the oxidation of reduced inorganic compounds such as H_2S[1]. Chemotrophic bacteria can also be used in bio-trickling filter to produce elemental sulfur instead of sulfate under anaerobic conditions. They can convert H_2S to elemental sulfur and sulfate, according to reaction scheme below. In this process, nitrate (NO_3^-) acts as an electron acceptor.

Anoxic biological reaction[8]: $H_2S + CO_2$ + Nutrients + Nitrate → Cells + Sulfur and/or Sulfate + Water + Nitrite and/or Nitrogen

In aerobic conditions biodegradation of H_2S by chemotrophs occurs with O_2 as an electron acceptor and in anaerobic conditions with alternative electron acceptors (*e.g.* nitrate), depending on the type of bacteria[9,10].

In this paper Chemotrophic bacteria *Denitrification thiobacillus* were chose to remove H_2S due to not needing light resource. Activated sludge from WWTP of Tianjin University dealing with domestic waste has been used to inoculate a bio-trickling filter. Removal efficiency was investigated under different conditions, the transformed form of element S under *Denitrification thiobacillus* under anaerobic conditions was checked and the degradation mechanism of H_2S was predicted.

EXPERIMENTAL

Culture media: The culture media for the microorganisms consisted of $MnSO_4$ (0.02 g L^{-1}), Na_2HPO_4 (1.2 g L^{-1}), KH_2PO_4 (1.8 g L^{-1}), NH_4Cl (0.5 g L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.4 g L^{-1}), $CaCl_2 \cdot 2H_2O$ (0.05 g L^{-1}), $FeCl_3$(0.05 g L^{-1}), KNO_3(5 g L^{-1}), $Na_2S_2O_3$ (10 g L^{-1}) was also supplied as the electron donor.

Experimental setup: The experimental schematic diagram for continuously removing H_2S is presented in Fig. 1. The reactor is a bio-trickling filter made of a glass column with a diameter of 50 mm and a height of 0.5 m and equipped with 1 L of plastic cascade ring which was chose as the packing material due to its high specific surface area and pore volume, which allows it to retain more biomass and water. Prior to starting the experiment, the packing material in the bio-trickling filter was soaked with the activated sludge taken from the sewage treatment plant of Tianjin University. The bio-trickling filter was inoculated initially with 200 mL of the activated sludge and 800 mL fresh nutrient medium. The initial pH of the recirculation liquid in the bio-trickling filter was 7. The culture liquid was recirculated from the bottom to the top of the column using a booster pump (flow rate 10 L h^{-1}) to provide the bacteria with moisture and minerals necessary for growth. Circulation liquid of 300 mL was replaced by fresh nutrient medium every week. After microorganisms on the packing materials grew to balance, NaS_2O_3 was canceled and replaced by H_2S. Hydrogen sulfide was continuously produced by the 0.1 M sodium sulfide solution dropping into the 0.5 M sulfate solution and was carried by N_2 from gas cylinder into the bio-trickling filter in counter-current with the recirculation medium.

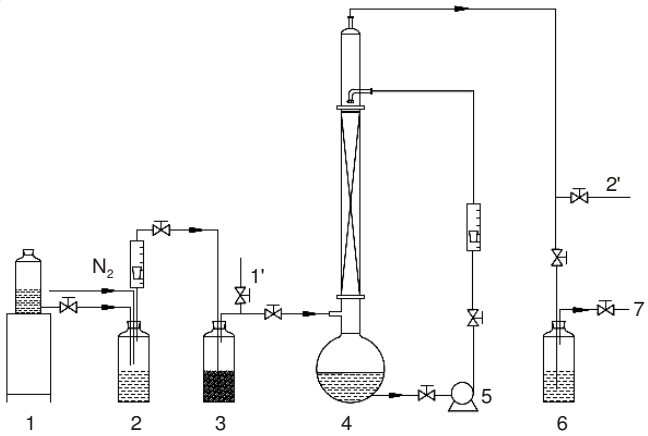

Fig. 1. Schematic diagram of the H_2S removal bio-trickling filter system: 1. storage tank; 2. generator of H_2S; 3. mixer; 4. bio-trickling filter; 5. pump; 6. odor absorption fluid; 7. off-gas; 1' H_2S sampling; 2' H_2S sampling.

In order to calculate the total amount of element S after H_2S was degraded, some of the culture was taken from the bio-trickling filter and an experiment of H_2S degrading was carried out in flasks. The schematic diagram of the H_2S removal for calculating sulfur balance is shown in Fig. 2. As a result, all the forms of element S could go into the solution and the gas, which avoided the troubles of measuring caused by S^0 and other relevant ions adhering to the biofilm.

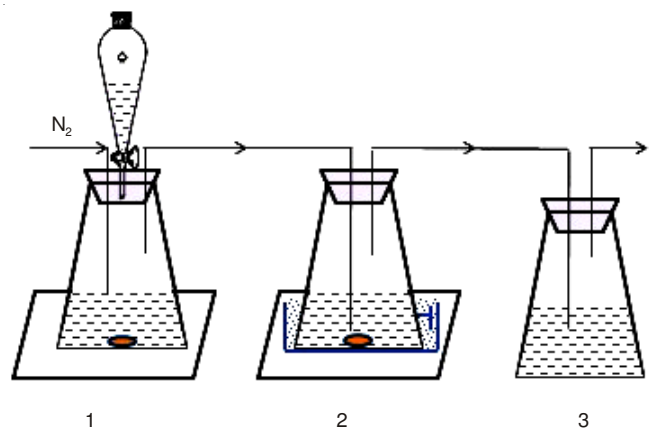

Fig. 2. Schematic diagram of the H_2S removal for calculating sulfur balance. 1. generator of H_2S; 2. H_2S and NO_3^- removal in the culture; 3. odor absorbent

Nutrient solution must be supplied for the microorganisms to maintain their metabolism. The pH of the fresh nutrient solution was adjusted to approximately 7 with a phosphoric acid solution immediately when starting the experiment because the optimum pH for growth of *Denitrification thiobacillus* is 7. Potassium nitrate was added to act as the

electron acceptor source. At pH of 7, it can be concluded that the removal of H_2S from the mixed gas was mainly the result of biological process but not the physical or chemical process.

The gas samples were taken from the inlet and the outlet of the bio-trickling filter. Concentrations of H_2S in N_2 in gas phase were measured using a gas detector. The pH of the nutrient solution was determined using a permanently immersed pH electrode. The growth rhythm of microorganisms on the packing materials during the experiment could be obtained by detecting the total quantity of sulfur in the nutrient solution. Therefore, sulfur particles in the nutrient solution produced during degrading H_2S was excreted directly from sulfur particles suspend. Simultaneously, in order to measure the element sulfur in cells of microorganisms, cells needed to be separated firstly. After the nutrient solution was centrifuged, the sulfur precipitate was washed with distilled water and then mixed with acetone, so the residual water was evaporated by boiling acetone. Then dried sulfur was dissolved in a certain amount of *n*-hexane to measure the concentration with a spectrophotometer (UV-2100 Spectrophotometer, UNICO, China) at 260 nm.

Microscopy pictures of the microorganisms were obtained using a microscope Olympus (Model BX61TRF) coupled to a photo camera. Plate experiments were performed on 50 mm × 15 mm Petri dishes.

Experimental methodology: Continuous monitoring the concentration of H_2S inlet and the outlet of the bio-trickling filter during anaerobic degradation was carried out for approximately a few months. In order to determine the performance of the bio-trickling filter, after the biomass on the packing materials grew to achieve balance, the H_2S concentration and the mixture gas flowrates were increased by adjusting the rate of Na_2S solution dropping into H_2SO_4 solution and the flowrate of N_2. The empty bed residence time of mixed gas were tested: 5, 10, 20, 30 and 40 s and the nutrient solution was circulated counter-currently at a flow rate of 10, 15, 20, 25 and 30 L h^{-1}. The pH of the culture nutrient was detected every day. The nutrient was renewed by replacing half of it using the fresh one if the pH no longer changed.

RESULTS AND DISCUSSION

Effect of the inlet concentration of H_2S, empty bed residence time (EBRT) and liquid flow rate on the removal efficiency of H_2S

As can be seen in Fig. 3, the initial H_2S concentration was 100 ppm with the empty bed residence time of 30 s, pH was 7, the inlet mixed gas flow rates was 40 L h^{-1} and the flow rate of the nutrient solution was circulated counter-currently at 20 L h^{-1}, the H_2S concentrations in the outlet were approximately 2 ppm which indicated that the removal efficiencies of H_2S were close to 100 %. After 5 days, the H_2S concentration in the inlet increased to 200 ppm and the H_2S concentration in the outlet were about 4 ppm. At day 26, when the inlet H_2S concentration was increased to 500 ppm, the removal efficiency decreased to 93 %. The removal efficiency decreased with the inlet H_2S concentration increasing. Moreover, it is shown that pH fell firstly and then increase with H_2S continuously entered into the system with final value of above 7. The inflection points on the curve in Fig. 3 were the time when fresh nutrient solution was added and pH was adjusted to 7. The reason for the change of pH is that, at the beginning, the reactor was in a H_2S under loading condition and the formation of H_2SO_4 was caused, so pH decreased; with H_2S continuously entered into the system and the reactor was in a H_2S overloading conditions gradually, H_2S was oxidized to element S^0 in the reactor which was a process of producing alkali, so pH increased.

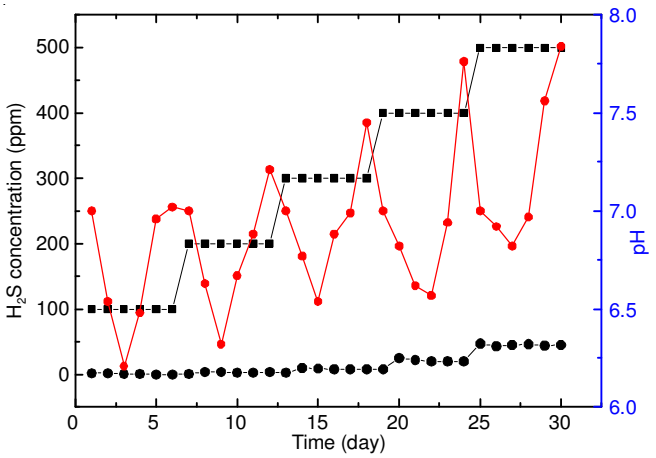

Fig. 3. Influence of the inlet concentration of H_2S on removal of H_2S and pH of the nutrient solution in the bio-trickling filter, H_2S inlet concentration (■), H_2S outlet concentration (●), pH (●)

As shown in Fig. 4, when the inlet H_2S concentration was 100 ppm with empty bed residence time of 40 s, H_2S removal efficiency was 100 %. When empty bed residence time was decreased to 30 s, H_2S removal efficiency can be up to 99 %. With empty bed residence time was shortened to 5 s, H_2S removal efficiency was decreased to 62.4 %. When the inlet H_2S concentration was increased to 500 ppm and empty bed residence time was set of 40 s, H_2S removal efficiency was 95.7 %. With empty bed residence time was shortened to 5 s, H_2S removal efficiency was decreased to 39.1 %. It was suggested that the removal efficiency decreased with the inlet concentration increased and the removal efficiency decreased with the empty bed residence time was shorten.

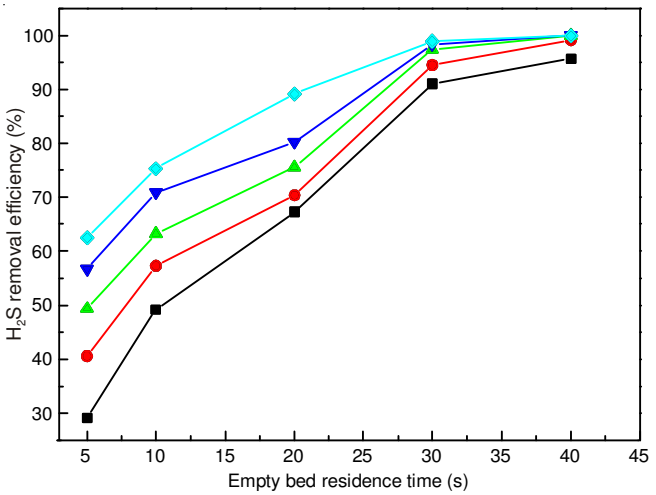

Fig. 4. Influence of the empty bed residence time (EBRT) on removal of H_2S, the H_2S inlet concentration of 500 ppm (■), 400 ppm (●), 300 ppm (▲), 200 ppm (▼), 200 ppm (●)

As can be seen in Fig. 5, the H_2S removal efficiency increased firstly and then decreased with the liquid flow increased and decreased with the inlet concentration increased. So there was a best liquid flow for the mixed gas of any H_2S inlet concentration. The reason for that the best liquid flow increased with the H_2S inlet concentration increased may be associated with mass transfer.

H_2S removal was caused by biological degradation or chemical reaction ?

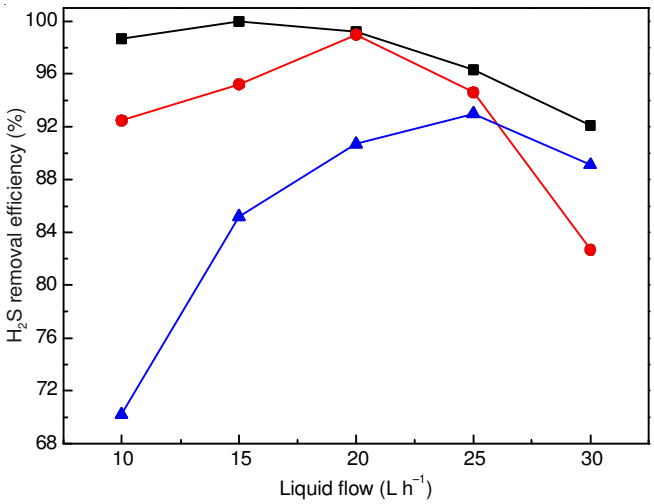

Fig. 5. Influence of the liquid flow on removal of H_2S, the H_2S inlet concentration of 100 ppm (■), 300 ppm (●), 500 ppm (▲)

Based on the results above, in order to verify the H_2S removal was caused by biological degradation or physical absorption or chemical reaction, an experiment was carried out by detecting the amount of the product S^0 and SO_4^{2-} when there were cultures in the nutrient solution or no in a set of device as shown in Fig. 2. Results are shown in Table-1.

Table-1 shows the amount of the product S^0 and SO_4^{2-} before and after reaction when there were cultures in the nutrient solution or no. As can be seen, under anaerobic conditions, when there was no culture in the nutrient solution, trace amounts of elementary substance S^0 and sulfate were produced. It was suggested that during the process of removing H_2S, biological degradation was indeed accompanied by a slight chemical reaction, but chemical reaction was negligible relative to the biological function.

What is the influence of the initial H_2S/NO_3^- (mol/mol) on final SO_4^{2-}/S^0 (mol/mol) and what is the reaction mechanism?

Fig. 6 presents the variation of SO_4^{2-} concentration, S^0 concentration and SO_4^{2-}/S^0 (mol/mol) as a function of H_2S/NO_3^- (mol/mol). As can be observed, SO_4^{2-} concentration increased firstly and then decreased with H_2S/NO_3^- (mol/mol) increasing, maximized at the initial H_2S/NO_3^- (mol/mol) approximately 3.5. S^0 concentration increased all the time with the initial H_2S/NO_3^- (mol/mol) increasing. As a result, SO_4^{2-}/S_0 firstly increased and then decreased with the initial H_2S/NO_3^- (mol/mol) increasing, also maximized at H_2S/NO_3^- (mol/mol) approximately[3,5].

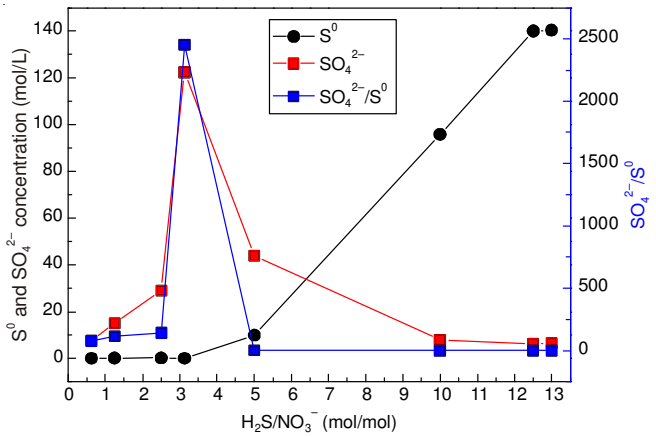

Fig. 6. Relationship between H_2S/NO_3^- (mol/mol) on SO_4^{2-}/S^0 (mol/mol)

The reaction mechanism for H_2S degradation and the formation of S^0 and SO_4^{2-} under anaerobic conditions was supposed. Due to *Denitrification thiobacillus*, this process is H_2S dependent. When the initial H_2S/NO_3^- (mol/mol) was very small, in other words, H_2S concentration was far less than NO_3^- concentration, H_2S was almost completely converted into SO_4^{2-}; the excess H_2S was transferred into S^0 with H_2S/NO_3^- (mol/mol) increasing by the reaction with SO_4^{2-} under *Denitrification thiobacillus*. The experimental results were in accordance with the reports in literatures, the biological response equations between H_2S and NO_3^- are as follows:

$$5H_2S + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{2-} + 4N_2 + 4H_2O$$

$$5H_2S + 2NO_3^- + 7H^+ \rightarrow 5S^0 + N_2 + 6H_2O$$

Under *Denitrification Thiobacillus,* where did the H_2S go?

In order to know if the total amount of element S before and after H_2S was degraded was equal, an experiment was designed in a set of device as Fig. 2. All the forms of element S were detected. When the initial H_2S/NO_3^- was 1.25/2, the result is shown in Table-2.

From Table-2, it was found that the total amount of element S before and after H_2S was degraded was equal. When S^{2-}/NO_3^- was 1.25/2, nearly half of the H_2S was transferred into $S_2O_3^{2-}$ and SO_3^{2-}, 1/5 of the H_2S was transferred into SO_4^{2-} and element S^0 production was very little.

Microbial growth curve: Some of the culture was taken from the bio-trickling filter and its growth curve was measured (Fig. 7).

TABLE-1
AMOUNT OF THE PRODUCT S^0 AND SO_4^{2-} BEFORE AND AFTER REACTION WITH OR WITHOUT CULTURE IN THE NUTRIENT SOLUTION OR NO

	Before reaction SO_4^{2-} concentration (g L^{-1})	After reaction SO_4^{2-} concentration (g L^{-1})	Difference of SO_4^{2-} between after and before reaction (g L^{-1})	Before reaction S concentration (g L^{-1})	After reaction SO_4^{2-} concentration (g L^{-1})	Difference of S between after and before reaction (g L^{-1})
Without culture	0.155	0.161	0.006	0	0.00062	0.00062
With culture	0.155	0.677	0.522	0	0.01400	0.01400

TABLE-2
ALL THE FORMS OF ELEMENT S AND THE TOTAL ELEMENT S AMOUNT BEFORE AND AFTER H_2S WAS DEGRADED

Amount of element S in the system (mg)	$Na_2S·9H_2O$			H_2SO_4			The total S	
	190.04			304.08			494.12	
Amount of sulfur after the reaction was finished (mg)	The amount of H_2S in the effluent	S^{2-} in the solution	SO_4^{2-} in the solution	S^0 in the solution	$S_2O_3^{2-}$ and SO_3^{2-} in the solution	S^0 produced in the H_2SO_4 solution	H_2S dissolved in the H_2SO_4 solution	Total S
	24.67	23.91	99.76	1.60	208.94	141.18	2.64	502.69

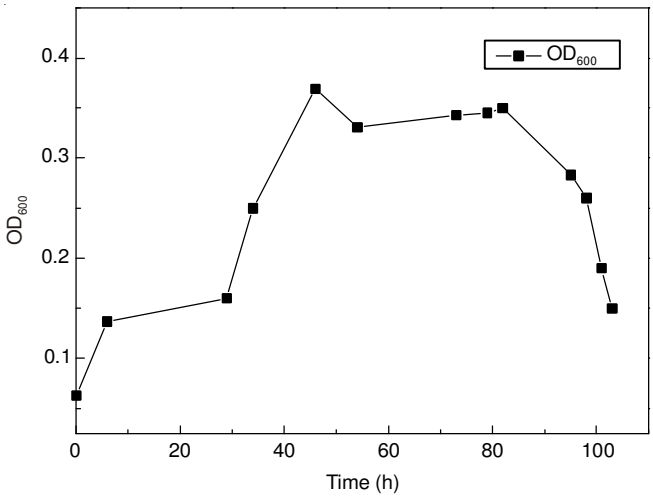

Fig. 7. Growth curve of microorganism degrading H_2S (pH of 7)

The morphology of microorganisms are shown in Fig. 8.

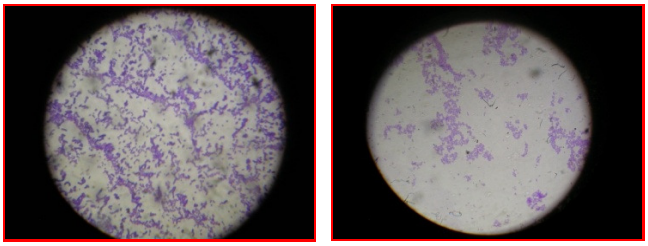

Fig. 8. Morphology of microorganisms from opticalmicroscope

As is shown in Fig. 8, after rinsed with alcohol, the bacteria still kept violet. So we inferred that the bacterial flora are Gram-positive bacterium.

Conclusion

H_2S was degraded by *Denitrification thiobacillus* under anaerobic conditions. Results showed that:

• When H_2S concentration was 500 ppm, the inlet mixed gas empty bed residence time was 30 s, pH was 7 and the flow rate of the nutrient solution was circulated counter-currently at 25 L h^{-1}, the H_2S the removal efficiency could achieved 93 %.

• Results showed that almost all the H_2S was transferred by biological degradation. Although H_2S was transferred to all kinds of forms, the total element S amount after H_2S was degraded conserved.

• With H_2S/NO_3^- (mol/mol) increasing, SO_4^{2-}/S^0 increased first and then decreased and remained constant finally. When S^{2-}/NO_3^- was 1.25/2, nearly half of the H_2S was transferred into $S_2O_3^{2-}$ and SO_3^{2-}, 1/4 of the H_2S was transferred into SO_4^{2-} and element S^0 production was very little.

• The bacteria used in the experiments were Gram-positive bacterium.

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