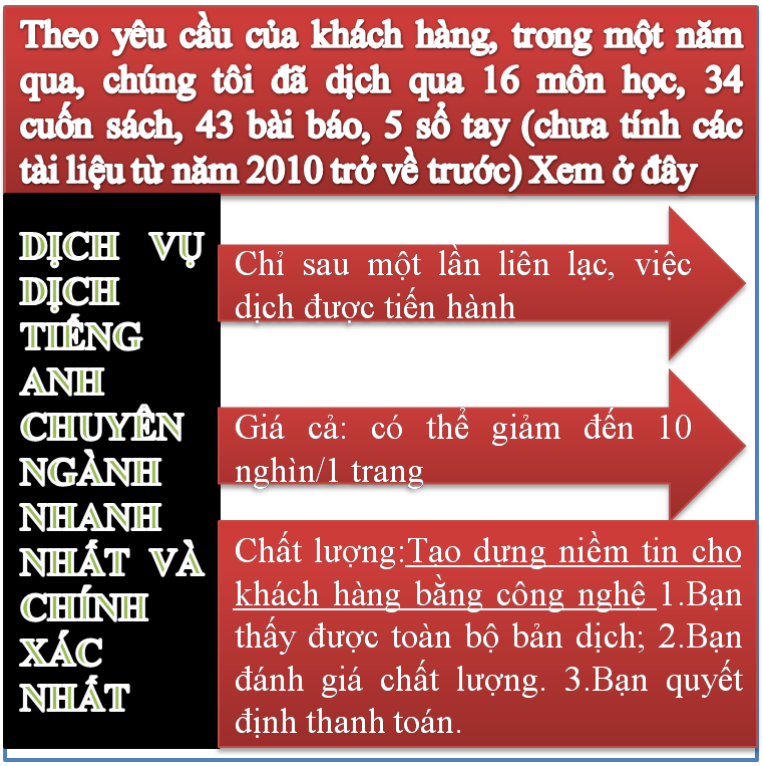

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Characterization of Sulfur Oxidation by an Autotrophic Sulfur Oxidizer, Thiobacillus sp. ASWW-2	Đặc tính của quá trình oxy hóa lưu huỳnh bằng tác nhân oxy hóa lưu huỳnh tự dưỡng, Thiobacillus sp. ASWW-2	
Abstract An autotrophic sulfur oxidizer, Thiobacillus sp. ASWW-2, was isolated from activated sludge, and its sulfur oxidation activity was characterized. Thiobacillus sp. ASWW-2 could oxidize elemental sulfur on the broad range from pH 2 to 8. When 5-50 g/L of elemental sulfur was supplemented as a substrate, the growth and sulfur oxidation activity of Thiobacillus sp. ASWW-2 was not inhibited. The specific sulfur oxidation rate of strain ASWW-2 decreased gradually until sulfate was accumulated in medium up to 10 g/L. In the range of sulfate concentration from 10 g/L to 50 g/L, the sulfur oxidation rate could keep over 2.0 g-S/g-DCW-d. It indicated that Thiobacillus sp. ASWW-2 has tolerance to high concentration of sulfate.	Tóm tắt Tác nhân oxy hóa hóa lưu huỳnh tự dưỡng, Thiobacillus sp. ASWW-2, được phân lập tự bùn hoạt tính, và hoạt tính oxy hóa của nó được nghiên cứu. Thiobacillus sp. ASWW-2 có thể oxy hóa lưu huỳnh nguyên tố trên khoảng PH rộng từ 2 đến 8. Khi 5-50 g/L lưu huỳnh nguyên tố được thêm vào dưới dạng chất nền, sự tăng trưởng và hoạt tính oxy hóa lưu huỳnh của Thiobacillus sp. ASWW-2 không bị ức chế. Tốc độ oxy hóa lưu huỳnh đặc trưng của dòng ASWW-2 giảm dần cho đến khi sunfat được tính lũy trong môi trường lên đến 10 g/L. Trong khoảng nồng độ sunfat từ 10 g/L đến 50 g/L, tốc độ oxy hóa lưu huỳnh có thể nằm ở giá trị 2.0 g-S/g-DCW-d. Người ta đã chứng tỏ rằng Thiobacillus sp. ASWW-2 có khả năng chịu nồng độ sunfat cao.	
INTRODUCTION Hydrogen sulfide (H2S) is one of the malodorous compounds most widely emitted from wastewater treatment, petrochemical	[redacted]	

refining, food preparation, paper and pulp manufacturing, and fuels treatment [1,2]. H2S not only aggravates the neighborhood but can also cause adverse effects to health [3]. Besides, H2S has a corrosive property to create a damage and danger of collapse because of the corrosion of pipe and concrete construction [4,5]. To remove this gas, biofiltration is attracting more attention because of low capital and operating costs, low energy requirements and an absence of residual products requiring further treatment or disposal. Biofiltration units are microbial systems incorporating microorganisms grown on a porous solid media like soil, peat, compost or porous ceramics, etc [6-8]. Therefore, it is very important to select effective microorganisms to remove hydrogen sulfide. It has become a common practice to inoculate the filter bed with pure cultures of microorganisms capable of biodegrading H2S to reduce the adaptation time of the biofilter. For instance, the inoculation of a biofilter with Thiobacillus species remarkably reduced the

acclimatization period for biodegradation of H2S [7,9,10].

Diverse autotrophic and heterotrophic microorganisms have been employed to remove H2S in biofilter systems. In microbial treatment of H2S, most of microorganisms belong to Thiobacillus spp. [7,9-13]. Some chemolitoheterotrophic bacteria such as Thiothrix, Beg- giatoa, and Hyphomicrobium can oxidize H2S to ele-mental sulfur that will be further oxidized to sulfate [14-16]. Photoautotrophic bacteria including Chloro- bium, Chromatium, Ectothiorhodospira, and Rhodobacter have been used to convert H2S to elemental sulfur under anaerobic conditions [17-20].
The major disadvantages of the practical use of photoautotrophic bacteria lie in their anaerobic nature and their need for radiant energy. As for the chemoorganoheterotrophic bacteria, Pseudomonas sp. and Xanthomonas sp. have also been reported to oxidize H2S [21,22].

Generally, autotrophic biofilter, employing the autotrophic and sulfur-oxidizing bacteria such as Thiobacillus spp., has shown high affinity for H2S. Besides, it is not necessary to supplement any carbon or/and energy sources into autotrophic biofilter because the autotro- phic and sulfur-oxidizing bacteria can utilize CO2 and H2S as a carbon and energy source, respectively. However, the final oxidation product of H2S by the autotro- phic sulfur oxidizer is sulfate, and the resulting acidity has adverse effects on microbial activity [7,9]. Therefore, it is important to screen an autotrophic sulfur oxidizer having tolerance to high concentration of sulfate for stable long-term continuous operation of biofilter system.	[redacted]	
In this study, an autotrophic sulfur oxidizer was isolated from activated sludge, and its sulfur oxidation activity was characterized. In addition, the effect of sulfate concentration on the sulfur oxidation	[redacted]	

activity of the isolate was studied.

[redacted]

MATERIALS AND METHODS

[redacted]

Isolation of a Sulfur-oxidizing Bacterium ASWW-2

[redacted]

Activated sludge, sampled at a wastewater treatment plant, was used as a source for the isolation of sulfur- oxidizing bacteria. Ten grams of wet activated sludge was inoculated in 100 mL of MW medium in a 500 mL flask, and incubated at 30°C in a shaking incubator with 180 rpm. MW medium is composed of 10 g of So, 3 g of KH_2PO_4, 0.1 g of NH_4Cl, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of $CaCl_2 \cdot 2H_2O$ in 1 L of distilled water. The pH of medium was adjusted to 4 by using 2N HCl. When the pH of culture broth declined below 1.5 due to the accumulation of sulfate that was the final product of sulfur oxidation, 10 mL of the culture broth was inoculated into 90 mL of fresh medium. After this process was repeated several times, the acclimated culture broth was

[redacted]

spread on the MW-agar plate. One dominant colony appearing on a MW-agar plate was isolated, and purified by repeated transfer of the cells to fresh MW medium. The purified bacterium was named as ASWW-2.	[redacted]	
Characterization of Sulfur Oxidation Several factors affected sulfur oxidation activities of the isolate, ASWW-2, were investigated: initial pH of medium, inoculum size, and concentration of substrates. Cells grown in 1 L of MW medium for 3 days at 30°C were harvested by centrifugation (7,500 x g, 20 min), washed and suspended in 100 mL of basal mineral medium containing 3 g of KH_2PO_4, 0.1 g of NH_4Cl, 0.01 g of $FeSO_4 \cdot 7H_2O$, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of $CaCl_2$	[redacted]	
• $2H_2O$ in 1 L of distilled water (pH 4). The cell suspen-sion was used as inoculum for each experiment, and the amount of initial inoculum was adjusted to 0.05-0.07 of	[redacted]	

optical density at 660 nm after the cell suspension added into the culture medium. Experiments were carried out in 500 mL shake flasks. The flasks were charged with 100 mL of the basal mineral medium. To investigate the effect of initial pH on sulfur oxidation rate, 10 g/L of So was supplemented in the medium as a substrate, and the initial pH of the medium was adjusted to 1, 2, 3, 4, 5, 6, 7, 8 with 2N NaOH or 2N HCl. Also, the effects of substrate concen-tration (5-50 g/L of So and 1.8-7.4 g/L of S2O32-) on sulfur oxidation rate were investigated. All experiments were carried out at 30°C and 180 ppm on a rotary shaker. Three mL of culture broth from each flask was sampled every 24 h, and the pH, cell density, and sulfate concentration were measured. Analysis To monitor the cell growth of strain ASWW-2, the optical density of culture broth was measured by spectrophotometer (Spectronic 20, Milton Roy Company, U.S.A.) at 660 nm.	[redacted]	

Before the protein assay, the cells were digested with 2N NaOH solution at 100°C for 60 min. The concentrations of thiosulfate and sulfate were analyzed by ion chromatography (Waters 510, U.S.A.). IC-PakTM anion column (4.6 mm ^ x 50 mm L, Waters, U.S.A.) and conductivity detector (Waters 432, U.S.A.) were used.

Table 1. Characteristics of strain ASWW-2

Items	Characteristics
Colony on MW-agar plate	Whitish-yellow with sulfur
	Deposited 1-1.5 mm in diameter
Morphology	Short rod, 0.5 x 1-1.5
Motility	Positive
Gram-staining	Negative
Intracellular sulfur	Negative
Autotrophic growth with	
Hydrogen sulfide	Positive
Elemental sulfur	Positive
Thiosulfate	Positive
Tetrathionate	Positive
Hetrotrophic growth	Negative
Ferrous iron oxidation	Negative
Nitrate respiration	Negative

RESULTS AND DISCUSSION

Morphological and Physiological Characteristics of Strain ASWW-2

The morphological and physiological properties of strain ASWW-2 were summarized in Table 1. The cell of strain ASWW-2 was short rod, gram negative, and motile. The colony on the thiosulfate-agar medium was small, with a diameter of 0.5-1 mm and whitish yellow by extracellular deposition of sulfur. Fig. 1(a) shows pH and sulfate concentration profiles with cultivation time in the MW medium (pH 4) supplemented with So as a sole energy source. During the cultivation, the increase of sulfate concentration was accompanied by an increase in the cell mass. The pH value of the culture gradually decreased according to the accumulation of sulfate. The

final sulfate concentration was approximately 30 g/L, indicated that elemental sulfur (initial concentration, 10 g/L) was stoichiometrically oxidized to sulfate by strain ASWW-2. In the experiment with MW medium, CO2 and elemental sulfur were the sole carbon and energy source for the growth of strain ASWW-2, respectively. Therefore, strain ASWW-2 has chemolithotrophically grown by utilizing energy from the oxidation of elemental sulfur. When 8 g/L of Na2S2O3 • 5H2O was supplemented into the MW medium as an energy source instead of elemental sulfur, strain ASWW-2 could chemolithotrophically grow (Fig. 1(b)). In this medium, visible accumulation of elemental sulfur was found. These results suggested that this strain oxidized thiosulfate to sulfate via the accumulation of elemental sulfur as an intermediate [23]. This bacterium could also utilize tetrathionate as an energy source, and oxidize it to sulfate. The strain ASWW-2 could not heterotrophically grow in organic medium (data not shown). Stain ASWW-2 was negative in the oxidation of ferrous and in	[redacted]	

nitrate respiration. Major cellular fatty acids for stain ASWW-2 were analyzed as uniqinone-8, non-hydroxy 16:0, hydroxy 3-OH 14:0 (data not shown). Based on the description in Bergey's manual [24] and Katayama-F et al. [25], strain

Initial pH Sulfur oxidation rate (g-S/L • d)

Table 2. Sulfur oxidation rate of strain ASWW-2 at different initial pH of medium

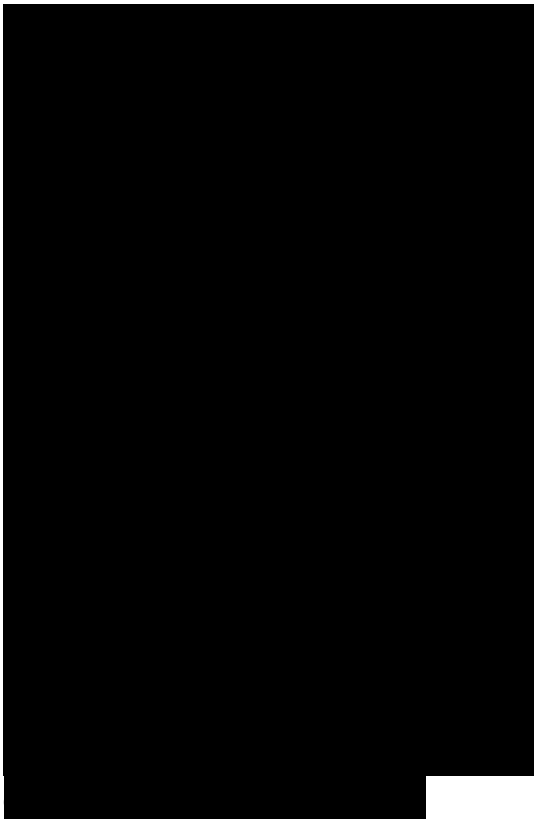
Effect of the Substrate Concentration on Sulfur Oxidation Rate

Sulfur oxidation rates were compared on each condition that 5-50 g/L of elemental sulfur was supplemented as a substrate. Fig. 2 shows the typical patterns of growth and sulfur oxidation of strain ASWW-2 at elemental sulfur of 50 g/L. The sulfate concentration, the final product of sulfur oxidation, increased with constant rate

until the accumulated sulfate concentration became to be 45 g/L, but the accumulation rate of sulfate decreased when sulfate accumulated in the medium over 45 g/L. It was considered that the reduction of sulfur oxidation rate was caused by a high ionic strength of sulfate. Although the growth of strain ASWW-2 was reached the stationary phase after 8 days of cultivation and pH in the culture medium dropped to 0.7, the sulfur oxidation activity was not diminished. Compared with the specific sulfur oxidation rates calculated from the respective sulfur concentration, the rate was increased with increasing sulfur concentration upto about 30 g/L (Fig. 3). There was no inhibition of substrate to the growth and sulfur oxidation activity of strain ASWW-2 when elemental sulfur was used as a substrate. The specific sulfur oxidation rate well describes as follow a Monod equation (Fig. 3) and the values for Km and Vm determined by a Lineweaver-Burk plot were 3.8 g-S/g-DCW-d and 14.3 g/L, respectively. ……..	[redacted]	

Fig. 2. Growth and sulfur oxidation patterns of strain ASWW-2 in MW medium supplemented with 50 g/L of elemental sulfur: 0, pH; ■, optical density at 660 nm; A, sulfate concentration.

The strain ASWW-2 could have used elemental sulfur as an energy source and oxidized it to sulfate. The attack of sulfur particles by this strain is believed to be initiated by the adhesion of the cells to the sulfur surface [26,27]. The outer surface of the sulfur particle is available for bacterial colonization and subsequent metabolism. Therefore, higher sulfur concentration is considered to facilitate faster sulfur oxidation because of higher surface area of the sulfur. The activity of sulfur oxidation of strain ASWW-2 is similar to those of several strains of T thiooxidans (0.53 to 0.95 g-S/L - d) [28, 29].



The effect of thiosulfate concentration on the sulfur oxidation rate of strain

ASWW-2 was also investigated (Fig. 4). The sulfur oxidation rate was increased with increasing thiosulfate concentration below 3.6 g-S2O32- /L. However, the sulfur oxidation rate was remarkably decreased at 5.4 g-S2O32-/L. The maximum specific sulfur oxidation rate were 16.9 g-S2O32-/g-DCW-d at 3.6 g-S2O32-/L of thiosulfate concentration. It was considered that high concentration of thiosulfate had suppressed the growth and sulfur oxidation rate because thiosul- fate could be dissolved easily. However, as can be seen in Fig. 3, elemental sulfur did not display inhibitory effect due to its insoluble property in water.

Sulfur Oxidation Rate at Various Sulfate Concentration

Fig. 5 shows the specific sulfur oxidation rate as the function of the concentration of sulfate formed in each condition of the concentration of elemental sulfur, 30, 40, and 50 g/L. In the range of sulfate concentration from 10 g/L to 50 g/L, the specific sulfur oxidation rate gradually decreased with

increasing concentration of sulfate accumulated, and the sulfur oxidation rate could be maintained over 2.0 g-S/g-DCW-d although the sulfur oxidation rate was very variable. When sulfate was accumulated in medium over 50 g/L, the sulfur oxidation rate remarkably decreased and the strain ASWW-2 was completely inhibited. On the other hand, as can be seen in Fig. 2, the sulfur oxidation activity of strain ASWW-2 could be maintained at low pH below 0.8. Therefore, the decrease in the sulfur oxidation activity of strain ASWW-2 at the sulfate concentration over 50 g/L is may be due to a high ionic strength of sulfate. These results indicated that strain ASWW-2 could oxidize the reduced sulfur compounds such as elemental sulfur and H2S at the strong acidic condition. Generally, it gives rise to the trouble to decrease the efficiency of deodorization, because the activities of deodorizing microorganisms were inhibited by pH decline. Therefore, strain ASWW-2 is considered to be an adequate candidate for the improvement of removal	[redacted] [redacted] [redacted] Do đó, chủng ASWW-2 được xem là ứng cử viên thích hợp để cải thiện hiệu suất loại bỏ của hệ thống khử mùi sinh học.	

efficiency in biodeodorization system.		