EFFECT OF H₂S AND AIR FLOW RATES ON PURE AND CONSORTIUM CULTURE OF SULFIDE OXIDIZING BACTERIA BIOFILTRATION FOR H₂S REMOVAL

Cheerawit Rattanapan*1, Piyarat Boonsawang1 and Duangporn Kantachote2

1ASEAN Institute for Health Development, Mahidol University, Nakhonpathom, 73170, Thailand.
2Department of Industrial Biotechnology, Faculty of Agro-Industry, 3 Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, 90112, Thailand.

ABSTRACT: A novel biofiltration system using granular activated carbon (GAC) as a carrier for sulfide oxidizing bacteria immobilization achieves a good performance of H₂S removal. The effect of H₂S and air flow rates were investigated using pure culture (Alcaligene faecalis T 307) and consortium culture. The results showed that H₂S flow rate of 15-35 L/h into pure and consortium culture biofiltrations had the little influence on performance of H₂S removal. Moreover, air flow rate of 5.83 L/h supplied into pure and consortium culture reactors gave the complete H₂S removal (0 ppm of H₂S concentration). Finally, the results showed that consortium culture was better performance than pure culture for H₂S removal using biofiltration in acid condition.

Keywords: Biofiltration; Hydrogen sulfide; Alcaligene faecalis T307; Consortium; Granular activated carbon;

INTRODUCTION: Hydrogen sulfide is extremely toxic to living organisms and plants. At a level of 0–5 ppm in the air, it can be detected easily. Levels greater than 10 ppm, can affect human health, while levels more than 600 ppm can cause death. Physicochemical methods for H₂S removal have relatively high energy requirements or high chemical and disposal cost. Biological treatment by biofiltration has been proposed as a convenient alternative for treating gaseous emissions containing hydrogen sulfide and reduced sulfur compounds.

Biofiltration is a process in which air containing pollutants is passed through a bed of moist, porous material in order to remove the pollutants. This technology, based on microbiological degradation of compounds from a gas stream, is considered to be an attractive alternative when compared to chemical and physical treatment. Nevertheless, using biofiltration for hydrogen sulfide control showed the technical merits and economics and found that total annual costs for the biofiltration system were 46% lower than an activated carbon system and 40% less than a chemical scrubber.

It has been reported that various used microorganism in H₂S biofiltration as sulfide oxidizing bacteria (SOB), including Thiobacillus, Xanthomonas and Pseudomonas have great potential to metabolize H₂S effectively for its low acid production and fast oxidation rate. Most researches studied H₂S removal using pure culture immobilized on carrier but there are limitations on employing pure cultures for industrial application to remove H₂S using biofiltration. Therefore, some researches used consortium culture from compost and wastewater from treatment plant sludge to removal H₂S. In addition, there is no study in the reported about the performance comparison of biofiltration inoculated with pure and consortium culture for biofiltration removal of H₂S. Moreover, the degradation of H₂S by microorganisms is affected by various environmental factors. Biofiltration for H₂S removal is also affected by input flow rate consisted of H₂S and air for this case. Therefore, maintenance of H₂S biofiltration conditions suitable for microbial activity is essential for successful biofiltration operations. Despite a large number of publications on biofiltration, further report on the effect of H₂S and air flow rates is needed. The objectives of this research is to...
study the effect of biofiltration system using granular activated carbon (GAC) as solid support for pure and consortium culture to treat a gaseous stream on H₂S gas and air flow rate.

MATERIALS AND METHODS: Organism cultivation and medium preparation

Pure culture stain of sulfide oxidizing bacterium used in this research was *Alcaligene faecalis* T307, which was isolated from the concentrated latex wastewater and was effective H₂S removal in wastewater from previously experiments. The optimal pH and temperature range at 7 and 25-35 °C, respectively. This strain was maintained on medium A agar slant (1.5% w/v of agar) and transferred to fresh slants every 2 months. The composition of the medium A contained the following (per liter): 2.0 g KH₂PO₄, 2.0 g K₂HPO₄, 0.4 g NH₄Cl, 0.2 g MgCl₂.6H₂O, 0.01 g FeSO₄.7H₂O, 8.0 g Na₂S₂O₃.5H₂O and 1.0 g Yeast extract. The final pH of the medium was adjust to 7 using 2 N NaOH to the medium.

Consortium culture of sulfide oxidizing bacteria was stimulated from influent wastewater of the sulfate reduction rector of concentrated latex industry with 2.21 g sulfur supplement under aerobic for 3 days. After stimulation process for about 3 days, the bacteria seeds were ready for inoculating onto GAC.

For all continuous experiments, the Thiosulfate mineral medium was used for humid and nutrient supply to biofiltration. The composition of medium contained the following (gram per liter): 2.0 g KH₂PO₄, 2.0 g K₂HPO₄, 0.4 g NH₄Cl, 0.2 g MgCl₂.6H₂O, 0.01 g FeSO₄.7H₂O and 8.0 g Na₂S₂O₃.5H₂O. The final pH was adjusted to 7 by using 1 N NaOH.

Cell immobilization procedure

*A. faecalis* T307 was grown in 1 L medium A for 3 days and then harvested by centrifugation (8000 rpm for 10 min). Then the cell pellet was put into a 5L plastic tank containing 3L medium. At the same time, 500 g GAC was added to be mixed with the above solution for microbial attachment and cultivation. During the cultivation period, medium was exchanged every 3 days until the cell number of *A. faecalis* T307 was estimated about 4.0 x 10⁸ cfu/g dry GAC by the traditional plate-counting method. After 15 days, the GAC in the tank was then transferred into the biofiltration.

Consortium of sulfide oxidizing bacteria were stimulated for growth in concentrated latex wastewater with 2.21 g sulfur supplement under aerobic conditions for 3 days. The precipitate was then put into a 5 L plastic tank containing 3 L sterile concentrated latex wastewater. At the same time, 500 g GAC was added and mixed with the above solution for microbial attachment. During the cultivation period, sterile concentrated latex wastewater was transformed with new sterile concentrated latex wastewater every 3 days until the cell number of microorganisms reaching about 4.0 x 10⁸ cfu/g dry GAC by the traditional plate-counting method. After 15 days, the GAC in the tank was then transferred into the biofiltration system.

Apparatus and H₂S removal for continuous operation

Figure 1 shown a schematic diagram of the GAC biofiltration designed to remove H₂S emission and this system were performed in two laboratory-scale biofiltrations. Each biofiltration was made of stainless steel, with 0.055 m inner diameter and 0.6 m height (a working volume of 1 L). Two biofiltrations were packed with different type of microorganisms. The packed bed volume of biofiltration was 40 cm or 0.67 L (about 400 g weight of GAC). Firstly, air from compressor was passed through an air filter (pore size of 0.2 μm) to leach nonessential impurities and the air flow rate was controlled by the mass flow meter. Then, the air was passed through a humidification bottle that contains the Thiosulfate mineral medium for humidification and for providing nutrient to biofiltration. Humid air was then mixed with H₂S gas, which was generated from a PVC pipe (0.05 m ID and 0.6 m length). The flow rate of H₂S gas was controlled by mass flowmeter. The inlet mixture gas was fed to the top of biofiltration at a constant flow rate. Biofiltration were operated at room temperature throughout all the experimental runs.

Analytical method

Inlet and outlet H₂S gas concentration of biofiltration was analyzed by Cadmium sulfide method. In additional, GAC samples were regularly taken out from
biofiltration for further identification. The 0.5 g GAC collected was mixed with 10 mL distilled water and was vortexed for 2 min. Then, the solution was determined for pH, total sulfur and sulfate using pH meter, gravimetric method\(^1\)\(^8\) and turbidimetric method\(^1\)\(^9\), respectively.

Fig. 1 Laboratory-scale experimental biofiltration system.

**RESULTS: Effect of H\(_2\)S flow rate**

Effect of H\(_2\)S flow rate on two biofiltration performances was investigated at various flow rates of 15, 25 and 35 L/h. Inlet H\(_2\)S concentration was fixed at 200 ppm according to optimum condition from previous study\(^2\)= and the air flow rate was maintained at 0.75 L/h. Concentrations of inlet and outlet H\(_2\)S from two biofiltrations were analyzed everyday for 21 days. It was found that outlet H\(_2\)S concentrations of pure and consortium culture reactors were less than 3 and 2 ppm, respectively (Shown in Figure 2). From this results showed that the H\(_2\)S removal performances were not significantly different at various H\(_2\)S flow rates. Nevertheless, the certain of H\(_2\)S concentration outlet observed here at varied H\(_2\)S flow rate (15-35 L/h) can be explained by the microorganisms which adapted themselves to the new environment at the slow diffusion of H\(_2\)S gas\(^2\)=.

The metabolic products on GAC biofiltration were investigated. pH values, sulfur and sulfate concentrations were measured and showed in Table 1. The results showed that the pH values of GAC in pure and consortium culture reactors were 2.40 to 1.94 and 2.60 to 2.04, respectively. The results of pH values of GAC in two biofiltrations were low and had the little variation. Moreover, the sulfate concentrations of GAC in pure and consortium culture reactors increased from 1.64 to 3.10 g/L and 1.36 to 2.66 g/L, respectively. Finally, the sulfur concentration of GAC in pure and consortium culture reactors increased from 3.86 to 4.04 g/L and 3.60 to 3.80 g/L, respectively. From this results showed that sulfide oxidizing bacteria is the key microorganisms for H\(_2\)S removal, which related to bacteria population, and can oxidize H\(_2\)S or element sulfur (S\(_0\)) for energy and produce sulfuric acid. The oxidation of H\(_2\)S occurs in two main stages and the first oxidation step results in the formation of element sulfur. Here H\(_2\)S is used as electron donors by the microorganism. When the supply of H\(_2\)S has been depleted, additional energy can be obtained from oxidation of sulfur to sulfate. The final product of oxidation in most case is sulfate\(^2\)=.

Fig. 2 Outlet concentration of H\(_2\)S in two biofiltrations at various H\(_2\)S flow rates.

**Effect of air flow rate**

Effect of air flow rate on the performance of two biofiltrations was investigated at changing air flow rates of 0.75, 1.34 and 5.83 L/h. The inlet H\(_2\)S concentration and H\(_2\)S flow rate were set at 200 ppm and 35 L/h, respectively. Concentrations of inlet and outlet H\(_2\)S from two biofiltrations were analyzed everyday for 21 days. The results showed that outlet H\(_2\)S concentrations of pure and consortium culture reactors were less than 3 and 1 ppm, respectively (shown in Figure 3). From this results showed that H\(_2\)S removal for two biofiltrations increased with the increasing...
air flow rate. The complete H₂S removal (0 ppm of outlet concentration) was found in two biofiltrations at 5.83 L/h of air flow rate. So, the complete hydrogen sulfide removal can occur when the oxygen is sufficient for the oxidation reaction. The activity of immobilized bacteria on GAC may be enhanced when air flow rate increased. Generally, sulfuric acid is the end-product of H₂S oxidation when the oxygen is implied. However, elemental sulfur may become the end product when the oxygen is consumed and apparently decreased. Oxygen is the key parameter that controls the level of oxidation. Insufficient oxygen may cause a shift in the proportion of the end-products and prevent complete removal of H₂S. Therefore, the metabolic products on GAC biofiltration at 5.83 of air flow rate were investigated. The pH values sulfur and sulfate concentrations were measured and showed in Table 1. The results showed that the pH values of GAC in consortium reactor decreased from 1.90 to 1.42, respectively. Then, the sulfate concentrations of GAC in consortium culture reactor increased from 2.66 to 5.50 g/L but sulfur concentrations of GAC in consortium culture reactor insignificantly increased from 3.80 to 4.02 g/L due to complete oxidation (high air flow rate) led to transformation of H₂S to sulfate instead of sulfur production from the partial oxidation by oxygen limitation (low air flow rate).

Moreover, the pH values of GAC in pure culture reactor insignificantly decreased from 1.94 to 1.90. Then, the sulfate concentrations of GAC in pure culture reactor decreased from 3.10 to 2.50 g/L but sulfur concentrations of GAC in pure culture reactor increased from 4.04 to 5.58 g/L. It was found that the main product of H₂S oxidation by A. faecalis T307 with high air flow rate (high oxygen) was sulfur compound instead of sulfate. It indicated not only the amount of oxygen was supplied but also type of microbial strain affected on type of metabolic products.

**Comparison of pure and consortium culture**

Comparison of pure and consortium culture was investigated by effect of H₂S and air flow rate on two biofiltrations. The results found that H₂S removal performance in each reactors related pH and sulfate concentration in GAC. Lower pH was originated form the biodegradation of H₂S which produces sulfuric acid. Since there is no biotransformation consuming the acid, the latter will accumulate very fast, and the pH will to the drop point where the microbial ecosystem is inhibited. Higher sulfate concentration originated form the process of H₂S biodegradation which H₂S is used as electron donor by the bacteria. In the experiment, pH in GAC of pure and consortium culture reactors were dropped at 1.90 and 1.42, respectively and sulfate concentration in GAC were increased at 2.50 g/L and 5.50 g/L, respectively after operations. This results showed that consortium culture was lower pH and sulfate but H₂S removal performance was higher than pure culture reactor because the autotrophic Thiobacillus group in consortium culture includes both acidophilic bacteria that grow at low pH values but optimum pH of pure culture (Alcaligenes faecalis T307) is neutral. Therefore, consortium culture was good performance for H₂S removal using biofiltration in acid condition.

![Fig. 3 Outlet concentration of H2S in different reactors at various air flow rates.](image)

**CONCLUSION**

The performance of pure (Alcaligenes faecalis T307) and consortium culture immobilization on GAC biofiltration showed that the suitable for the treatment of H₂S on various H₂S and air flow rate. Also, it was found that H₂S flow rate of 15-35 L/h into pure and consortium culture reactor had the little influences on H₂S removal, which can be explained by the microorganisms which adapted themselves to the new environment at the slow diffusion of H₂S gas. Moreover, air flow rate into pure
and consortium culture reactors gave the complete H$_2$S removal (0 ppm of H$_2$S concentration) at 5.83 L/h due to sufficient oxygen for the oxidation reaction. Finally, the results showed that consortium culture was better performance than pure culture for H$_2$S removal using biofiltration in acid condition.

Table. 1 The pH values, sulfur and sulfate concentrations of two biofiltrations on GAC

<table>
<thead>
<tr>
<th>Reactors</th>
<th>pH</th>
<th>Sulfur (g/L)</th>
<th>Sulfate (g/L)</th>
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<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
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<tr>
<td>Pure culture</td>
<td>2.40</td>
<td>1.94</td>
<td>1.90</td>
</tr>
<tr>
<td>Consortium culture</td>
<td>2.60</td>
<td>2.04</td>
<td>1.42</td>
</tr>
</tbody>
</table>

Note: A = Before experiment
B = At inlet H$_2$S concentration of 200 ppm and H$_2$S gas flow rate of 35 L/h
C = At inlet H$_2$S concentration of 200 ppm, H$_2$S gas flow rate of 35 L/h and air flow rate of 5.83 L/h

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