

The Exploratory Study on Sulfide Removal Ability of Sulfur-Oxidizing Bacteria in Biofilm on Salak Fruit Seeds

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Abstract. Sulfur-oxidizing bacteria were isolated from biofilm on salak fruit seeds. The bacteria were used for the removal of sulfide was taken from sludge of the municipal waste water treatment plant in Srandakan of Bantul of Yogyakarta (Indonesia). The results were 14 isolates of bacteria. Their performances in sulfide removal were experimentally tested. Each isolate was mixed with SOB medium containing approximate 25 ppm sodium sulfide and additives. Liquid samples were taken at the time of 4.5 hours and 9 hours. The sulfide contents in the samples were then analyzed with spectrophotometer at 664 nm. The isolates showing good performances in sulfide removal (effectively reducing sulfide concentration) were selected. Among 14 samples, there were 6 isolates that can reduce the sulfide concentration by 99 % for 9 hours at 30 °C. The batch experiments for cultivation of the isolated bacteria on salak fruit seeds (SFS) can reduce the sulfide concentration 91 % for 24 hours by isolate 3 on SFS without SOB medium, and 99.5 % for 31 hours by isolate 3 on SFS with SOB medium.

Keywords: Sulfur-oxidizing bacteria, isolate, biofilm, salak fruit seeds, sulfide removal

1. Introduction

Biogas is the result of the anaerobic digestion process of biodegradable substances. Anaerobic digestion is the process in which organic materials are degraded by anaerobic bacteria completing methanogenesis. The resulting biogas contains 55-70 % CH₄, 30-45 CO₂, and 0-1.5 % H₂S. Hydrogen sulfide is present in biogas produced from the degradation of proteins and other sulfur containing compounds present in the organic feed stock to the digester. The concentration of hydrogen sulfide in the biogas depends on the feedstock and varies between 0.1 to 2% [1]. Biogas used in heating and in turbines for electricity production has limited use in energy generation due to the presence of H₂S. Hydrogen sulfide as a nuisance compound is one major problem in biogas utilization. H₂S in biogas is corrosive to the engines and pipe lines especially when used as fuel for producing electricity. H₂S has a rotten egg odor, colorless, toxic, flammable and generally recognized as a hazardous gas [2]. By removing the H₂S, the use of biogas can be enhanced.

A number of methods including absorption, combustion, masking and scrubbing have been used to remove H₂S from industrial waste gas streams. But those physicochemical methods have high energy requirements, high chemical disposal, high costs and result in the production of secondary pollutants [3]. Compared to those physical and chemical processes, bio-filtration is considered economical, cleaner and greener because of its low operation costs, absence of secondary pollutants and emission of lower amounts of environmentally unfriendly gases compared to other methods [4]. Bio-filtration was investigated for this application. This method uses microorganisms living in a support matrix to degrade the pollutant present into a secondary form and is an inexpensive and robust alternative to chemical purification techniques. Bio-filters

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have been demonstrated to be effective in treating odorous compounds. The removal of hydrogen sulfide in biogas by bio-filter on packed bed was investigated. Several different packing materials have been used in biofiltration for the removal of H₂S. Activated carbon have been recognized as the most extensively used material, due to its capacity for adsorbing substrates quickly and then slowly releasing them for microbial degradation [5].

2. Objectives

This research focuses on isolating the sulfur-oxidizing bacteria from biofilm on salak fruit seeds, then testing the performances in sulfide removal.

3. Materials and Methods

3.1. Materials

Salak fruit seeds were harvested from Sleman, Yogyakarta, Indonesia. The salak fruit seeds were washed and then dried using oven at 55 °C to eliminate all impurities and the water. Sludge was taken from the municipal waste water treatment plant in Srandakan of Bantul of Yogyakarta (Yogyakarta, Indonesia). Sludge sample was used as inoculum seed. For the growth of mixed culture, the SOB medium of 0.4 g/l NH₄Cl, 0.2 g/l MgCl .6H₂O, 0.2 g/l KH₂PO₄, 0.2 g/l K₂HPO₄, 0.2 g/l yeast extract and 8 g/l Na₂S₂O₃ .5H₂O was used [6]. Medium and equipment were autoclaved and sterilized at 121 °C at 1.2 atm for 15 min.

3.2. Cultivation of Microorganism

The mixed microbial culture used for the removal of sulfide was taken from sludge of the municipal waste water treatment plant in Srandakan of Bantul, Yogyakarta, Indonesia. Approximately 25 g of sludge was suspended in 50 ml of sterile distilled water, and shaken vigorously. The resulting cell suspension was enriched with SOB medium. The enrichment cultures were started by seeding 5 ml of cell suspension into 250 ml of SOB medium. At the same time, 25 g of salak fruit seeds (SFS) was added into 250 ml of inoculum. The cultivation was conducted under aerobic condition. All enriched culture flasks were incubated at 30 °C on a rotary shaker at 180 rpm for 9 days. The cell number of microbial immobilization on SFS was estimated every day by the traditional plate-counting method using SOB medium.

3.3. Isolation of Sulfur-Oxidizing Bacteria

After 4 days, the microorganisms immobilized on SFS reached 1.26 x 10⁷ cfu/gr dry SFS. Fiveteen colonies, which had different shapes and color, were isolated and then to be tested to find which one having the highest sulfur oxidation capability. The selected isolates were then cultivated in fresh SOB + YE liquid medium with the same manner as described above. Growth and sulfur oxidation activity of the isolate was examined in the SOB + YE medium. The control test was performed by conducting sulfur removal using supplement medium containing sodium thiosulfate without the isolate.

3.4. Activity Test for Sulfur Oxidizing Bacteria

The SOB activity test was carried out in Erlenmeyer flask with 5 % of isolate in thiosulfate mineral salt medium and sodium sulfide of approximately 40 ppm. The samples were incubated at 30 °C for 8 hours. All experiments were performed in duplicate. The controls were also performed by supplement medium containing sodium thiosulfate without isolate. During the incubation, the samples were collected to determine the decrease of sulfide concentration after 4 hours and 8 hours. Samples (15 ml) were collected to determine the sulfide concentration by spectrophotometer on 664 nm wavelenght.

3.5. Batch Experiments

Batch culture of each isolated organism was grown in a 500 ml erlenmeyer flask at atmospheric pressure. The erlenmeyer containing 250 ml of liquid media was inoculated with 25 ml of culture. At the same time, 200 g of salak fruit seeds (SFS) was added into 250 ml of inoculum. The cultivation was conducted under aerobic condition. All enriched culture flasks were incubated at 30 °C for 6 days. Period for acclimation of metabolizing H₂S microorganisms is 4-5 days [7]. After about 6 days, salak fruit seeds were immobilised bacteria were transferred to the flask erlenmeyer and was added Na₂S concetrating about 97 ppm. To teste

the performance bacteria on salak fruit seeds, it compared the effect isolate 3 and isolate 13 on SFS were input in erlenmeyer flask with SOB medium also without SOB medium to the decreasing of sulfide concentration. The soluble sulfide has been found to be inhibitory to microorganisms at concentration of higher than 100 mg/l [8]. The removal of sulfide was investigated in a time range of 0 to 48 hours for each isolate. The liquid samples were taken in every 3 hours and were analyzed their sulfide concentration using spectrophotometer at wavelength of 664 nm.

4. Results and Discussion

4.1. Degradation of Sulfide by Isolates

The experimental results of the sulfide removal by 14 isolates in the form of the sulfide concentration in the liquid at various time are shown in Fig. 1. Significant sulfide removal can be observed in that figure. From the 14 isolates the best seven will be chosen. Within 15 hours, the best six isolates can remove 97.22% - 98 % of the sulfide respectively. Alemian et al. (2005) reported the experiment of removal of hydrogen sulfide from mixed gases in a batch bioreactor using the isolated microorganism from Ramsar hot spring. The maximum removal of hydrogen sulfide at 35 °C in batch culture in 0.06 h were 72% [9]. It shows that this exploratory study achieved better performances. The performance of the six isolates attached on salak fruit seeds in sulfide removal will be further tested.

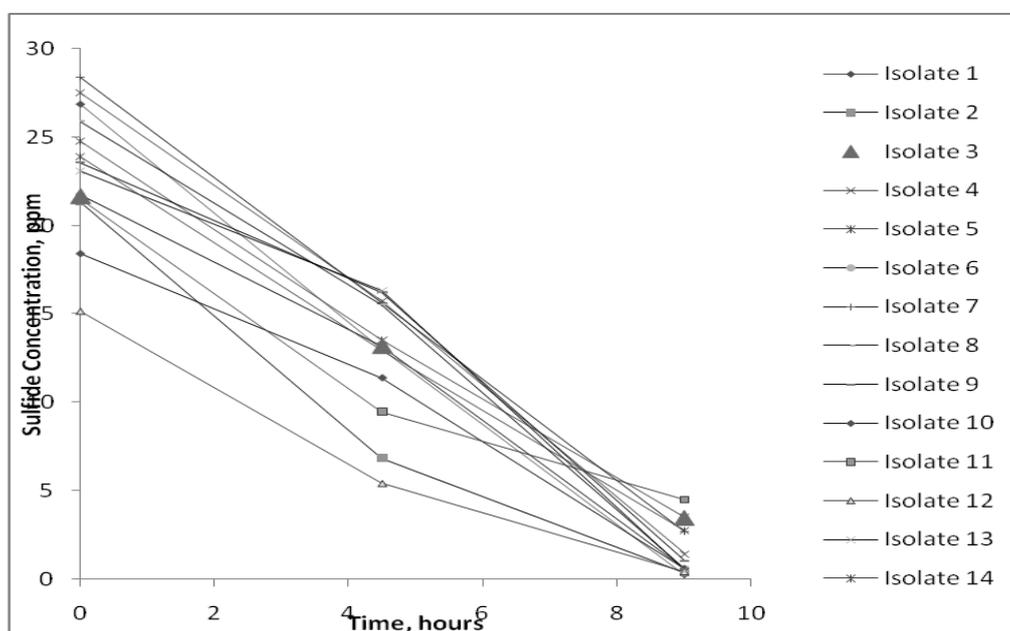


Fig. 1: Sulfide Concentration and respect to time for each isolate

4.2. Degradation of Sulfide by Bacterias on Salak Fruit Seeds (SFS)

Batch experiments were carried out using sodium sulfide as an inorganic sulfur compound for cultivation of the isolated bacteria on salak fruit seeds (SFS). Fig. 2 reports the performances of the sulfide-oxidizing bacteria (SOB) of isolate 3 and isolate 13 on SFS for 48 hours. The sulfide concentration input was about 97 ppm, but the sulfide concentration total in erlenmeyer flasks with SOB medium was about 50 ppm. According Fig. 2, sulfide concentration for control (no isolate and no SOB medium) was constant (no respect to time). The isolate 3 on SFS without SOB medium decreased the sulfide from 90.97 ppm to 8.1 ppm, so it can eliminated sulfide until 91 %. The isolate 13 on SFS without SOB medium decreased the sulfide from 97.83 ppm to 8.97 ppm, and it can eliminated sulfide until 90.83 %. Minimum sulfide concentration was obtained to be 0.517 ppm, which achieved by the isolate 3 on SFS with SOB medium, but it remove the sulfide concentration from 50 ppm, so it can remove the sulfide until 99.5 % for 31 hours. The isolate 13 on SFS with SOB medium can remove the sulfide until 71.11 %, from the sulfide concentration 53.43 to 14.88 ppm. The performance of the isolates on SFS chosen in sulfide removal will be further tested in packed bed column. This study is currently in progress. Even though the bacteria in each isolate has not been identified yet, but it is expected that those can be identified soon.

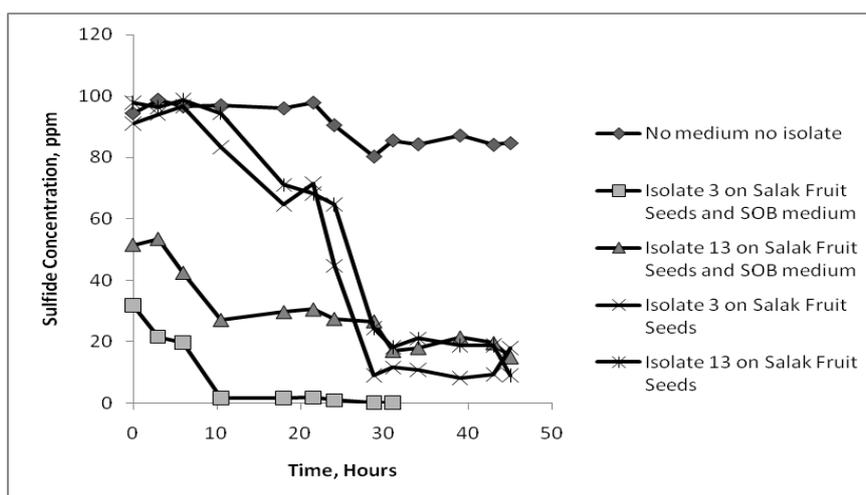


Fig. 2: Sulfide Concentration and respect to time for bacteria on SFS

5. Conclusion

It is found that the sulfur-oxidizing bacteria from sludge of the municipal waste water treatment plant in Srandakan, Bantul, Yogyakarta can grow on the surface of salak fruit seeds. The isolates of the sulfur-oxidizing bacteria can remove sulfide until 98 % for 9 hours at 30 °C. The batch experiments carried out using sodium sulfide as an inorganic sulfur compound showed that isolated bacteria on salak fruit seeds (SFS) can remove the sulfide until 91% in 48 hours by isolate 3 on SFS without SOB medium, and 99.5 % in 31 hours by isolate 3 on SFS with SOB medium.

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