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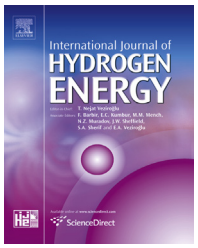

Hydrogen sulfide removal in biotrickling filter system by *Halothiobacillus neapolitanus*

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ABSTRACT

Hydrogen sulfide (H_2S) in biogas, is both toxic and damaging to the environment, to the human health, and also damages machines and engines by contributing substantially towards corrosion. It can be removed from the biogas before being used by utilizing biological or physiochemical processes. A new bacterial strain of obligately chemolithoautotroph, *Halothiobacillus neapolitanus* NTV01 (HTN) with ability to remove H_2S from gas stream was screened, purified, and inoculated in a biotrickling filter system with counter current gas/liquid flows. Maximum sulfur oxidation activity and cell growth were found in the culture medium consisting of 10 g/L of thiosulfate and 52 mM phosphate buffer pH 7. HTN is able to tolerate higher sulfate concentrations (8.35 g/L) than reported previously. In the biotrickling filter operation with biogas fed to the system, pH appeared to be a highly important factor to affect the H_2S removal. Liquid recirculation required a fresh replacement every 48 h and controlled pH 7 to achieve the optimum performance of H_2S removal. H_2S was efficiently removed 95–100% when the initial concentration in the range of 45–255 ppmv.

Introduction

H_2S is produced from proteins and other sulfur-containing compounds during the production of biogas under an anaerobic environment. In a biogas stream, even a trace of H_2S could lead to the substantial corrosion of machines, combustion engines, and other similar equipment. The tolerance levels of H_2S also differ depending on the machine. For example, kitchen ovens have a low threshold of H_2S < 10 ppmv; internal combustion engines are at H_2S < 100 ppmv; and boilers tolerate H_2S < 1000 ppmv [1–5]. Based on chemical and physical treatments, there are numerous processes for the removal of H_2S from biogas [6]. However, all of these methods posse some disadvantages, such as secondary waste production and elevated costs. Therefore, a different solution to combat these issues is the biological treatment process. To achieve this, biotrickling filters, biofilters, and bioscrubbers are the key biological technology [7]. Working in a similar way to biofilters, the

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biotrickling filters and fixed-film bioscrubbers have the advantage of working with higher concentrations of H_2S and at higher flow rates, because the recirculating medium in the biofilter/biotrickling filter improves the removal efficiency by controlling sulfate content, nutrients, and pH levels. As a result of this, size of reactors and construction costs are relatively low [8].

The microorganisms that are utilized in these bioprocesses can change H_2S into either elemental sulfur or sulfate. Chemotrophs and photoautotrophs are the two key bacteria types, which are able to remove H_2S from the biogas, but light sources is one of limitation of utilizing the photoautotrophs [9]. Chemoautotrophs, light independent microorganisms, are able to grow by acquiring the carbon source from carbon dioxide and energy sources from the oxidation of inorganic compounds such as hydrogen sulfide and thiosulfate [10]. Consequently, this microbial group is favorable for H_2S removal in the biological processes. With respect to the inoculum microorganisms in the biotrickling filter process, microbial consortia such as activated sludge seem to have disadvantage concern about the inconstant of its removal capacity and long start-up time. Pure culture strains containing only microorganisms capable of H_2S oxidation is more preferential regarding its relatively higher removal capacities and shorter start-up time [11].

The key objective of this study was to focus on using chemoautotrophic microorganisms in a biotrickling filter system for the removal of H_2S from a gas stream. This study utilized a potential source of the chemoautotrophic bacteria for screening and characterizing pure strains of sulfur oxidizing bacteria. Before applying into the biotrickling filter system, the thiosulfate and phosphate buffer concentrations were optimized for sulfur oxidation activity and microbial growth. The immobilization and operation processes of H_2S removal from the biogas by a pure culture were evaluated to derive the optimum process.

Materials and methods

Inoculum

The Sludge collected from a full-scale activated sludge system (Siriraj Hospital, Bangkok, Thailand) was used as the microbial seed for screening hydrogen sulfide-removing microbes. The isolated microbe was tested sulfur oxidation ability and was identified species by 16S rDNA method [12]. Total genomic DNA from isolated microbe was successfully extracted. Approximately 1500 bp 16S rDNA was amplified by PCR. For sequencing, representative clones were selected. The findings demonstrated that the microbe that had been isolated was a closely related species to *Halothiobacillus neapolitanus* NTV01 (HTN) (KJ027464). This sulfur oxidizing bacteria is an obligately chemolithoautotroph that is able to metabolize and tolerate high sulfide concentrations [13]. Temperature and pH ranges of 28–32 °C and 4.5–8.5 are optimum conditions for HTN growth. Additionally, this bacteria can tolerate high concentrations of NaCl over 860 mM [14]. In this work, HTN was cultured under 30 °C and pH 7 throughout the experiments.

Culture media

Thiosulfate mineral medium (TMM) was used for screening and culturing; and as the recirculating medium in biotrickling filter process. TMM contained the following (g/L): 2.0 KH_2PO_4, 2.0 K_2HPO_4, 0.4 NH_4Cl, 0.2 $MgCl_2 \cdot 6H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$ and 8.0 $Na_2S_2O_3 \cdot 5H_2O$ [15]. The medium was sterilized by autoclaving at 15 psi and 121 °C for 15 min. TMM agar was prepared by adding bacto agar (16 g/L) to TMM. TMM was later modified to optimized HTN growth and sulfur oxidation activity by evaluating the proper phosphate buffer (K_2HPO_4 and KH_2PO_4) and thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) concentrations. The buffer concentration varied between 26 and 52 mM (pH 7) in the initial test. Once the ideal buffer concentration was obtained, the thiosulfate concentration was evaluated (varied 6–20 g/L). The colony forming unit (CFU/ml) determined the growth of HTN at 30 °C, 180 rpm.

Biotrickling filter system

Biotrickling filter column was made from glass (0.475 m inner diameter and 0.72 m height). The column was packed with random packing media (GEA2H Water Technologies GmbH) by its working height of 0.282 m. The packing media has random structure and made from high-density polyethylene (HDPE), which has 12 mm diameter, surface 859 m^2/m^3 and weight 150 kg/m^3. Prior to use, this media were weighed and sterilized by autoclaved at 15 psi and 121 °C for 15 min and dried.

Biomass immobilization

TMM containing HTN biomass grown at logarithmic phase (1.87×10^{10} CFU) was fed into the reactor via a peristaltic pump with a liquid recirculation and air flow rates of 3.6 L/h and 0.5 L/min. These flow rates were fixed throughout experiments. During the immobilization process, three strategies were used to optimize the cell attachment and sulfur oxidation regarding the recirculating medium as following; (1) fresh TMM medium was replaced when pH dropped lower than pH 4.5; (2) there was no replacement of fresh TMM medium after inoculation but pH was adjusted to 7 every 24 h; (3) fresh TMM medium was replaced every 48 h and pH was adjusted to 7 every 24 h. Abiotic control was tested by using TMM broth without HTN inoculation. The reactor was operated under room temperature (30–32 °C). The attached packing media was weighted following each of the experiments after the system reached steady state with respect to sulfate production. The growth of microorganism was determined by calculation of the difference of weight between before and after process. The recirculating medium was sampled periodically to determine sulfate concentration, colony forming unit (CFU/mL), and pH.

Hydrogen sulfide removal

Mixed gas containing CH_4, CO_2, H_2S and air was utilized as an inlet gas to the biotrickling filter and the level of H_2S was varied in a range of 0–255 ppmv. In order to manage gas flow rates effectively, all gas tanks have valves, pressure gages, and flow meters to control a gas flow rates, while the air was