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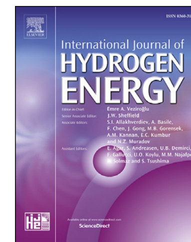

# Performance of *Paracoccus pantotrophus* for $H_2S$ removal in biotrickling filter

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ABSTRACT

*Paracoccus pantotrophus* NTV02 (PCP), a novel strain of sulfur oxidizer isolated from a full-scale leather industry wastewater treatment plant, was used for hydrogen sulfide ($H_2S$) removal from synthetic biogas in a biotrickling filter system. Its growth and sulfur oxidation activity were initially evaluated in batch reactors with the culture media containing 10 g/L thiosulfate and varying glucose concentrations (0.5–2.0 g/L) prepared in 52 mM phosphate buffer (pH 8). Its maximum growth rate ($0.57 \pm 0.00\ h^{-1}$) was observed at 1 g/L glucose, while the maximum sulfate production rate ($159.21 \pm 2.48$ mg/L·h) was found at 0.5 g/L glucose. The replacement of fresh nutrient containing-recirculation medium every 48 h, and pH adjustment (pH 8.0) every 24 h was the optimum practice for $H_2S$ removal in the biotrickling filter operation. At the optimum operation, $H_2S$ was removed up to 96% when the initial $H_2S$ concentration was in the range of 150–400 ppmv.

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## Introduction

Hydrogen sulfide ($H_2S$), a hazardous gas, presents in the biogas during the reduction of sulfur-containing organic compounds in anaerobic digestion process [1]. $H_2S$ concentrations in the biogas were reported in range 50–10,000 ppmv depending on the types of feedstock [2] such as organic waste (10–2000 ppmv), sewage (10–40 ppmv), and landfill (50–300 ppmv) [3]. Many reports showed that $H_2S$ has directly caused the human health including eye irritation (5.0 mg/m$^3$), olfactory paralysis (140 mg/m$^3$), and death (700 mg/m$^3$) [4]. The presence of $H_2S$ can limit the applications of the biogas since it causes the corrosion of metal pipe, equipment, machines, and combustion engines. Specific threshold $H_2S$ in the biogas is varied according to the applications such as 1000 ppmv (heating boiler) and 50–500 ppmv (internal combustion) [5,6]. Many biogas purification techniques are developed and investigated for $H_2S$ removal. Biotrickling filtration has accepted to be one of a major biological technique for air pollution control [7]. Trickling liquid play the important role in the control of the system performance [8]. In the biotrickling filter system, the polluted gas is passed through packing media in a reactor

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column, in which the liquid media is continuously recirculated through the packing media [9]. The recirculation media provides moisture, nutrients, pH control to the biofilm, and allows the removal of inhibiting products and several trace impurities also [7].

Two main types of bacteria commonly used in the bioprocess for $H_2S$ removal systems are photoautotrophs and chemotrophs. Interestingly, some chemotrophs can heterotrophically grow by using the organic carbon such as glucose or inorganic compound, such as thiosulfate and $H_2S$, as an energy source. These bacteria are called mixotrophic bacteria [10]. *Paracoccus pantotrophus* (PCP), which was isolated from wastewater of the leather industry from the previous study [11]. It is a mixotroph [12] that can grow on sulfide and thiosulfate under aerobic and denitrifying conditions [12]. The use of pure bacterial strains in the biotrickling filter process is attractive and has many advantages over the use of mixed cultures source including shorter start-up time easy operation, which leads to high $H_2S$ removal efficiency [13].

The potential use of PCP in a biotrickling filter process for $H_2S$ removal was tested. The immobilization of PCP and the $H_2S$ removal efficiency in biotrickling filter system were evaluated to determine the timing for the replacement of the fresh recirculation medium and the operating time of process.

## Materials and methods

### *Paracoccus pantotrophus*

*Paracoccus pantotrophus* NTV02 (PCP) (KJ027465), a sulfur oxidizing bacteria, was isolated and purified from wastewater of leather industry (Ked Prakobkarn Autsahakam Foknang KM.30 km Co., Ltd., Samut Prakarn province, Thailand). PCP was preserved in 15% glycerol at −20 °C. Its pH range is 6.5–10.5 (optimum pH is 8.0) and the temperature range is 15–42 °C (Optimum growth temperature is 37 °C) [11]. PCP was activated by culturing in thiosulfate mineral medium (TMM) at 37 °C, 180 rpm and transferred 10% v/v to fresh medium every 5 d.

### *Culture medium*

Thiosulfate mineral medium (TMM) contained the following (g/L): 4.0 $KH_2PO_4$, 4.0 $K_2HPO_4$, 0.4 $NH_4Cl$, 0.2 $MgCl_2 \cdot 6H_2O$, 0.01 $FeSO_4 \cdot 7H_2O$ and 10.0 $Na_2S_2O_3 \cdot 5H_2O$ [14]. pH was adjusted to 8.0 by 1 M NaOH and 1 M HCl. This medium was used for culturing, and used as the recirculation medium in the biotrickling filter system. The medium was sterilized by autoclaving at 15 psi and 121 °C for 15 min. The medium agar was prepared by adding bacto agar (16 g/L) to TMM.

### *PCP growth and sulfur oxidation activity*

In the previous study, 52 mM phosphate buffer, pH 8.0 and 10 g/L thiosulfate were optimized to increase the microbial growth and sulfur oxidation activity of PCP [11]. In the current study, the addition of glucose as a carbon source into TMM in the range of 0.5–2.0 g/L was evaluated if it can enhance the cell growth and sulfur oxidation activity of PCP. The cultivation was setup in 1 L batch reactors and incubated at 37 °C and 180 rpm for 5 d. The initial cell density was $2.5 \times 10^6$ CFU/mL. The samples were periodically collected to analyze the growth (CFU/mL), pH, sulfate concentration, and reducing sugar.

### *Immobilization of PCP on packing media in biotrickling filter*

The biotrickling filter reactor, made from glass with 0.48 m diameter and 0.72 m height. Random packing media, made from high-density polyethylene (HDPE) with 12 mm diameter, 859 $m^2/m^3$ specific surface area, and 150 $kg/m^3$ specific density, was used for microbial immobilization with 0.5 m height (1-L working volume). The packing media were weighed and sterilized at 15 psi and 121 °C for 15 min and dried before used.

In the immobilization process, PCP was reactivated from freezing stock by culturing in TMM containing 1 g/L of glucose at 37 °C, 180 rpm and transferred 10% v/v to the fresh medium every 48 h for 6 d. When PCP reached logarithmic phase ($2.9 \times 10^9$ CFU/mL), 2-L PCP containing medium was sprayed on the top of the packing media in the reactor and the medium was continuously recirculated for 48 h prior to the replacement of the fresh medium (glucose free TMM). 0.5 LPM airflow and 3.6 L/h medium recirculation rates were used to operate the system.

Three strategies regarding the fresh medium replacement and pH adjustment were evaluated to optimize the PCP growth and its sulfur oxidation activity as follows: (1) replacement of fresh medium every 48 h when pH was dropped below 6.5; (2) pH adjustment to 8.0 every 24 h operation without the replacement of the fresh medium; (3) pH adjustment to 8.0 every 24 h operation and the replacement of the fresh medium every 48 h. The fresh medium in this experiment was glucose-free TMM. During the system operation, the liquid medium was sampled to monitor pH, temperature, suspended cells (CFU/mL), and sulfate concentration.

### *$H_2S$ removal in gas stream*

After the immobilized PCP reached steady state, $H_2S$ (150–400 ppmv) mixed with nitrogen gas and air (3% v/v) was introduced upward into the biotrickling filter. The gas flow rate of 0.5 LPM, and the empty bed residence time (EBRT) of 120 s were used to operate the system. Abiotic control experiment was setup with 90 ppmv $H_2S$ concentration and operated for 48 h.

Recirculation medium in this experiment contained no $Na_2S_2O_3 \cdot 5H_2O$ and was continuously recirculated in the system with the rate of 3.6 L/h. This experiment was operated at room temperature, and pH was daily adjusted to 8.0. Gas and recirculation medium samples were collected every 6 and 12 h. The analytical parameters of this experiment were inlet and outlet $H_2S$ concentrations, pH, temperature, cell density, and sulfate concentration.

### *Analysis methods*

Cell density in the recirculation medium was monitored by colony forming units (CFU/mL) [15]. Sulfate and reducing sugar

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