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ScienceDirect
Energy Procedia 79 (2015) 629 – 633
Energy Procedia

2015 International Conference on Alternative Energy in Developing Countries and Emerging Economies

Optimizing Sulfur Oxidizing Performance of *Paracoccus pantotrophus* Isolated from Leather Industry Wastewater

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Abstract

Biogas has been used as alternatives for renewable energy in many applications. Hydrogen sulfide in the biogas is a significant factor to limit its usages. This research focused on using a pure bacterial strain for hydrogen sulfide removal from the biogas in a biotrickling filter process. The pure bacterial strain was isolated from a full-scale leather industry wastewater treatment plant. 16S rDNA sequence of the isolated bacterium is closely related to *Paracoccus pantotrophus*. *P. pantotrophus* is able to use sulfide and thiosulfate as energy sources for growth under aerobic conditions. The optimum concentrations of phosphate buffer (26 - 78 mM, pH 8) and thiosulfate concentrations (5 – 20 g/L) were evaluated in order to maximize microbial growth and sulfur oxidation activity before applying in the biotrickling filter system. The result showed that 52 mM buffer concentration and 10 g/L thiosulfate were suitable for growth and sulfur oxidation activity. The research findings suggest that *P. pantotrophus* has the potential application in the biotrickling filter process of hydrogen sulfide removal for upgrading biogas quality.

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Keywords: Paracoccus pantotrophus, thiosulfate, hydrogen sulfide, biogas

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Peer-review under responsibility of the Organizing Committee of 2015 AEDCEE
doi:10.1016/j.egypro.2015.11.544

1. Introduction

Biogas has been accepted to be one of the green alternatives for renewable energy. Hydrogen sulfide is generated in the biogas production during the anaerobic digestion of proteins and other sulfur containing compounds present in the organic feed stock. One of the biggest factors limiting the use of biogas is related to the presence of hydrogen sulfide exerting corrosive to internal combustion engines and equipments. Besides, there are reports on health effects to human from hydrogen sulfide. Since hydrogen sulfide is a gas, inhalation is the major route of exposure to hydrogen sulfide. Single inhalation exposures to high concentrations of hydrogen sulfide cause health effects in many systems [1]. Nowadays, there are many treatment processes base on physical and chemical methods for hydrogen sulfide removal in biogas. However, these processes have many disadvantages concerning about its costs, and secondary waste production. The biotrickling filter is one of alternative biological techniques of alternative solution for these problems. Chemotrophic bacteria can grow by using inorganic carbon as a carbon source and obtain chemical energy from the oxidation of reduced inorganic compounds such as hydrogen sulfide. Some of these microbes, which were called mixotrophic microorganisms, can heterotrophically grow by using the organic carbon as carbon source (such as glucose, etc.) and using an inorganic compound as an energy source. Chemotrophic bacteria can degrade hydrogen sulfide under the aerobic conditions by using oxygen (O_2) as an electron acceptor, while they used alternative electron acceptors (such as nitrate, etc.) in anaerobic conditions that depend on bacteria species [2]. Therefore, these microbes have potential to degrade hydrogen sulfide from biogas by applying in biotrickling filter.

This research focuses on the screening and characterizing a pure culture strains capable of hydrogen sulfide removal in biogas from high sulfide source such as waste water from leather industry. The medium was evaluated the optimal buffer (phosphate buffer) and thiosulfate concentrations for microbial growth and sulfur oxidation activity before using in biotrickling filter system.

2. Materials and methods

2.1 Isolation of sulfur oxidizing bacteria

Mixed cultures obtained from the wastewater treatment plant of leather industry, Samut Prakarn province, Thailand was isolated and purified by repeatedly transferring the cells to fresh medium [3]. The different colonies morphology were isolated by picking a single colony and inoculated on thiosulfate agar by a streak plate technique. Its sulfur oxidation ability was tested prior to strain identification.

The pure isolate was stored in 15% glycerol at -20 °C. Prior to use, it was activated by culturing in 200 mL thiosulfate mineral medium (TMN) at 37 °C, 180 rpm and transferred 10 % v/v to fresh medium every 5-7 days.

2.2 Cultural medium

TMN was used for screening, culturing, and testing microorganism. This medium 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 $Na_2S_2O_3 \cdot 5H_2O$ [4]. The medium was autoclaved at 15 psi and 121 °C for 15 min before use. The medium agar was prepared by adding bacto agar (16 g/L) to into TMN broth.

2.3 Optimization of buffer and thiosulfate concentration

Concentration of phosphate buffer (K_2HPO_4 and KH_2PO_4) and sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) in TMN were varied in order to find the optimum concentration for microbial growth and sulfur oxidizing activity. The buffer concentrations were varied in the range of 26 - 52 mM (pH 8). The concentrations of sodium thiosulfate were varied in the range of 5-20 g/L at the optimum buffer concentration. Each experiment, HTN was cultivated at 37 °C, 180 rpm. The growth of HTN was observed by colony forming unit (CFU/ml). Liquid samples were periodically collected for analysis of pH and sulfate content.

2.4 Analytical techniques

Growth of microorganisms was monitored with colony forming unit (CFU/mL) by drop plate technique [5]. Sulfate (SO_4) content was determined by turbidimetric method according to standard method [6].

2.5 16S rDNA sequence analysis

Total genomic DNA was extracted from the isolated microorganism using the benzyl chloride method [7]. The purified DNA was used as the template for amplification of the full-length 16S rDNA using bacterial specific primers BSF8/20 (5'agagtttgatcctggctcag 3') and REVB (5' ggttaccttgttacgactt 3') with DyNAzyme EXT DNA polymerase (Finnzyme, Espoo, Finland), according to Kanokratana et al. [8]. The PCR products were gel-purified using a QIAGEN Gel Extraction kit, ligated to pTZ57R/T vector (Fermentas, Vilnius, Lithuania) and transformed into *Escherichia coli* DH5α by the heat-shock method. Transformants were selected on Luria-Bertani agar plates containing ampicillin (100 μg/ml), supplemented with 40 μg/mL 5-bromo-4-chloro-3-indolyl-β-d-galactopyranoside (X-GAL) and 20 μg/mL isopropyl-β-d-thiogalactopyranoside (IPTG). Sequences were initially compared to the available databases using the BLAST network services to determine the approximate phylogeny.

3. Results and discussions

3.1 Strain of isolated sulfur oxidizing bacteria

Pure culture strain capable of oxidizing thiosulfate was isolated, purified, and identified by 16S rDNA sequence analysis. The results of its 16S rDNA sequence showed that it is closely related to *Paracoccus pantotrophus* NTV02 (PCP) (99% identity) (KJ027465). PCP is mixotrophic bacteria that can use mix of different carbon and energy sources such as carbon dioxide, glucose, fructose, acetate, and lactate. PCP can also grow on sulfide and thiosulfate under aerobic and denitrifying conditions. The optimum temperature and pH for its growth are 37 degree Celsius (range 15-42 degree Celsius) and pH 8 (range 6.5-10.5) [9]. Therefore, PCP is a bacterium that is challenged to use for upgrading the biogas quality in term of hydrogen sulfide removal.

3.2 Optimum buffer concentration

Higher ability to control pH was found in higher buffer concentrations (Fig. 1A) but the growths of the PCP are not significant different at all buffer concentrations (Fig 1B). The results of growth founded that the highest growths of 26 mM, 52 mM, and 78 mM buffer are 1.0×10^8, 1.2×10^8, and 1.3×10^8 CFU/mL,

respectively. The results indicated that the increase of buffer concentrations have no effect to the PCP growths.

The highest sulfate production of each concentration is 3373.40, 4402.53, and 4043.30 mg/L, respectively (Fig 1C) after 120 hours incubation. The highest sulfate production rate was found in 52 mM buffer concentration (97.29±3.72 mg/L·h). Therefore, 52 mM buffer concentration is suitable for the growth and sulfur oxidation activity of PCP in TMN medium.

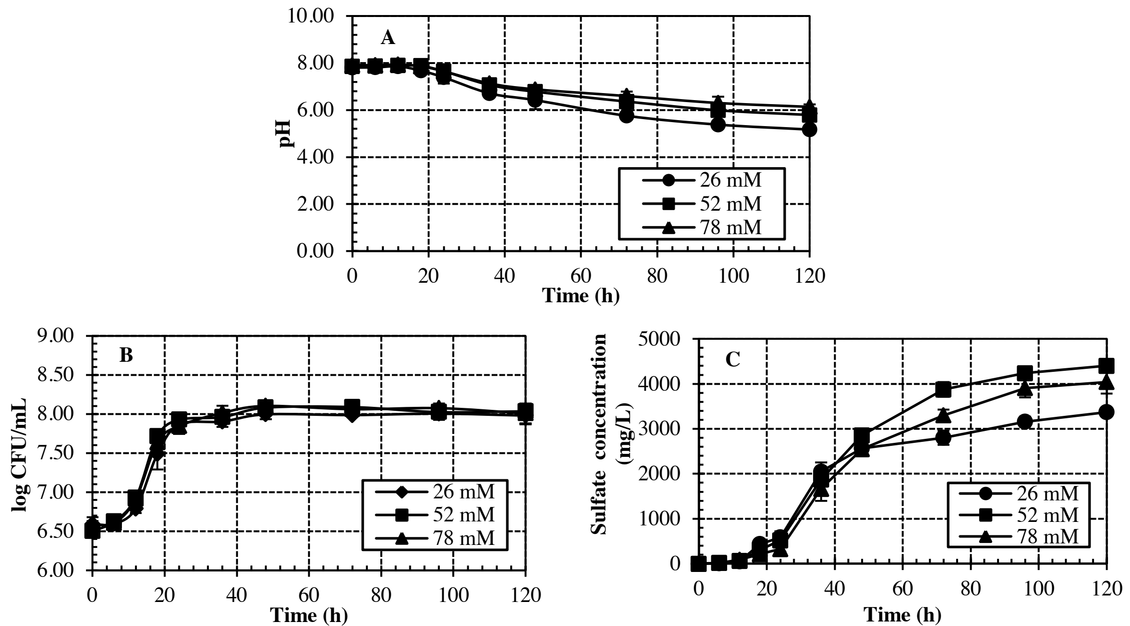

Fig. 1. (A) pH, (B) growth, and (C) sulfate production of PCP in TMN medium with varying buffer concentrations. Symbols represent mean values of duplicate experiments; error bars represent one standard deviation.

3.3 Optimum thiosulfate concentration

The thiosulfate concentrations have effect to the growth and sulfate production of PCP (Table 1). Thiosulfate concentrations over 15 g/L can inhibit the growth and sulfate production. Whereas, 5 and 10 g/L thiosulfate concentration provided similarly specific growth rates, but 10 g/L thiosulfate concentration had the highest sulfate production rate. The growth of PCP was apparently corresponded with sulfate production.

Table 1. Specific growth rate and sulfate production rate of PCP under varying thiosulfate concentrations

Thiosulfate (g/L)	Specific growth rate (h^{-1})	Sulfate production rate (mg/L·h)
5	0.14±0.00	95.50
10	0.14±0.00	148.20
15	0.11±0.00	26.54
20	0.10±0.01	7.35

The sulfate concentration of 5 and 10 g/L thiosulfate concentrations were 3,918.42 and 5,169.12 mg/L after 36 hours incubation (50.61 and 66.77 % sulfur conversion), while the sulfate concentration of 15 and 20 g/L thiosulfate concentrations were 2694.74 and 2123.68 mg/L after 120 hours incubation (34.81 and 27.43 % sulfur conversion). Therefore, the suitable thiosulfate concentration is 10 g/L for PCP growth and sulfur oxidation activity.

4. Conclusion

Paracoccus pantotrophus is mixotrophic bacteria that can grow on sulfide and thiosulfate under aerobic and denitrifying conditions. The optimum temperature and pH for its growth are 37 degree Celsius and pH 8. 52 mM Phosphate buffer and 10 g/L thiosulfate concentration in TMN medium is suitable for growth and thiosulfate oxidation activity of PCP. The current findings indicate the potential use of PCP in a biotrickling filter process of hydrogen sulfide removal to upgrade biogas quality.

Acknowledgements

The authors gracefully acknowledge the financial support from the Royal Golden Jubilee (RGJ) Scholarship of Thailand Research Fund (TRF, Grant No. PHD/0139/2553), the Joint Graduate School of Energy and Environment (Grant No. JGSEE 522), King Mongkut's University of Technology Thonburi, the Faculty of Applied Science (grant no. 5844103), King Mongkut's University of Technology North Bangkok. The author also would like to thank Ms. Natnaree Promduang and Ms. Sirinun Tungtondee for their assistants.

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