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Comparative Performance of *Halothiobacillus Neapolitanus* and *Paracoccus Pantotrophus* in Sulphur Oxidation

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Abstract

The presence of hydrogen sulfide (H_2S) in biogas is one of the biggest factors limiting the use of biogas since related it can cause corrosion in internal combustion engines. This study investigated and compared the properties and sulfur oxidizing activities of *Halothiobacillus neapolitanus* (HTN) and *Paracoccus pantotrophus* (PCP) in their suitable conditions for apply in biotrickling filter to remove hydrogen sulfide in biogas. These bacteria were screened and characterized from different wastewater treatment plants. The results indicated that HTN had higher specific growth rate than PCP. However, the sulfate production rates of HTN and PCP are not significantly different, but HTN can produce higher sulfate concentration, and can tolerant high sulfide and sodium chloride concentration and low pH, which are advantages to apply in biotrickling filter in term of preventing contaminations. This study demonstrated that HTN is better option than PCP for application in the hydrogen sulfide removal in the biogas. However, PCP has challenge to apply for hydrogen sulfide removal in the other conditions such as denitrifying condition.

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Keywords: Paracoccus Pantotrophus, thiosulfate, hydrogen sulfide, biogas

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1. Introduction

Hydrogen sulfide presents in biogas production from the degradation of proteins and other sulfur containing compounds present in the organic feed stock during the anaerobic digestion. The hydrogen sulfide concentration depends on the types of material substrates [1]. One of the biggest factors limiting the use of biogas is related to the hydrogen sulfide composition, which is very corrosive to internal combustion engines and other equipments. However, it can be used for many applications designed for natural gas, assuming sufficient purification. Besides, there are reports on health effects to human from hydrogen sulfide [2].

Many physical and chemical processes for hydrogen sulfide removal in biogas have the disadvantages concerning about high costs, and secondary waste productions. Biotrickling filter process is one of biological process, which is an alternative solution to solve these problems. Many types of chemotropic bacteria have property to use for the hydrogen sulfide degradation. 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, elemental sulfur, thiosulfate, etc. Moreover, some of chemotrophs can use both the organic and inorganic carbon as a carbon source and using an inorganic compound as an energy source. These bacteria were called mixotrophic bacteria.

Therefore, this study investigated and compared the properties and sulfur oxidizing activities of HTN (Chemotroph) and PCP (Mixotroph), which were screened and characterized from different aerobic wastewater treatment plants, in their suitable conditions previous to apply in biotrickling filter application for hydrogen sulfide removal in biogas.

2. Materials and methods

2.1 Microorganisms

Halothiobacillus neapolitanus NTV01 (HTN) (KJ027464) was screened and purified from activated sludge system collected from a full scale wastewater treatment process of Siriraj Hospital, Bangkok, Thailand. Whereas, *Paracoccus Pantotrophus* NTV02 (PCP) (KJ027465) was isolated and purified from an aerobic wastewater treatment process of leather industry (Ked Prakobkarn Autsahakam Foknang KM. 30 km Co., Ltd., Samut Prakarn province, Thailand).

These pure isolated cultures were kept in 15% glycerol at -20 °C. Prior to use, it was activated by culturing in TMN medium and transferred 10 % v/v to fresh medium every 5-7 days.

2.2 Cultural medium

Thiosulfate mineral nutrient (TMN) contained the following (g/L): 4.0 KH_2PO_4, 4.0 K_2HPO_4, 0.4 NH_4Cl, 0.2 $MgCl_2.6H_2O$, 0.01 $FeSO_4$ $.7H_2O$ and 10.0 $Na_2S_2O_3.5H_2O$ [3]. This medium was used for maintaining microorganisms and experiments. Previous to use, this medium was sterilized by autoclaving at 15 psi and 121 °C for 15 mins. The medium agar was prepared by adding bacto agar (16 g/l) to TMN medium broth.

2.3 Sulfur oxidizing test

HTN and PCP were tested in their optimal pHs and temperatures conditions by shaking at 180 rpm for 120 hours. Previous study, TMN medium has the suitable buffer and thiosulfate concentration for growth

and sulfur oxidation activities for these microbes. Liquid samples were periodically collected for analysis of the growth, pH, and sulfate content.

2.4 Analytical techniques

Growth of microorganisms was monitored with colony forming unit (CFU/mL) by drop plate technique [4]. Sulfate (SO_4) content was determined by turbidimetric method according to standard method [5].

3. Results and discussions

3.1 Isolation of sulfur oxidizing bacteria

HTN and PCP were investigated their ability in order to compare advantages and disadvantages of these microbes for applied in biotrickling filter for hydrogen sulfide removal. Both pure culture strains are chemotrophic bacteria and able to degrade hydrogen sulfide in biogas and gas stream to be elemental sulfur and sulfate.

HTN is an obligately chemolithoautotrophic bacterium, which can tolerate and utilize high sulfide concentrations energy sources, and use carbon dioxide as sole carbon source [3, 4]. Whereas, PCP can mixotrophic growth by using the mix of organic and inorganic carbon sources (carbon dioxide, glucose, etc.), and it can use sulfide and thiosulfate as energy sources under aerobic and denitrifying conditions [5]. The optimum temperature and pH of these microbes were showed in table1.

Table 1. The ranges of pH and temperature for *Halothiobacillus neapolitanus* and *Paracoccus pantotrophus* cultivation [6, 7]

Microbial strains	HTN	PCP
Growth pH range	4.5-8.5	6.5-10.5
Optimum pH	6.9	8
Growth range of temperature (°C)	8-39	15-42
Optimum temperature (°C)	28-32	37

3.2 Comparative performance on sulfur oxidizing activities

HTN was tested in pH 7 and at 30 °C, whereas PCP was tested in pH 8 and at 37 °C. This experiment used carbon dioxide from air as sole carbon source in order to compare in same condition. The results showed that HTN had higher specific growth rate than PCP (Table 2), but HTN growth was dropped after 36 hours because pH dropped lower than the pH growth range from high sulfate production (Fig 1). However, PCP growth reached stationary phase after 36 hours incubation also. For the sulfate production, the sulfate production rates of these microbes are not significantly different, but HTN can produce higher sulfate concentration.

Moreover, there is report that HTN can tolerate high concentration of sodium chloride (4 M). The properties of HTN, which are tolerant high sulfide concentration, low pH and high sodium chloride, are advantage to apply in biotrickling filter in term of preventing contaminations. Besides, HTN has the optimal growth in room temperature that is not necessary to supplied heat as PCP. Therefore, this study suggested that HTN higher properties than PCP for using in biotrickling filter in this condition. However, PCP has challenge to apply in biotrickling filter for hydrogen sulfide removal in biogas in the other

conditions. Since, PCP can mixotrophic growth in both of aerobic and also denitrifying conditions, so the hydrogen sulfide removal from biogas in absent air is interesting to investigate in future experiment.

Table 2. Comparison of kinetic parameters of *Halothiobacillus neapolitanus* and *Paracoccus pantotrophus* at the optimal condition.

Microbial strains	HTN	PCP
Specific growth rate (h-1)	0.15±0.01	0.14±0.00
The highest growth (CFU/mL)	9.0×10^8	1.8×10^8
Sulfate production rate (mg/L.h)	147.7±9.8	148.2±21.4
Maximum sulfate concentration (mg/L)	6,120.00	5,679.60

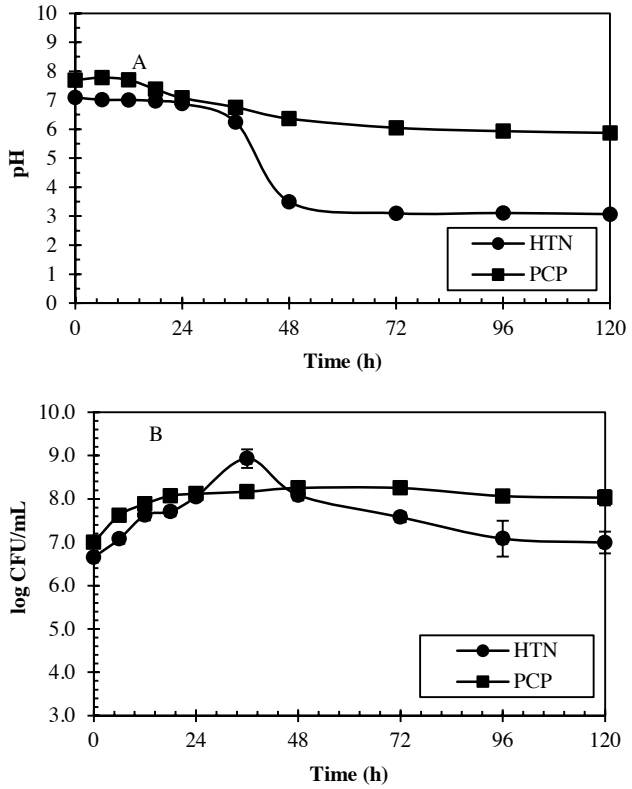

Fig. 1. pH (A) and Growth (B) of HTN and PCP in TMN medium at optimal conditions. Symbols represent mean values of duplicate experiments; error bars represent one standard deviation.

4. Conclusion

HTN and PCP were investigated and compared the properties and sulfur oxidizing activities in their suitable conditions for apply in biotrickling filter to remove hydrogen sulfide in biogas. These bacteria were screened and characterized from different wastewater treatment plants. The results showed that the properties of HTN had higher than PCP in this study. However, PCP has challenge to apply for hydrogen sulfide removal in the other conditions such as denitrifying condition.

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