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Potential Application of *Halothiobacillus neapolitanus* for Hydrogen Sulfide Removal in Biogas

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Abstract

Biogas has been extensively used for many applications. The amount of carbon dioxide and hydrogen sulfide in the biogas directly affects its energy content and corrosive property. Many processes were used to remove these gases from the biogas stream. The current research aimed to isolate a pure bacterial strain capable of simultaneous removal both gases, and apply it in the biotrickling filter designed for carbon dioxide and hydrogen sulfide removal. Pure isolate of bacteria capable of thiosulfate oxidation was isolated from an activated sludge seed, and was identified by 16S rDNA method. The isolated microbe was closely related to *Halothiobacillus neapolitanus* (HTN), an obligately chemolithoautotrophic bacteria able to utilized carbon dioxide as a carbon source, and oxidize sulfide as a energy source. Optimum buffer concentration for thiosulfide oxidation activity was evaluated by varying phosphate buffer concentration in the range of 26 - 78 mM (pH 7.3). The optimum buffer concentration for the growth and thiosulfate oxidation was 52 mM. At this buffer concentration, HTN can remove thiosulfate up to 69.8 % within 36 h, and maximum thiosulfate removal was 79.1 % after 120 h incubation at the initial concentration of 10 g/L thiosulfate. The sulfate production rate was 147.7 ± 9.76 mg/L·h. The current findings indicate the potential use of HTN in a biological process of hydrogen sulfide and carbon dioxide removal to upgrade biogas quality.

Keywords: *Halothiobacillus neapolitanus*; thiosulfate; hydrogen sulfide; biogas

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1. Introduction

Biogas, an alternative renewable energy, can be produced from various organic containing sources such as animal manure, agricultural and industrial wastewaters, garbage and agriculture-residues by anaerobic process. The use of biogas has been increased for many applications such as boilers and internal combustion engines. The presence of a trace amount of hydrogen sulfide (H₂S) in the biogas is harmful to human health and cause significant corrosive damage to the equipments or machines contacting the biogas. The previous reports revealed that hydrogen sulfide concentration above 10 ppm can affect human health, while levels exceed 750 ppm can immediately cause unconsciousness or death [1]. Physical and chemical processes for hydrogen sulfide removal have many disadvantages regarding its high costs, and secondary waste production. The biotrickling filter, which is biological treatment process, is the one of alternative solution for these problems. The microorganisms used in these bioprocesses are able to convert hydrogen sulfide into elemental sulfur and sulfate. Chemoautotrophic bacteria are microorganisms that obtain the carbon sources for growth from carbon dioxide (CO₂) and obtain energy from the oxidation of inorganic compounds as hydrogen sulfide. Applying these microorganisms in the biotrickling filter can eliminate both hydrogen sulfide and carbon dioxide at the same time [2].

Potential sources of chemoautotrophic bacteria were selected for screening and characterizing pure bacterial strains capable of hydrogen sulfide and carbon dioxide removal in biogas stream. Optimum buffer concentration (phosphate buffer) and thiosulfate concentrations were evaluated for microbial growth and sulfur oxidation activity before applying the pure strain into biotrickling filter system.

2. Materials and methods

2.1 Isolation of sulfur oxidizing bacteria

Mixed cultures obtained from a full scale activated sludge system treating wastewater generating from Siriraj Hospital, Bangkok, Thailand were 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 activity 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 30 °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 maintaining microorganisms. This medium contained the following (g/L): 2.0 KH₂PO₄, 2.0 K₂HPO₄, 0.4 NH₄Cl, 0.2 MgCl₂·6H₂O, 0.01 FeSO₄·7H₂O and 8.0 Na₂S₂O₅·5H₂O [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 (K₂HPO₄ and KH₂PO₄) and sodium thiosulfate (Na₂S₂O₅·5H₂O) 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 7.3) at fixed 10 g/L sodium thiosulfate concentration. The concentrations of sodium thiosulfate were varied in the range of 6-10 g/L at the optimum buffer concentration. Each experiment, HTN was cultivated at 30 °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 (SO₄) 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 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 Isolated microbe

A pure bacterial strain capable of oxidizing thiosulfate was obtained after the isolation and purification processes. Analysis of its 16S rDNA sequence found that it is closely related to Halothiobacillus neapolitanus (HTN) (99% identity). HTN is an obligately chemolithoautotrophic bacterium, a sulfur oxidizing bacterium, that tolerate high sulfide concentration; and utilize as inorganic compounds as energy sources, and carbon dioxide as a sole carbon source [9]. Its metabolic characteristics suggested that HTN is a challenging bacterium that can be used for upgrading the biogas quality in term of simultaneous sulfide and carbon dioxide removal.

3.2 Optimum buffer concentration

Higher buffer concentrations showed greater ability to control pH (Fig. 1A), but not the growths of the HTN (Fig. 1B). pH rapidly dropped below the optimum pH range (4.5-8.5) for HNT [10] after 24 h incubation in the 26 mM buffered medium and 36 h incubation in the 52 mM buffered medium. Cultural time to reach maximum growth seemed to relate to the time where pH started to drop. The result suggested that suitable buffer (K₂HPO₄ and KH₂PO₄) concentration for HTN growth and sulfur oxidation activity is 52 mM in TMN medium.
3.3 Optimum thiosulfate concentration

The higher thiosulfate concentrations resulted in higher growth in 52 mM buffer medium (Table 1). The highest sulfate production was observed in 10 g/L thiosulfate concentration. The sulfate concentration was 5,400 and 6,120 mg/L after 36 and 120 h incubation (69.8 and 79.1 % sulfur conversion). The growth of HTN was apparently corresponded with sulfate production and pH drop.

Table 1. Growth and sulfate production rate of HTN under varying thiosulfate concentrations

<table>
<thead>
<tr>
<th>Thiosulfate (g/L)</th>
<th>Specific growth rate (h⁻¹)</th>
<th>Biomass (CFU/mL)</th>
<th>Sulfate production rate (mg/L.h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.12±0.01</td>
<td>3.23 x 10⁸</td>
<td>118.3±1.91</td>
</tr>
<tr>
<td>8</td>
<td>0.13±0.00</td>
<td>3.92 x 10⁸</td>
<td>130.6±2.12</td>
</tr>
<tr>
<td>10</td>
<td>0.15±0.01</td>
<td>9.00 x 10⁸</td>
<td>147.7±9.76</td>
</tr>
</tbody>
</table>

4. Conclusion

Halothiobacillus neapolitanus, a chemolithoautotrophic bacterium, was successfully isolated and tested for its ability to oxidize thiosulfate. TMN containing 52 mM phosphate buffer pH 7.3, and 10 g/L thiosulfate was the optimum medium for HNT growth and thiosulfate oxidation. The research findings suggest the potential application of this bacterium in the biotrickling filter process of simultaneous hydrogen sulfide and carbon dioxide removal from biogas.

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References


**Biography**

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