Thesis Title Acetic Acid Utilization between Mixed Culture and Enriched

Acetoclastic Methanogens in Biogas Production

Thesis Credits 12

Candidate Miss Phuangthong Mangked

Supervisors Associate Professor Dr. Morakot Tanticharoen

Dr.Pawinee Chaiprasert

Degree of Study Master of Science

Department Biotechnology

Academic Year 1998

## Abstract

More than 70 percent of methane gas generated in anaerobic digestion process are attributable to acetic acid. Due to its slow growth rate, a population size of methanogens in the mixed culture is relatively small. The conversion from acetic acid to methane gas, hence, becomes the rate-limiting step of the biogas production from various types of wastes. The objective of this research was to enrich methanogenic population for enhancement of acetoclastic methanogenesis. The utilization of various acetic acid concentrations by enriched culture was observed and compared with mixed culture from the anaerobic digester.

The enrichment of acetoclastic methanogens from anaerobic digester was succeeded by transferring the mixed culture into medium containing 15 mM acetic acid as sole carbon source. The transfer was conducted every 2 weeks. After 9 transfers, the number of acetoclastic methanogens in enriched culture increased to  $1\times10^8$  cells/ml compared with  $2\times10^4$  cells/ml in the mixed culture. In addition, the enrichment culture was unable to utilize glucose, butyric acid, propionic acid, and lactic acid. The enriched culture was mainly rod shaped with flat ends and utilized acetate to methane and carbondioxide. These characteristics were similar to Methanosaeta sp. (Methanothrix sp.).

Rate of acetic acid utilization by mixed culture and enriched culture were examined. It was found that the rate of acetic acid utilization was faster by the enriched culture than by the mixed culture due to a number of methanogens in the inoculum. Acetic acid utilization rate of mixed culture containing  $2 \times 10^4$  cells of methanogen/ml reduced when acetic acid concentration

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exceeded 70 mM. Whereas, rate of acetic acid utilization by acetoclastic enrichment culture containing  $1\times10^8$  cells of methanogen/ml still increased with increasing acetic acid concentration to 120 mM. However, the rate decreased at 250 mM acetic acid concentration and methanogenesis was lower. Acetic acid was not totally utilized. When cell concentration of enriched culture was increased from 1 to 5 milligram/litre, an increase in acetic acid utilization was observed from 0.76 mM to 4.32 mM within 6 hours.

Not only faster, the methanogenesis from acetic acid was more efficient by the enriched culture with 0.72-0.85 mmole methane/mmole acetic acid utilized compared to 0.54-0.69 mmole methane/mmole acetic acid utilized by the mixed culture. More carbondioxide was produced by the mixed culture at the first 2-3 day. Besides acetoclastic methanogens, the activity of sulfate reducing bacteria was observed with the mixed culture in media containing acetic acid.

Keywords: Acetoclastic methanogenesis / methanogenic enrichment bacteria /

Methanosaeta sp. / acetic acid