D115 Biogas Methane Production Utilizing *Clostridium formicoaceticum* and *Methanosarcina mazei*

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Introduction

Elimination of large quantities of byproduct glycerol is the challenge of the current bioethanol and biodiesel refinery plants. The present study was undertaken to develop a biotechnological method to convert glycerol to biogas methane utilizing an anaerobic mixed culture digestion system of *Clostridium formicoaceticum* and *Methanosarcina mazei* (**Figure 1**).

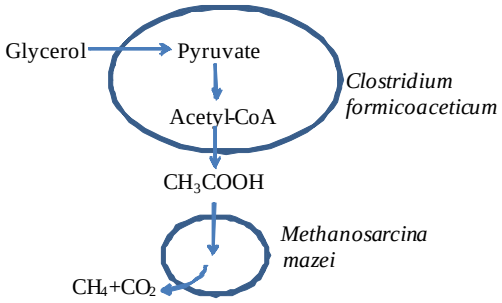

Figure 1 Generation of methane from glycerol in the mixed culture

1. Experimental

Microorganisms

C. formicoaceticum ATCC 27076 and *M. mazei* NBRC 101201 were used in this work.

Medium

The basal medium contained KH_2PO_4, 0.75 g; K_2HPO_4, 10 g; $MgCl_2 \cdot 6H_2O$, 0.36 g; NH_4Cl, 1 g; yeast extract (Difco), 1 g; polypepton, 5 g; sodium acetate, 2.5 g; trimethylamine·HCl, 8 g; $NaHCO_3$, 0.8 g; $Na_2S \cdot 9H_2O$, 0.5 g; cysteine-HCl, 0.5 g; resazurin, 1 mg per 1 litter. Trace mineral and vitamin were also added as following the recipe of ATCC and NBRC. The pH of medium was adjusted between 7.0- 7.2.

Mixed culture

The anaerobic batch culture experiments were performed at 150 rpm by two 250 mL shaking flasks (Run A: basal medium (control), Run B: basal medium added with 2.5 $g \cdot L^{-1}$ glycerol) at 310 K under 20% CO_2 and 80% N_2 without pH control. First, 10 mL pre-cultures of *M. mazei* were inoculated into flasks containing above medium and cells were grown for 1 day. Then 5 mL pre-cultures of *C. formicoaceticum* were added. The OD_{660} of culture was determined by spectrophotometer. Concentration of glycerol was determined by enzymatic method. Total pressure in gas phase was adjusted to 101.3 kPa by removing the overhead gas with the syringe at 144, 216, 288, 360 and 432 h. Volume of the removed gas was monitored. Amount of methane in the removed gas was analyzed by GC.

2. Results and discussion

Figure 2 shows the time courses of OD_{660}, glycerol concentration (c_{Gly}), and total amount of produced methane (N_P). The pHs of sample solution were

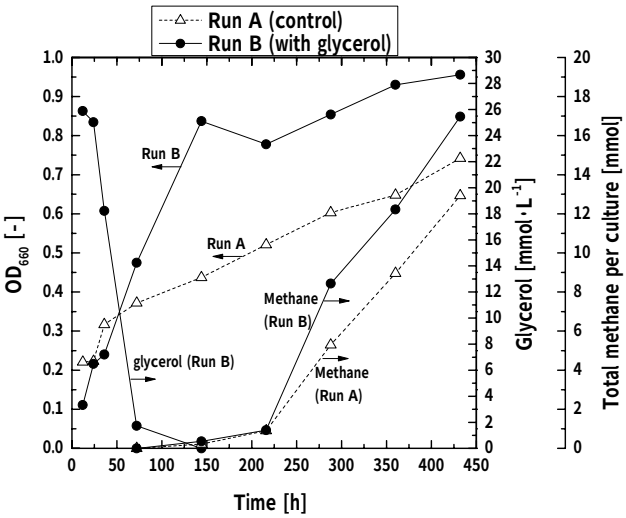

Figure 2 Time courses of OD_{660}, glycerol concentration and total methane per culture

monitored at 298 K. The range of pH was 7.0-7.9 in Run A and 7.0-8.5 in Run B. The highest pH value of 8.5 was observed at 288 h in Run B. Glycerol digestion rate in Run B was so high. The average consumption rate ($=dc_{Gly}/dt$) from 24 h to 72 h was 0.49 $mmol \cdot L^{-1} \cdot h^{-1}$. Complete conversion was observed within 144 h.

In both Run A and B, active productions of methane were observed after 216 h. Approximately 45-50% of the gas produced was methane in Run B. The average methane production rate ($= (dN_P/dt)/V_L$) from 216 h to 432 h was 0.056 $mmol \cdot L^{-1} \cdot h^{-1}$ in Run A. The average methane production rate in Run B was 0.10 $mmol \cdot L^{-1} \cdot h^{-1}$ from 216 h to 288 h and 0.059 $mmol \cdot L^{-1} \cdot h^{-1}$ from 288 h to 432 h. Glycerol was successfully converted to biogas methane in this system.

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