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An interesting correlation between methane production rates and archaea cell density during anaerobic digestion with increasing organic loading

Kiyohiko Nakasaki [a,*], Sang Hagk Kwon [a], Yuji Takemoto [b]

[a] Department of International Development Engineering, Tokyo Institute of Technology, 2-12-1, Ookayama, Meguro-Ku, Tokyo 152-8550, Japan

[b] Department of Applied Chemistry and Biochemical Engineering, Graduate School of Engineering, Shizuoka University, 3-5-1, Johoku, Naka-Ku, Hamamatsu-shi 432-8561, Japan

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ABSTRACT

Changes in the cell density of bacteria and archaea during anaerobic digestion producing methane gas with increasing the organic loading were determined using real-time polymerase chain reaction (PCR). It was confirmed that the methane production rate (MPR) was proportional to the cell density of archaea, whereas no clear relationship was observed between the cell density of bacteria and MPR. When the organic loading rate (OLR) increased, the bacteria activated quickly to produce volatile fatty acids (VFAs). However, the enhancement of archaeal activity to consume VFAs and produce methane gas was relatively small. This was because the high oxidation-reduction potential (ORP) and VFA concentration associated with the high OLR were harmful to the archaea. Consequently, when the OLR was very high, VFAs accumulated at high concentrations, the cell density of archaea was reduced, and finally the fermentative methane production deteriorated.

1. Introduction

Biogas is an ideal alternate fuel made from biomass [1]. Extensive studies have been conducted on anaerobic digestion using different organic wastes as substrates [2–4]; therefore, the process by which organic waste is fermented to produce methane is well characterized. During anaerobic digestion, bacteria decompose organic waste to produce intermediate compounds such as volatile fatty acids (VFAs), which are further digested anaerobically by archaea to form methane. Moreover, the characteristics and role of each microorganism involved in the complex microbial consortium has been elucidated by the recent development of molecular biology. In fact, several studies succeeded to identify the microorganisms that contribute to anaerobic digestion producing methane and revealed the shift in the type of microorganisms during the acclimation stages of fermentative methane production [5–11].

Based on the mechanism discussed, both the bacteria and archaea play important roles in methane production during

* *Corresponding author*. Tel./fax: +81 3 5734 3169.
E-mail address: nakasaki@ide.titech.ac.jp (K. Nakasaki).
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anaerobic digestion. The archaea are responsible for the final step, i.e., the production of methane from degradation intermediates, in the multi-step reactions of methane production. Therefore, it can be deduced that the MPR is proportional to the cell density of the archaea. However, to the best of the authors' knowledge, there have been no previous studies that have reported the correlation between MPR and the cell density of archaea quantitatively during fermentative methane production. Consequently, this study attempts to show the changes in the MPR and cell density of microorganisms during methane production in anaerobic digestion, as the organic loading rate increases. Moreover, the study aims to quantitatively elucidate the correlation between MPR and cell density of bacteria and archaea.

2. Materials and methods

2.1. Substrates and inoculum

In this study, 2 types of wastewater were used as substrates for the production of methane gas by anaerobic digestion: synthetic peptone wastewater and wastewater containing syrup (syrup wastewater), which consisted mainly of sucrose and was obtained from a fruit-canning factory, Yamanashi Kanzume Co. Ltd., Shizuoka, Japan. The CNP ratio was adjusted to 100:15:1 (mass ratio) by using urea and K_3PO_4 before use, while $NiCl_2$ and $CoCl_2$ were added to the syrup wastewater so that the final concentration was 1 g m^{-3} each [12].

The synthetic peptone wastewater prepared in our laboratory was composed of trypticase peptone, 17 g L^{-1}; KH_2PO_4, 0.3 g L^{-1}; $KHCO_3$, 4.0 g L^{-1}; NH_4Cl, 1.0 g L^{-1}; NaCl, 0.6 g L^{-1}; $MgCl_2$ $6H_2O$, 0.82 g L^{-1}; $CaCl_2$ $2H_2O$, 0.08 g L^{-1}; cysteine–HCl H_2O, 0.1 g L^{-1}; $NiCl_2$ $6H_2O$, 0.001 g L^{-1}; $CoCl_2$ $6H_2O$, 0.001 g L^{-1}; 1.0×10^{-4} L of the trace element solution DSMZ medium 318, and 1.0×10^{-4} L of the vitamin solution DSMZ medium 318 without B12 [10,13].

The chemical oxygen demand (COD) of the syrup wastewater and synthetic peptone wastewater were 126.7 and 20.1 g L^{-1}, respectively. The COD value was determined by using potassium permanganate as an oxidizer. The wastewater was thoroughly diluted before being transferred into the methane reactor. The pH value of both wastewaters was adjusted to 8.0 with 1 mol L^{-1} sodium hydroxide. The inoculum source, which is the granular sludge, was obtained from an up-flow anaerobic sludge blanket reactor used to treat brewery wastewater. The details can be found in previous papers [10,12].

2.2. Operating conditions

Two types of anaerobic sequencing batch reactors (ASBRs) made from Pyrex glass with different sizes, 1.8 and 3 L, were used for the production of methane gas through anaerobic digestion from syrup wastewater and synthetic peptone wastewater, respectively [10,12]. The temperature of both reactors was maintained at 39 °C in a thermostated water bath, and the solution in the reactor was agitated slowly with a stirrer at 0.83–1.67 Hz. Prior to the start of the operation, the reactor was filled with the granular sludge and distilled water; the pH was adjusted to 8.0 and the reactor was flushed with N_2 for 10 min to ensure anaerobic conditions. In the present repeated-batch operation, a specific volume of the liquid in the reactor was withdrawn and the same volume of the influent solution was introduced. The exchanging volume of the liquid was decided according to the hydraulic retention time (HRT). The organic loading rate (OLR), defined as a quotient of the substrate concentration, and HRT increased in a step-wise manner for both types of wastewater. Details of OLR, HRT, and substrate concentration for anaerobic digestion used to produce methane from both syrup and synthetic peptone wastewaters are shown in Table 1 [10,12].

2.3. Analytical methods

The pH value was monitored using a pH electrode (PH-3P, Mettler Toledo, Tokyo, Japan) installed inside the reactor and the oxidation-reduction potential (ORP) was measured immediately after sampling the outflow sample using an ORP meter (FPH92, Tokyo Garasukikai Co., Ltd., Tokyo, Japan). The exhaust gas from the reactor was collected into a plastic bag (Tedlar bag, Omi Odoair Service Co., Ltd., Omihachiman, Japan), and the bag was replaced daily. Gas compositions were analyzed by GC–MS. GC–MS analysis was performed on a Shimadzu QP-5050A mass spectrometer with a CP-Polar Bond Q fused silica capillary column (25 m × 0.25 mm i.d., film thickness 3 mm, Varian Instruments, CA, USA). The gas volume was measured using a dry test gas meter (DC-1, Shinagawa Co., Ltd., Tokyo, Japan). The liquid sample was centrifuged for 10 min at 17700× *g* at 4 °C, and the supernatant was filtered through a membrane filter (DISMIC-25 JP, Advantec Co., Ltd., Tokyo, Japan). The filtered supernatant was subjected to VFA analysis. Organic acids were analyzed by HPLC (LC-2000 plus HPLC system, Jasco Corp., Tokyo, Japan). Analysis details have been reported in previous papers [10,12].

2.4. Measurement of the cell density

The DNA of the microorganisms contained in the liquid sample was extracted using DNA extraction kit, ISOIL for Beads Beating (Nippon Gene Co., Ltd., Toyama, Japan). After the purification of the DNA, real-time PCR was performed to quantify the microbial DNA in the liquid sample. The primer sets used for total bacteria were Eu27F, 5′-AGAGTTTGATCCTGGCTCAG-3′ and Eu518R, 5′-GTATTACCGCGGCTGCTGG-3′; the primer sets used for total archaea were Ar28F, 5′-TGGTTGATCCTGCCAGAGG-3′ and ARC915, 5′-GTGCTCCCCCGCCAATTCCT-3′ for amplification of the respective 16S rRNA gene regions [14]. Real-time PCR was performed using the Smart Cycler system (Cepheid, California, USA) and SYBR1premix Ex Taq™ (Takara Biomedicals, Shiga, Japan). The reaction mixture (25 mm^3) was prepared according to the manufacturer instructions and consisted of 1 SYBR1premix Ex Taq™, forward and reverse primers (200 nmol L^{-1} each), extracted DNA (2 mm^3), and sterilized distilled water. The real-time PCR cycling conditions were as follows: initial denaturation and polymerase activation at 95 °C for10 s followed by 40 cycles of denaturation at 95 °C for 5 s and a combined annealing and extension step at 60 °C for