Journal of Industrial and Engineering Chemistry 21 (2015) 443–450

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Relationship between changes in microbial community and the deterioration of methane fermentation which treats synthetic peptone wastewater

Sang Hagk Kwon, Kiyohiko Nakasaki *

Department of International Development Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro-ku 152-8550, Tokyo, Japan

ARTICLE INFO

Article history:
Received 21 December 2013
Accepted 4 March 2014
Available online 12 March 2014

Keywords:
Methane fermentation
Peptone wastewater
Microbial community
Ammonium ion
Volatile fatty acid
Sulfate-reducing bacterium

ABSTRACT

Synthetic peptone wastewater was used as a substrate in a laboratory-scale methane fermentation reactor. Methane production ceased when the hydraulic retention time (HRT) was changed from 4 d to 1 d. A DNA-based method revealed that the amount of acidogenic bacteria increased, while methanogenic archaea decreased under these conditions. The short HRT resulted in a high concentration of dissolved oxygen in the wastewater, which adversely affected the archaea by stimulating bacterial production of high concentrations of volatile fatty acids and ammonium ion, and growth of the bacterium belonging to *Desulfoglaeba* that competes with methanogens for reducing power.

1. Introduction

The world's population continues to grow, increasing its energy demand and fossil fuel consumption. This contributes to negative environmental consequences, such as global warming, ozone depletion, and acid rain. The eventual depletion of fossil fuel has motivated the development of renewable energy, especially from biomass [1].

Biomass can be converted to various energy sources, including ethanol, methanol, and methane. Among them, methane is an ideal fuel, because it is easily produced and emits few atmospheric pollutants. In contrast, other fuels, such as ethanol and methanol, are not well suited for commercial production and are technically more difficult to generate from biomass [2]. Another advantage of fermentative methane production is that it can be achieved using biomass waste as a raw material. To date, many studies have investigated methane fermentation using various types of biomass waste. Several of these studies have taken a microbiological perspective and have provided valuable findings.

Bacteria decompose organic matter to produce intermediate compounds, which are anaerobically digested to form methane by archaea. The entire process involves a series of reactions requiring the cooperative action of several microorganisms. Specifically, in the first stage of protein fermentation to form methane, various fermentative bacteria break down protein into amino acids. In the second stage, acetogenic bacteria produce acetate, hydrogen, and carbon dioxide. Finally, these compounds are converted to methane by methanogenic archaea.

As described above, the process by which proteins are fermented into methane has been well characterized. However, the roles of microorganisms remain poorly characterized, because the process involves a complex microbial system, where many types of microorganisms co-exist and interact complexly. Recent developments in molecular biology have elucidated microbial communities and their roles in methane fermentation. The role of the microbial community in methane production has been described in detail for the fermentation of olive mill solid residues [3], pig and cattle slaughterhouse waste [4], and carbohydrates [5]. In particular, Wagner et al. [6] reported differences in the microbial community structure associated with a change in raw material from skim milk to sunflower seed oil.

These studies identified the microbial communities that contribute to favorable methane fermentation and accumulated useful knowledge for understanding the detailed mechanisms of methane fermentation; however, only a few studies have considered the changes in the microbial community when methane production suddenly decreased to extremely low levels as a result of changes in operational conditions [7–9]. Kundu et al. [7] investigated the effects

* Corresponding author. Tel.: +81 3 5734 3169; fax: +81 3 5734 3169.
E-mail address: nakasaki@ide.titech.ac.jp (K. Nakasaki).

http://dx.doi.org/10.1016/j.jiec.2014.03.003
1226-086X/

of operating temperatures on the microbial community and found that acetoclastic methanogenic activity was higher at 37 °C than that at 55 °C, resulting in decreased methane production and decreased diversity of acetoclastic methanogens at 55 °C. Akarsubasi et al. [8] reported that sudden changes in wastewater composition resulted in a decrease in microbial activity. Similarly, Nakasaki et al. [9] reported that the microbial community changed with the deterioration of methane fermentation when syrup wastewater was used as the raw material. When the HRT was shortened, intermediate volatile fatty acids (VFAs) increased to high concentrations, and there was an increase in iron-reducing bacteria that compete with methanogens for hydrogen sources, i.e., reducing power.

Using synthetic wastewater containing protein as a raw material, the present study was designed to investigate the microbial community associated with the decline in methane production resulting from a shortened HRT. The microbial community structure depends on substrate type; thus, it is expected that changes in the microbial community associated with decreased methane production will be characteristic for each substrate. During the acclimation period, decreasing the HRT increases the organic loading rate (OLR), thus changing the microbial community and causing a sudden decrease in methane production. This phenomenon can be used to study the deterioration of methane fermentation. To our knowledge, no study has reported the relationship between changes in methane production using peptone wastewater and changes in the microbial community.

2. Materials and methods

2.1. Inoculum and synthetic wastewater

The inoculum source was obtained from an upflow anaerobic sludge blanket reactor used to treat brewery wastewater. Synthetic wastewater with peptones was used as the sole source of carbon and was composed of trypticase peptone (17 g/L), KH_2PO_4 (0.3 g/L), $KHCO_3$ (4.0 g/L), NH_4Cl (1.0 g/L), NaCl (0.6 g/L), $MgCl_2{\cdot}6H_2O$ (0.82 g/L), $CaCl_2{\cdot}2H_2O$ (0.08 g/L), cystein–$HCl{\cdot}H_2O$ (0.1 g/L), $NiCl_2{\cdot}6H_2O$ (0.001 g/L), $CoCl_2{\cdot}6H_2O$ (0.001 g/L), 10 mL of the trace element solution DSMZ medium 318 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH: Catalogue of Strains 2007), and 10 mL of the vitamin solution DSMZ medium 318 without B_{12}. The synthetic wastewater in the present study was modified from the previously described BSA-synthetic wastewater [10], which contained albumin, while the present synthetic wastewater contained peptone. The chemical oxygen demand (COD) of synthetic wastewater was 20.1 g/L, and its pH was adjusted to 8.0 with 1 N sodium hydroxide.

2.2. Operation of the anaerobic sequencing batch reactor

The schematic diagram of experimental apparatus is shown in Fig. 1. The anaerobic sequencing batch reactor (ASBR) was made of Pyrex glass and had a working capacity of 3.0 L. The head space volume of the reactor was approximately 1.0 L. The temperature in the reactor was maintained at 39 °C in a thermostated water bath (HT-90D, As one, Tokyo, Japan), and the solution was agitated with a stirrer (NZ-1300, Eyela, Tokyo, Japan) at 100 rpm. The influent substrate was stored at 4 °C before feeding and was fed into the reactor using a peristaltic pump (MP-3N, Eyela, Tokyo, Japan). Prior to the operation, the reactor was filled with 1.5 L of granular sludge and distilled water; the pH was adjusted to 8.0 with 1 N sodium hydroxide, and the reactor was flushed with N_2 for 10 min to ensure anaerobic conditions. The injection volume of N_2 in the reactor was approximately 300 L.

The organic loading rate (OLR) was adjusted by changing both the substrate concentration of synthetic wastewater and the HRT.

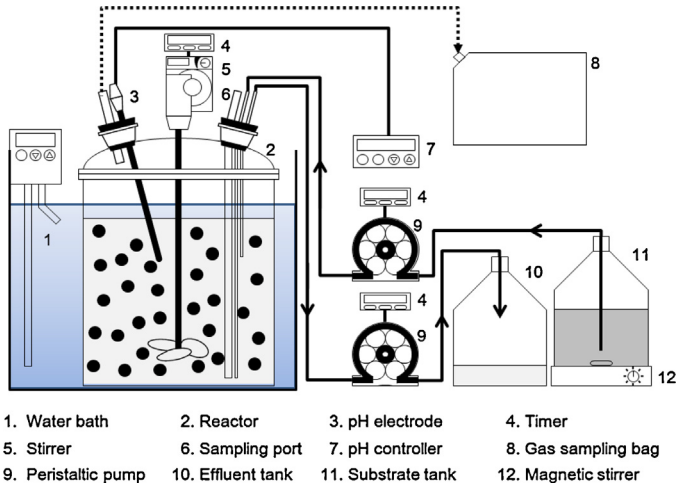

Fig. 1. The schematic diagram of experimental apparatus.

The substrate concentration of synthetic wastewater was set at 20.1 g COD/L from days 0 to 161 and then decreased to 5.0 g COD/L from days 162 to 197. The HRT was set at 8 d from days 0 to 17, 4 d from days 18 to 161, and 1 d from days 162 to 197. The withdrawing of the fermentation solution and the feeding of the substrate were conducted daily to achieve the designed HRT. Before withdrawing the fermentation solution from the reactor, we stopped the agitation for 2 hours to allow the settling down of the granule sludge. The numbers of feedings per day were 1 for an HRT of 8 d, 1 for an HRT of 4 d, and 3 for an HRT of 1 d. The OLR was set at 2.5 g COD/L/d until day 17 and was then maintained at 5.0 g COD/L/d until the end of the fermentation process.

2.3. PCR-DGGE method and DNA sequencing analysis

The DNA of the granule sample was extracted using DNA extraction kit, ISOIL for Beads Beating (Nippon Gene Co., Ltd., Toyama, Japan). After purification of the DNA, TaKaRa Ex Taq Hot Start Version (Takara Bio Inc., Shiga, Japan) was used for PCR amplification by using TaKaRa PCR Thermal Cycler Dice (TP600; Takara Bio Inc., Shiga, Japan) with the following primer sets deriving from the 16S rRNA gene: 357FGC, 5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′, and 518R, 5′-ATT ACC GCG GCT GCT GG-3′ for bacteria, and PRA46F, 5′-(C/T)TA AGC CAT GC(G/A) AGT-3′, PREA1100R, 5′-(T/C)GG GTC TCG CTC GTT (G/A)CC-3′, PARCH 340F, 5′-CGC CCG CCG CGC GCG GGC GGG GCG GGG GCA CGG GGG GCC CTA CGG GG(C/T) GCA (G/C)CA G-3′, and PARCH519R, 5′-TTA CCG CGG CKG CTG-3′ for archaea. PRA46F and PREA1100R were used for nested PCR. DGGE analysis was carried out using D-code DGGE Complete System (BioRad Laboratories, CA, USA). The PCR product was mixed with an equal volume of 2× gel loading dye (10 mM Tris–HCl at pH 8.0, 20 mM EDTA at pH 8.0, 0.05% [w/v] bromophenol blue, and 70% glycerol) and loaded onto a 10% (w/v) polyacrylamide gel in a 1× TAE buffer (40 mM Tris–acetate at pH 7.4; 20 mM acetate, 1 mM Na_2EDTA) with a denaturing gradient ranging from 30% to 60%. Using the DGGE image as a reference, the bands of interest were excised from the gel on a Dual Intensity Ultraviolet Transilluminator (Model TDS-20; UVP, CA, USA). Sequencing reactions for the purified DNA bands were carried out with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA), while sequencing was performed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, CA, USA). The results of the sequencing analyses were compared to the sequences of the 16S rRNA genes available in the common databases (DDBJ, and GenBank) and the affiliation, including the