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Research paper

Effects of shock loading versus stepwise acclimation on microbial consortia during the anaerobic digestion of glycerol

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ABSTRACT

Sludge from a brewery was used to produce methane by feeding glycerol (propane-1,2,3-triol) at an organic loading rate (OLR) of Chemical Oxygen Demand (COD) at 2.5 g L^{-1} d^{-1}. Results from two different substrate-feeding approaches were compared: one was the shock loading of glycerol (Run A) and other was a gradual increase in the glycerol amount in a mixture with other carbon sources including glucose, sodium acetate, and lactate (Run B). Methane production rate was similar for both experiments (approximately 21 mmol L^{-1} d^{-1}). Dominant bacteria in Run A were closely related to *Mesotoga* sp., *Alkalibacter* sp., and *Garciella* sp., while the dominant bacteria in Run B were closely related to *Trichococcus* sp. Dominant archaea were similar for both experiments and were closely related to *Methanosaeta* sp. and *Methanobacterium* sp. From these results, it was confirmed that the microbial consortium, especially the bacterial consortium, was strongly dependent on the feeding approach of the glycerol in the anaerobic digestion system.

1. Introduction

Increasing concerns about fossil fuels depletion, air pollution, and greenhouse gas emissions from fossil fuel usage have necessitated the search for and utilization of alternative sources of energy worldwide. Biodiesel is a widely used option [1,2], with its production growing rapidly over the last decade and likely continuing to grow [3,4].

Biodiesel production generates by-products, with glycerol being the most abundantly produced one. For every tonne of biodiesel produced, 100 kg of glycerol by-product is generated [5]. Without expensive purification, it cannot be used in chemical industries since it has high concentrations of impurities. Anaerobic digestion of glycerol is a promising method to deal with this impure glycerol. During glycerol digestion, methane gas is produced, which can be used as an energy source [5]. The anaerobic digestion of glycerol is inexpensive, easy to implement, and generates small amounts of sludge that can be disposed of [6,7].

Anaerobic digestion is a complex process and involves the joint action of a consortium of microorganisms containing many species of bacteria and archaea. This consortium requires specific growth conditions, including temperature, pH, and substrate [8], and any change in these conditions may affect the dominant microorganisms present [8–11]. To achieve anaerobic digestion of a new substrate, the sludge from an established consortium that is anaerobically digesting one substrate is slowly adapted to the new substrate. This adaptation, or acclimation, generally involves a significant change in the microorganisms present in the sludge, because some microorganisms cannot adapt to the new conditions, while those with the ability to adapt to the new conditions and substrate become dominant.

During this acclimation process, the increasing capacity of the sludge to produce methane from glycerol corresponds to changes in the microbial consortia. Some microorganisms, including those that are able to consume glycerol, will proliferate and establish a new consortium that can interact to degrade the glycerol and produce methane. An understanding of these changes in the microbial populations would allow us to know which microorganisms enable higher production of methane during the anaerobic digestion of glycerol, which would allow the development of systems to monitor the presence and abundance of relevant microorganisms. Additionally, the identification of the microbial consortium that enables efficient methane production from glycerol digestion would help to determine the biological conditions that promote the growth and action of the microorganisms. As a consequence, methane production from glycerol could be further improved by

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adjusting the optimum conditions for the microorganisms.

Few efforts have been made to understand the microbial consortium present during the anaerobic digestion of glycerol [12,13]. A previous study investigated the changes in bacterial and archaeal communities over time that are associated with the stepwise acclimation of anaerobic sludge during a substrate change from brewery wastewater to glycerol [12]. The stepwise acclimation consisted of gradually increasing the proportion of glycerol relative to the glucose, sodium acetate, and lactic acid present in the substrate fed to the anaerobic digestion system. In addition, the established microbial communities in the stable process of anaerobic fermentation of glycerol were previously studied [13]. In this study some important microorganisms were identified; however, they did not address the changes in the microbial communities during the acclimation to glycerol as the sole source of carbon.

This study analyzes changes in the microbial consortia during the adaptation of granular sludge to glycerol after a shock loading of substrate in an anaerobic digestion process. Furthermore, effects of this shock loading of glycerol are compared to those of stepwise acclimation where the ratio of glycerol in the substrate is increased gradually.

2. Materials and methods

2.1. Raw granular sludge and acclimation approaches

Granular sludge was obtained from a full-scale upflow anaerobic sludge blanket (UASB) reactor from a brewery wastewater treatment plant treating mainly sugars, organic acids and ethanol at a concentration level equivalent to approximately of COD 1.5 g L^{-1}. The wastewater treatment plant is located in the province of Shizuoka prefecture, Japan. The sampling of granular sludge from the UASB reactor was made with a 500 μm diameter pipe. The granular sludge was stored at 4 °C until it was used in the fermentation experiments. The schematic diagram of the experimental apparatus was described previously [14]. An anaerobic sequencing batch reactor (ASBR) with 3 L of working volume was used to perform the fermentation processes. At the start of the experiment, the reactor was filled with distilled water and 300 cm^3 of granular sludge. The temperature was maintained at 39 °C throughout the fermentation by keeping the reactor in a water bath. The contents of the reactor were stirred at 1.7 Hz. A new batch was started daily by withdrawing 750 cm^3 of liquid medium from the reactor and introducing the same volume of fresh medium containing 4.5 g L^{-1} of $NaHCO_3$, 1.5 g L^{-1} of NH_4Cl, 0.2 g L^{-1} of $(NH_4)_2SO_4$, 0.25 g L^{-1} of K_2HPO_4, 0.3 g L^{-1} of KH_2PO_4, 0.2 g L^{-1} of $CaCl_2 \cdot 2H_2O$, 0.25 g L^{-1} of $MgSO_4 \cdot 7H_2O$. All components of the fresh medium were reagent-grade chemicals. The fresh medium contained also 8.25 g $L^{-1}$of reagent-grade glycerol as the sole source of carbon. The pH of the liquid medium in the reactor was adjusted daily to 7.6 using 1 mol L^{-1} sodium hydroxide. The hydraulic retention time (HRT) of the reactor was set at 4 days as it was found previously to be a good condition for anaerobic digestion using a similar experimental system [14]. The organic loading rate (OLR) was adjusted to a COD of 2.5 g L^{-1} d^{-1} considering the results of a previous study in which a good performance of methane production was observed in anaerobic digestion of glycerol [15]. The fermentation period of 97 days, was rather arbitrarily decided, but it does represent an amount of time after which a stable methane gas production was observed. This shock loading experiment was denoted as Run A.

The results from Run A were compared to those obtained using a stepwise acclimation approach, in which the amount of glycerol in the substrate fed to the reactor was increased in a stepwise manner from 0 to 100% on COD basis, while reducing the amount of GAL solution [12]. The GAL solution was composed of 5.0 g L^{-1} glucose, 2.5 g L^{-1} sodium acetate, and 2.2 g L^{-1} lactic acid. The organic loading rate in the stepwise acclimation approach was kept constant at a COD of 2.5 g L^{-1} d^{-1} as reported previously; this experiment was defined as Run B. In Run B, the influent solutions were designated as solution G0, G25, G50, G75 and G100. Solution G0 (containing 0% glycerol and 100% GAL based on the COD) was fed from day 0 to day 20; solution G25 (25% glycerol and 75% GAL) was fed from day 21 to day 36; solution G50 (50% glycerol and 50% GAL) was fed from day 37 to day 48; solution G75 (75% glycerol and 25% GAL) was fed from day 49 to day 60; and solution G100 (100% glycerol and 0% GAL) was fed from day 61 until the end of the experiment. Duration of the loading for each solution from G0 to G100 was arbitrarily decided; however, the methane production rate was monitored to be almost stable. Additional details can be found in the previous paper [12].

2.2. Analytical methods for physicochemical parameters

Samples of the liquid medium in the reactor were withdrawn daily. Before withdrawing the 750 cm^3 of liquid medium, the granular sludge inside of the reactor was allowed to settle for 2 h in order to avoid a washout of the granules. The pH and oxidation reduction potential (ORP) values were determined with a pH electrode (pH-3P, Mettler Toledo, Greifensee, Switzerland) and an ORP meter (FPH92, Tokyo Garasu Kikai Co., Ltd., Tokyo, Japan). Gas samples stored in a Tedlar bag were analyzed by Chromatography-mass spectrometry (GC–MS). The GC–MS used was a Shimadzu QP-5050A equipped with a CP7348 CP-PoraBOND Q column at 40 °C and 1 cm^3 min^{-1}. The volume of the produced gas was measured using a dry test gas meter (DC-1, Shinagawa Corporation, Tokyo, Japan). Volatile fatty acids (VFA) concentrations were measured by high pressure liquid chromatography (HPLC) system equipped with an L-3300 RI monitor (HITACHI, Tokyo, Japan) and a SUGAR SH1011 column (Shodex, Tokyo, Japan) at 40 °C with 5.0 mmol L^{-1} H_2SO_4 as the mobile phase and a flow rate of 1 cm^3 min^{-1}.

2.3. Molecular analysis of microbial communities

Sample of granular sludge was taken from the reactor at adequate time intervals. The DNA was extracted from 0.2 g wet weight of granular sludge using the ISOIL method for the Beds Beating kit (Nippon Gene Co. Ltd., Toyama, Japan). PCR was performed using the TaKaRa ex Taq kit and an automated thermal cycler (PCR thermal cycler dice, TaKaRa, Shiga, Japan). Primers that amplify the 16S rRNA gene were used. For the analysis of the bacterial consortium, the primers used were as follows: forward primer 357FGC [5′-CGC CCG CCG CGC GCG GCG GGC GGGGCG GGGGCA CGG GGG GCCTACGGGAGGCAG CAG-3′] and reverse primer 517R [5′-ATTACCGCGGCTGCT GG-3′]. PCR amplification of archaeal DNA was carried out by nested PCR using two sets of primers: forward primer PRA46F [5′-(C/T)TAAGCCATGC(G/A)AGT-3′] and reverse primer PREA1100R [5′-(T/C)GGGTCTCGCTCGTT(G/A)CC-3′] and PARCH 340F [5′-CGCCCGCCGCGCGCGGCGGGC GGG GCG GGG GCA CGG GGGCCCTACGGGG(C/T)GCA(G/C)CAG-3′] and PARCH519R [5′-TTACCGCGGC(G/T)GCTG-3′].

Denaturing gradient gel electrophoresis (DGGE) and DNA sequencing to identify the microorganisms were carried out as reported previously [14]. The sequences of DNA were compared to the sequences of the 16S rRNA genes available in databases (DDBJ, EMBL and GenBank) [16–18] in order to determinate the microorganisms present in the experiments. The number of bases read for identification of bacteria and archaea were expected to be 161 and 180, respectively. The actual length for each microorganism can be known by tracking the accession number given in this paper (cf.