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Changes in the microbial community during the acclimation stages of the methane fermentation for the treatment of glycerol

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ABSTRACT

Granular sludge from a full-scale methane reactor treating brewery wastewater was used as a seed for the treatment of glycerol in a laboratory-scale repeated-batch methane reactor, and the change in the microbial community during the acclimation stages was examined. Two types of substrate solutions, a glucose, sodium acetate, and lactic acid mixture, as well as glycerol, were prepared and fed by mixing the two solutions to increase the ratio, in a stepwise manner, of glycerol from 0% to 100%, while keeping a loading of COD at 2.5 kg m^{-3} d^{-1} throughout the fermentation process. Vigorous methane gas production, approximately 580 dm^3 m^{-3} d^{-1}, was observed during the acclimation stages. Microbial analysis revealed that both bacterial and archaeal communities changed significantly; bacteria (genus *Trichococcus* and family *Syntrophomonadaceae*) became dominant rapidly after the start of acclimation, and archaea belonging to the hydrogenotrophic methanogens (genera *Methanobacterium* and *Methanospirillum*), increased gradually with the progress of acclimation.

1. Introduction

To address the deficiency of fossil fuels, the development of new energy sources, such as biogas, bioethanol, and biodiesel, has been explored in recent years [1]. Because biodiesel is an attractive alternative fuel to replace petroleum-based fuel, the production of biodiesel is of interest in many countries. Biodiesel manufacturing has been rapidly expanding worldwide, including the European Union, the United States, Australia, Argentina, Brazil, Malaysia, Indonesia, and Thailand. From 2008 to 2010, biodiesel production in the world averaged 17.608 hm^3 y^{-1}, in which the European Union achieved the highest production amount with 9.184 hm^3 y^{-1}. It is predicted that the production of biodiesel in the world will be 41.917 hm^3 y^{-1} in 2020 [2]. During the production of biodiesel, however, huge amounts of glycerol, a waste by-product, is generated [1,3]; therefore,

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proper treatment of residue glycerol is required urgently. In the present study, glycerol was used as a raw material for the production of methane gas. By applying this method, issues of waste management and energy production can be solved simultaneously.

Previously, researchers have investigated methane fermentation of glycerol or by-product wastewater from the production of biodiesel [4–7]. López et al. [4] observed that the biodegradability and methane yield coefficient of by-product wastewater from the production of biodiesel were 100% and 306 $dm^3 kg^{-1}$ glycerol, respectively, if the wastewater was pretreated with acid. Astals et al. [5] elucidated that anaerobic co-digestion of glycerol and pig manure could increase the organic loading rate of the fermentor by adjusting the C/N balance of the nutrients and reducing the free ammonia emission. In addition, there have been some studies that analyzed the microbial community in methane-fermented glycerol. Yang et al. [6] treated glycerol-containing synthetic wastes using a fixed-bed anaerobic reactor at 55 °C, and elucidated that *Methanobacterium* sp. and *Methanosarcina* sp. were the dominant archaea, and *Bacillus* sp., *Clostridium* sp., *Desulfotomaculum* sp., and *Ruminococcus* sp. were the dominant bacteria. Luo et al. [7] investigated both bacterial and archaeal communities in the two-stage anaerobic hydrogen and methane production process of wastewater containing glycerol waste from the production of biodiesel. Luo and colleagues [7] discovered that some dominant bacteria and archaea maintained stable performance under conditions of fermentation; however, there seems to be no literature on the dynamic change of the microbial community during the acclimation stages of methane fermentation for the treatment of glycerol.

A long lag time is required to achieve stable performance of anaerobic digestion, and the balance in the activities of different groups of microorganisms in the fermentor is established during the lag time [8–10]. Therefore, the inoculation of the seed sludge from a methane fermentor, which has been operated stably, is often applied to accelerate the start-up of a new methane fermentor, even if the types of substrate in the previous and the new fermentors differ from each other. If the seed sludge is introduced into a fermentor treating different types of substrate, a microbial community may change with the progress of the acclimation stages from the previous substrate to the new substrate. There have been some studies that compared the microbial community in the initial seed sludge and in the sludge after acclimation to the new substrate [11–13]. Lee et al. [11] inoculated the seed sludge from an anaerobic digester treating municipal wastewater into the anaerobic digester of dairy-processing wastewater and determined the change in the cell density of total bacteria and total archaea. Moreover, a shift of members in the bacterial community associated with the change in the substrate was clarified. Lee et al. [12] successively analyzed methanogenic communities in three anaerobic digesters treating different types of wastewater by inoculating the seed sludge from municipal wastewater. In both studies, methane fermentation was conducted in the batch operation, and the substrate concentrations in the digester changed with the progress of the fermentation. Nelson et al. [13] investigated changes in a microbial community by introducing a granular sludge into a methane fermentor treating different types of substrate. Namely, initial sludge from an upflow anaerobic sludge blanket (UASB) reactor treating jam- and jelly-manufacturing wastewater (granule S) was used as the seed sludge for a second anaerobic reactor treating a high concentration of starch and lipid wastewater (granule N). Granule N was used as the seed sludge for treating wastewater from a cheese-manufacturing company (granule B). As a result, a unique microbial community was observed corresponding to a particular feedstock. In granule S, *Proteobacteria* and *Firmicutes* were the most dominant phyla (85%), while unclassified bacteria corresponded to 6% of the total phyla. *Proteobacteria*, *Firmicutes*, and the unclassified bacterial portions increased to one-third of all sequences, respectively, in granule N. The major sequences in the granule B sample were either unclassified *Chloroflexi* (47%) or unclassified bacteria (28%).

In the studies mentioned previously, microbial communities have been compared in samples collected at the initial and the final substrate acclimation stages of methane fermentation with new substrates; however, the time course of microbial-community change during acclimation stages to the new substrate has yet to be elucidated.

Moreover, there is limited research that refers to the members of both bacterial and archaeal communities coexisting during the acclimation stages. Therefore, the aim of the present study was to elucidate the shifts of both bacterial and archaeal communities over time that associate with the changes in the types of substrate (from brewery wastewater to glycerol) in the acclimation stages of repeated-batch methane fermentation.

2. Materials and methods

2.1. Reactor and operation

In order to activate microorganisms prior to feeding glycerol, the reactors were first fed with a synthetic solution composed of glucose, sodium acetate, and lactic acid (GAL solution). The solution was then changed to a mixture of the GAL solution and glycerol, and the amount of glycerol in the mixture was increased to 100% as in the previous study [4]. Briefly, two types of substrate solutions, the GAL solution and glycerol, were prepared and the reactor was fed by mixing the 2 solutions to increase the amount of glycerol, in a stepwise manner, from 0 to 100%, while keeping the loading of COD at 2.5 kg m^{-3} d^{-1} throughout the fermentation, except for the first 2 days, when the loading of COD was kept at 1.5 kg m^{-3} d^{-1} with the GAL solution. In addition to these organic fractions, inorganic salts and trace elements were added to the influent solution according to Ghangrekar et al. [14]. The influent solutions with different compositions of organic constituents were designated as solution I, G0, G25, G50, G75, and G100, and the compositions of these solutions are shown in Table 1.

A 3 dm^3 working volume reactor made of Pyrex glass (250 mm in height and 120 mm ID) was used. At the start of experiment, 300 cm^3 of granules were added to the reactor, and then 2700 cm^3 of solution I was introduced. Subsequently, a daily repeated-batch operation of fermentation to