Comparative study of changes in reaction profile and microbial community structure in two anaerobic repeated-batch reactors started up with different seed sludges

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HIGHLIGHTS

▶ Two anaerobic repeated-batch reactors differing in seed source performed similarly.
▶ H_2-Utilizing pathway was likely the main route for methanogenesis in both reactors.
▶ The reactor methanogen communities were likely dominated by *Methanospirillum hungatei* strains.
▶ Bacterial community structure changed dynamically over cycles in both reactors.
▶ Bacterial community shifts caused little change in methanogen community structure.

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ABSTRACT

Microbial community structure and dynamics were examined in two anaerobic reactors run in repeated-batch mode to treat whey permeate. Despite being started up using different seeding sources, the reactors showed generally similar reaction patterns and performances. During the repeated-batch operation for three cycles, the overall reaction rate increased with the increase in the initial population size of both bacteria and methanogens over cycles. *Clostridium*- and *Methanospirillum*-related microorganisms were likely the main acidogenic and methanogenic populations, respectively, in both reactors. Bacterial community structure shifted dynamically over cycles, while little change was observed in methanogen community structure throughout the operation. This means that the changes in bacterial community structure changes had little influence on the formation and evolution of methanogen community structure in the reactors. The increased methanogenesis rate with cycles seemed therefore more likely due to the effect of the increase in methanogen abundance rather than the alteration of community structure.

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1. Introduction

Anaerobic digestion (AD) has been widely applied to the treatment of organic pollutants due to its ability to produce combustible biogas (mainly methane) while reducing pollution load. Owing to the additional benefit of energy production, increasing attention is being paid to AD in these times of global energy and environmental crisis (Appels et al., 2011). AD is a multi-stage reaction conducted by diverse microbial populations which can be broadly grouped into acidogens and methanogens. Acidogens are a group of bacteria which hydrolyze and ferment complex organic molecules finally to hydrogen and acetate, the major substrates for methane formation, through various fermentation pathways. The acidogenic products are subsequently utilized for the growth of methanogens and converted to methane, whereby pollution load can be finally stabilized. Therefore, extremely diverse microorganisms of different physiological and biochemical types coexist in AD environments and their harmonized activity is necessary for complete decomposition of organic pollutants. The overall performance of an AD process is consequently dependent on the functioning and interactions of acidogens and methanogens involved.

With the development and application of culture-independent molecular techniques that typically target specific nucleic acid sequences, a large number of studies have recently been conducted to investigate microbial community structure and dynamics in anaerobic digesters. Microbial community analysis has been reported in various types of anaerobic digesters run in different operation modes, i.e., continuous and batch modes and their

modifications. Lee et al. (2010), for example, assessed the transition of methanogen community structure in a batch digester treating whey permeate. Bialek et al. (2011) comparatively investigated methanogen community structure and dynamics between two digesters of different configurations treating a synthetic dairy wastewater: inverted fluidized bed (IFB) and expanded granular sludge bed (EGSB) reactors. De Sanctis et al. (2010) studied the shifts in microbial community structure during the transition of biomass structure from flocculent to biofilm and granular sludge in a digester run in sequential batch mode. Anaerobic discontinuous processes, such as sequential batch, fed-batch, and repeated-batch reactors, semi-continuously treat waste feed by repeating a series of batch treatments through cyclic feeding and discharging. Discontinuous processes have several advantages over continuous ones due to their ability to retain biomass within the system for a desired period of time, for example, no need for solid or liquid recycling, higher treatment efficiency, easier process control, and greater methanogenesis activity (Zaiat et al., 2001). On this account, discontinuous processes can offer a more suitable treatment option in certain cases, for example, in treating intermittently produced wastewater. During the early cycles of a discontinuous process (i.e., acclimation period), reaction rate typically increases as the cycle number increases until the system reaches a quasi-steady state (Wilderer et al., 2001). Such changes in process performance could be the effect of changes over cycles at the microbial community level, e.g., changes in community structure or population size. Previous approaches to this question in environmental bioprocesses have, however, been limited due to the complexity of mixed-culture microbial system, and little information is so far available on the microbial community behavior and its effects on the overall performance in discontinuous processes. This knowledge vacuum seems to deserve more attention particularly in AD, given that slow reaction rate and long start-up period are among the major drawbacks of AD.

The aim of this study was to assess the changes in microbial community structure, along with the changes in physicochemical reaction profiles, over cycles in anaerobic digesters run in repeated-batch mode. For this purpose, two bioreactors seeded with separate sludges were operated over three cycles for a total period of 72 days, using whey permeate as feed. In order to obtain a comprehensive insight into the digester microbial ecosystems, both qualitative and quantitative approaches were conducted. Previous studies have been mostly limited to non- or partially quantitative approaches that potentially lead to difficulties in comparing community structures between different ecosystems. As the functional attributes of an anaerobic digester should be related to the abundance as well as the diversity of microorganisms working in the system (Akarsubasi et al., 2005), it is desirable to have both types of information for reliable linking between microbial and process data. Therefore, microbial communities were analyzed by a combination of denaturing gel gradient electrophoresis (DGGE), a qualitative technique, and real-time polymerase chain reaction (PCR), a quantitative technique. Shifts in microbial community structure over cycles were visualized using non-metric multidimensional scaling (NMS). To our knowledge, this is a rare study reporting qualitative and quantitative changes in microbial community structure over cycles in anaerobic repeated-batch reactors.

2. Methods

2.1. Bioreactor operation

Two identical completely mixed tank reactors of a 10-L working volume were operated under anaerobic conditions in repeated-batch mode to treat whey permeate, a high-strength organic wastewater produced from cheese-processing plants. Diluted whey permeate (5 g/L as soluble chemical oxygen demand (SCOD)) was used for the reactor experiments. The soluble to total COD ratio of the prepared wastewater was 93%, indicating that the organic compounds in the feed are mostly soluble. Carbohydrate (mostly lactose) was the major organic compound that contributed 80% of the substrate SCOD. More detailed physicochemical characteristics of the feed can be found in a previous publication (Lee et al., 2010). Whey permeate contains most of the essential nutrients for microbial growth and it has been frequently treated by anaerobic systems with no supplements added. Two reactors were respectively started up with different anaerobic sludges from two separate full-scale digesters: one treating domestic sewage sludge (D1) and the other treating industrial sewage sludge (D2). The reactor seeded with D1 sludge was designated R1 and the one with D2 sludge was designated R2. The seeding ratio was 1% (v/v) for both reactors. Microbial community compositions in D1 and D2 sludges analyzed by real-time PCR the 16S rRNA gene are given in Supplementary Fig. 1. The hydrogenotrophic order Methanomicrobiales, followed by the aceticlastic family Methanosaetaceae, was found to be the most abundant methanogen groups in both sludges, whereas the total population of methanogens was about 3-fold greater in D2 sludge than in D1 sludge. Complete anaerobic treatment (no more biogas production; residual SCOD <100 mg/L) was repeated for 3 cycles over a period of 72 days including starvation periods. Between each cycle, feed concentration (5 g SCOD/L) and working volume (10 L) were replenished to the initial levels. Throughout the reactor operation, temperature was maintained at 35 ± 1 °C and pH was kept over 7.0 with 6 N NaOH. Each reactor was monitored during the operation by sampling the mixed liquor at intervals of several hours to days (shorter in the early period for fast acidogenesis). Sampling periods were determined by the progress of reaction. Sampling was carried out in duplicate (2 × 25 mL) at each measuring point.

2.2. DNA extraction

Total DNA was extracted from reactor samples using an automated nucleic acid extractor (MagNa Pure Compact, Roche, Mannheim, Germany) according to the manufacturer's instructions. One milliliter of a sample was centrifuged at 12,000g for 5 min and the spun-down pellet was washed by repeated centrifuging (1-min × 12,000g), decanting (900 μL supernatant), and resuspending (in 1 mL distilled water) to remove cell debris and possible PCR inhibitors. A 100-μL portion of the suspension was loaded on the extractor with the MagNa Pure Compact Nucleic Acid Isolation Kit I (Roche). The purified DNA was eluted in 200 μL of elution buffer and stored at −20 °C until subsequent analysis. DNA was extracted in duplicate from each reactor sample.

2.3. Real-time PCR analysis

Real-time PCR was conducted using a LightCycler 480 machine (Roche) with six 16S rRNA gene-specific primers/probe sets targeting different microbial groups (Supplementary Table 1; Yu et al., 2005): the domain Bacteria, the order Methanobacteriales, the order Methanococcales, the order Methanomicrobiales, the family Methanosarcinaceae, and the family Methanosaetaceae. A 20-μL reaction mixture was prepared using the LightCycler 480 Probes Master kit (Roche) and amplified using a two-step thermal cycling procedure as previously described (Bialek et al., 2011). A quantitative standard curve was constructed for each primers/probe sets as previously described (O'Reilly et al., 2010). Standard plasmids carrying the nearly full-length 16S rRNA gene sequences of the representative target strains of the primers/probe sets were generously provided by Prof. S. Hwang, POSTECH, Korea, Rep. For each set, an