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Bioreactor performance and methanogenic population dynamics in a low-temperature (5–18 °C) anaerobic fixed-bed reactor

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ABSTRACT

The effect of temperature on the functionality of microbial community structure in a low temperature, anaerobic fixed-bed reactor was studied by decreasing the operating temperature from 18 °C to 5 °C. The reactor was productive within 20 days and produced stable methane content in biogas (above 77%) throughout the trial period. At 17 °C and 15 °C, chemical oxygen demand (COD) removal efficiency and biogas production of reactor were significantly reduced. These might be temperature thresholds when fixed-bed reactors are operated under low temperatures. The methanogen community composition was analyzed using 16S rRNA gene clone library screening and quantitative PCR. At low ambient temperatures, Methanomicrobiales were dominant methanogens, and they preferentially adhered to the carbon fiber carrier. The results indicated that 16S rRNA levels of Methanomicrobiales and Methanosaetaceae in adhering sludge were higher than in deposited sludge, and they all contributed to the efficient performance of the fixed-bed reactor at low operating temperatures.

1. Introduction

Anaerobic digestion is a stable and proven technology for highly efficient treatment of various types of wastewater. However, most full-scale anaerobic digesters are operated at mesophilic (24–45 °C) or thermophilic (45–65 °C) temperature ranges (McHugh et al., 2006; Ueno et al., 2007). Because of poor substrate-utilization rates, low biogas production, low microbial activity, increased gas solubility, and liquid viscosity under low-temperature conditions, psychrophilic anaerobic digestion has not been considered feasible (Kettunen and Rintala, 1997; Lawrence and McCarty, 1969; Lettinga et al., 1999; Lin et al., 1987). However, most domestic and industrial effluent is discharged at low ambient temperatures (⩽18 °C) (Connaughton et al., 2006). Heating of the wastewater for mesophilic or thermophilic anaerobic digestion increases energy consumption and decreases cost-efficiency, thus resulting in a marginal or negative overall energy yield.

In the present study, the feasibility of improved low-temperature anaerobic digestion was studied using cold-adapted sludge in modified upflow anaerobic sludge blanket (UASB), internal circulation (IC), and expanded granular sludge bed (EGSB) bioreactor systems for anaerobic treatment of synthetic and real wastewater at low-temperature (Akila and Chandra, 2007; Alvarez et al., 2006; McHugh et al., 2004; Rebac et al., 1995). Previous studies have reported on bioreactor performance and dynamic transitions in the microbial communities in improved reactors. They indicated that Methanomicrobiales populations are significantly involved in the mechanism of psychrophilic biomass granulation and noted a distinct shift from acetoclastic methanogenic dominance to hydrogenotrophic dominance in anaerobic reactors in response to a decrease in the operating temperature (Connaughton et al., 2006; McHugh et al., 2004; O'Reilly et al., 2009, 2010). Methanomicrobiales-related populations are therefore likely to play an important role in low-temperature anaerobic granular sludge systems or digestion under psychrophilic conditions, where hydrogenotrophic methanogenesis is the main pathway for methane production. Previous study reported that Methanomicrobiales preferentially adhere to the active carbon fiber carrier. In organic wastewater treatment, Methanomicrobiales-related populations are likely to be the dominant methanogens in fixed-bed reactors packed with active carbon fiber as the biofilm carrier (Zhang et al., 2011). Fixed-bed reactors may develop biofilms formed by sludge adherence to a supporting material, producing higher activity than suspended sludge (Arnaiz et al., 2006; Chen et al., 2007); hence, microbial activity is very high in fixed-bed systems.

The characteristics of Methanomicrobiales bacteria and fixed-bed systems might result in good performance of fixed-bed reactors at low ambient temperatures. Because there are few studies examining the operational performance and microbial dynamics of fixed-bed reactor treatment of organic wastewater under

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low-temperature conditions, it is difficult to evaluate the feasibility of this kind reactor. Greater insight into the performance of and population communities in fixed-bed reactors under low-temperature conditions is required to answer critical questions, such as whether a fixed-bed reactor packed with active carbon fiber can be effectively operated at low temperatures and how low temperature affect the biogas methane content.

The aim of this work was to monitor the effect of lower temperatures on wastewater treatment and microbial population community composition in a fixed-bed reactor with carbon fiber textiles. A fixed-bed reactor with an effective volume of 10 L was packed with active carbon fiber biofilm and used to treat molasses wastewater. The reactor was started under low-temperature conditions (18 °C), and the operating temperature was gradually decreased to 5 °C. The composition of the microbial population was investigated using 16S rRNA gene clone library screening and quantitative PCR during the 78-day operation period.

2. Methods

2.1. Bioreactor

A fixed-bed reactor packed with active carbon fiber (Japan Carbon Company, Tokyo, Japan) as the biofilm carrier was anaerobically operated with molasses wastewater (Fig. 1). The bioreactor was constructed from 10 mm thick synthetic glass, and the effective volumes was 10 L, with an outside diameter of 24 cm and a height of 27 cm. Six cylindrical carbon fiber textiles (inside diameter: 5.5 cm; height: 30 cm; thickness: 2 mm) bundled together by stainless steel wire were placed into the reactor as biofilm carriers. Molasses wastewater was pumped into the reactor using a peristaltic pump. Biogas was collected via a porthole located at the top of the reactor and measured using the water displacement method under standard conditions. Approximately 30–50 mL of effluent was periodically sampled from the reactor. During the experiment, bioreactor effluent and biogas were routinely sampled for COD, pH and methane (CH_4) content. The COD was determined using a water quality monitor (Lovibond 99731COD, Germany), pH was measured using a Horiba Compact pH meter (Model B-212, Japan), and CH_4 content was determined using biogas analyzer (Model ADG, Landtec, USA). Sampling for microbial analyzes was carried out on days 0, 20 and 78. The bioreactor was placed into a biochemical incubator (Model MIR 254, Sanyo, Japan) to control the operating temperature.

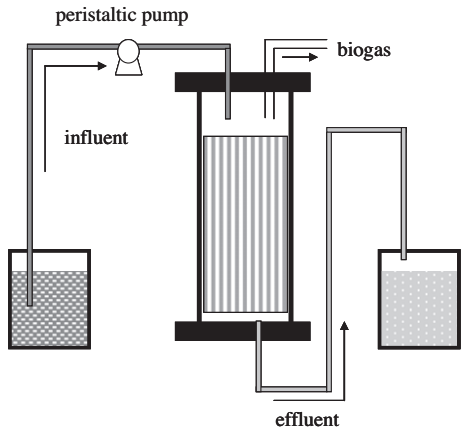

Fig. 1. Schematic diagrams of the fixed-bed reactor.

2.2. Feeding solution and seed sludge

Artificial wastewater was composed of 10 L of molasses (sag: 70%; brix: 45%), 800 g commercial cat food (Whiskas, Beijing, China), and 90 L of tap water, and its COD was about 100,000 mg/L. The wastewater was then diluted with water to the COD concentration required for the experiment, and the ratio of COD:N:P was maintained at 300–500:5:1 to supply microorganisms with adequate nitrogen and phosphorus.

The reactor was inoculated with 4 L mesophilically-grown granular sludge obtained from a full-scale treatment plant for Coca-Cola production wastewater. The total solid (TS) and volatile solid (VS) content of the inoculated sludge were 33.63% and 6.16%, respectively. After inoculation, the TS and VS content of sludge adhering to the carbon fiber and deposited fraction were 12.90%, 10.01% (TS) and 12.17%, 8.85% (VS), respectively. The values at 5 °C were 10.21%, 8.88% (TS) and 8.20%, 7.54% (VS), respectively.

2.3. Experimental procedure

The reactor was operated with molasses wastewater as the major carbon source for microbial growth, at a constant influent COD concentration of 5000 mg/L. The reactor was operated with a constant hydraulic retention time (HRT) and varying operating temperature (Table 1). The system was initialized at an operating temperature of 18 °C at a HRT of approximately two days. The temperature was only decreased when steady state conditions were obtained for the existing loading conditions and biogas production rate. The pH of the molasses wastewater was maintained at 7.0 ± 0.2 by automatic titration with 5 N NaOH.

2.4. DNA extraction and conventional PCR

Granular sludge samples were collected from the fixed-bed reactor on days 0, 20 and 78. The sludge adhering on the carbon fiber biofilm and the deposited sludge at the bottom of the reactor were sampled separately. The deposited sludge sampling ports were located on the bottom of reactor. Three sheets (15 mm × 30 mm × 2 mm) of active carbon fiber were retrieved directly from the reactor and the adhering biomass was collected (designated as adhering sludge). After 20 days of operation, a difference between the nature of the adhering sludge and deposited sludge was observed. Because of the low temperature operating environment, the bioreactor was filled with slurry like sludge deposited at the bottom of reactor (deposited sludge). In contrast, the adhering sludge contained granules which ranged in size between 2 mm and 5 mm. Even in the 5 °C operating environment, the adhering sludge still presented a dense, well-settling, spherical granular morphotype and maintained a thickness of 4 to 5 mm.

Table 1
Operation parameters of the fixed-bed reactor.

Operation stage	HRT[a]	T (°C)	Influent pH	Effluent pH	OLR[b]
1st stage (1–20 days)	2	18 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
2nd stage (21–26 days)	2	17 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
3rd stage (27–32 days)	2	16 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
4th stage (33–38 days)	2	15 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
5th stage (39–44 days)	2	13 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
6th stage (45–50 days)	2	11 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
7th stage (51–56 days)	2	9 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
8th stage (57–62 days)	2	7 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
9th stage (63–72 days)	2	5 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	2.5
10th stage (73–78 days)	2	5 ± 0.2	7.0 ± 0.2	6.8 ± 0.1	1.0

[a] Hydraulic retention time (days).
[b] Organic loading rate (kg COD m^{-3} day^{-1}).