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Organic loading rate shock impact on operation and microbial communities in different anaerobic fixed-bed reactors

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HIGHLIGHTS

- Stepwise increase of OLR concentration for four anaerobic reactors impact ability and threshold.
- Fixed-bed anaerobic reactor biological parameter dynamics using quantitative methods.
- Main bacteria functional groups identified as bacteroidetes bactericum and uncultured chloroflexi bacterium.
- Main archaea functional groups identified as methanomicrobiales.

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ABSTRACT

For the fixed-bed reactors in this experiment, during 40 days of stable operation and under different organic loading shocks, biogas production remained stable at 21 L, effluent pH remained between 6.8 and 7.5, and chemical oxygen demand (COD) removal efficiency and the biogas methane content were greater than 80% and 75%, respectively. The community was analyzed using denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene clone library screening, and quantitative PCR. Findings revealed that bacteria and methanogenic archaea were typically dominant in the adhering sludge. Methanomicrobiales was identified in carbon fiber carriers, they were breeding slowly, and attached easily. The 16S rRNA gene concentration of methanogenic archaea was higher in the adhering sludge than in the deposited sludge. Our results indicated that the colonization of the microorganism played a very important role in the carbon fiber carriers, as well as in the improvement of sludge activity and the shock resistance of the reactor.

1. Introduction

Anaerobic biological treatment technology has been the best choice for treating high-strength wastewater with high efficiency and low consumption in the wastewater treatment process. However, the anaerobic microorganism generation cycle is long and possesses a slow rate of proliferation, and the reactor start-up requires a relatively long time. On the other hand, current anaerobic fermentation technology possesses myriad problems, such as low biogas production rate and high investment cost (Ren and Wang, 2004; Sasaki et al., 2009). Therefore, it would mark a significant turning point for high strength wastewater treatment processes, if certain technological conditions could be met: a reactor with a quick start up and high efficiency; a reactor in which operating conditions were effectively controlled; and a reactor that could produce optimal start-up and gas production conditions. This will be major turning point for high concentration wastewater treatment processes.

Start-up constitutes an important step in the anaerobic biological treatment process. It has been reported that a poor start-up in a biological treatment system could lead to the ineffective removal of soluble nutrients and organic matters, or to a prolonged period of acclimation (Sowmeyan and Swaminathan, 2008; Wu et al., 2001). A successful start-up can be confirmed by the establishment of a stable microbial community, and is also related to HRT (hydraulic retention time), SRT (sludge retention time), and inoculated sludge sources. Studies have shown that a variety of carrier materials, including sand, waste tires, zeolite, glass beads, polyurethane foam, etc., can facilitate microorganism attachment on a carrier to form a biofilm system; these materials can also facilitate an enrichment of the diversity of the microbial community, and greatly improve the density of the active flora within the reactor (Garcia Calderon et al., 1998; Heinen and Lauwers, 1990; Sowmeyan and Swaminathan, 2008). On the other hand, biofilm attachment on the carrier allows for a longer average residence

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time (SRT), which provides beneficial acclimation conditions for slow-growing microbes. Sludge does not expand on the biomembrane and sludge wash out is reduced, which could improve the stability and shock resistance of the reactor to less advantageous environmental conditions (De Vrieze et al., 2012; Lin et al., 2009a). Anaerobic fixed-bed reactors with carbon fiber biofilm and with high shock resistance are better and produce a higher methane yield; this is because the methanomirobiales-related populations likely play an important role in anaerobic granular sludge systems or digestion under shock conditions, where hydrogenotrophic methanogenesis is the main pathway for methane production (Zhang et al., 2011, 2012). The microbe activity and shock resistance of an anaerobic fixed-bed reactor would likely be higher than those of other technologies. However, the effects of different OLR shocks on microbial communities for this type of reactor have not reported up to the present.

The aim of this work was to monitor the effects of different OLR shocks on reactor operation and microbial communities in a fixed-bed reactor. 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 effect of the reactor was investigated using the COD removal efficiency, biogas production, and methane concentration. The composition of the microbial population was investigated during the 40-day operation period using denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene clone library screening, and quantitative PCR technology.

2. Methods

2.1. Bioreactor

A fixed-bed reactor packed with a biofilm carrier of active carbon fiber (Japan Carbon Company, Tokyo, Japan) was anaerobically operated with molasses wastewater. The bioreactor was constructed from 10-mm-thick synthetic glass, and the effective volume was 10 L. The outside diameter was 24 cm and the height was 30 cm. Six cylindrical carbon fiber textiles (inside diameter: 5.5 cm; height: 27 cm; thickness: 2 mm), bundled together with 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, VFA, pH, and methane (CH_4) content. The COD was determined using a water quality monitor (Lovibond 99731COD, Germany); VFA concentration was measured using High Performance Liquid Chromatography (LC–MS2020, Japan); pH was measured using a Horiba Compact pH meter (Model B-212, Japan); and CH_4 content was determined using a biogas analyzer (Model ADG, Landtec, USA). The bioreactor was placed into a biochemical incubator (Model MIR 254, Sanyo, Japan) to control the operating temperature (Zhang et al., 2012).

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 100,000 mg/L. The wastewater was 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 meshophilically-grown granular sludge obtained from a full-scale treatment plant for Coca-cola production wastewater. The total solid (TS) and volatile solid (VS) contents of the inoculated sludge were 33.63% and 6.16%, respectively.

2.3. Experiment procedure

The reactor was operated at a constant temperature of 35 °C with molasses wastewater as the major carbon source for microbial growth. The COD and hydraulic retention time (HRT) were varied for the four reactors (Fig. 1). For R_1 and R_2, the system was initialized using a COD concentration of 5000 mg/L at an HRT of 3 days, and for R_3 and R_4, the system was initialized using a COD concentration of 5000 mg/L at an HRT of 7 days. For R_1 and R_2, the reactor was operated with a constant HRT (3 days), and the COD was increased 5000 mg/L every 10 days and 5 days, respectively. For R_3 and R_4, the reactor was operated with a decreasing HRT every 7 days, and the COD was increased 1000 mg/L every 2 days and 1 day, respectively. At the end of the operation period, the OLR of the four reactors was 6.7 kg/m^3 d, when steady state conditions were obtained for the existing loading conditions and biogas production. 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 and 40. The sludge adhering to 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 the 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 40 days of operation, a difference could be observed between the OLR shock of the adhering sludge and that of the deposited sludge. The deposited sludge was directly sampled at the bottom of reactor, and the adhering sludge maintained a thickness of approximately 5 mm.

The granular sludge samples were centrifuged at 8000 rpm for 10 min, and the supernatant was decanted carefully to obtain the sediment sample (0.3 g net weight) for DNA extraction. The VS concentration of each sediment sample was measured to estimate the amount of biomass used for DNA extraction. Genomic DNA was extracted using an automated nucleic acid extractor (Bioteke Biotech Co., Ltd., Beijing, China) for use as the PCR template. The

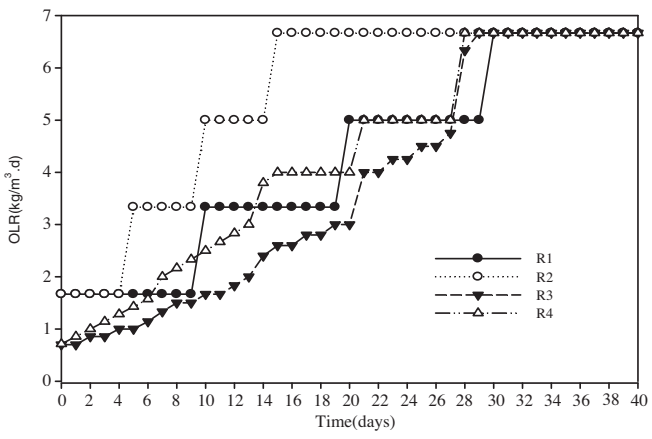

Fig. 1. The operation parameters of fixed-bed reactor.