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Optimization of illumination time for the production of methane using carbon felt fluidized bed bioreactor in thermophilic anaerobic digestion

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ABSTRACT

This paper reports the effects of illumination on methanogenic performance using acetate synthetic wastewater as the organic substrate. Several different illumination times such as 0 (continuous darkness), 15, 30, 45, 60, 90, 150, 240 and 480 min/day and continuous light were examined in a laboratory set-up under batch conditions. The reactors, when exposed to short illumination times such as 60 min/day, showed the greatest methane concentration, methane yield, and dissolved organic carbon (DOC) removal, which were 78.5%, 176 ml/g-VS, and 75.8%, respectively, in comparison to other conditions. Furthermore, the performances of three fluidized bed reactors operated in semi-continuous mode under conditions of continuous darkness, 60 min/day illumination, and continuous illumination were investigated. The methane yield and DOC removal was 794 ml/g-DOC added, 893 ml/g-DOC added, and 596 ml/g-DOC added, and 88.3%, 93.7%, and 60.5%, respectively. In the present investigation, illumination for 60 min/day for microbial treatment was found to be more effective than either continuous darkness or continuous illumination. Molecular and microscopic study revealed that the major methanogens in the bed reactors belonged to the genus *Methanosarcina* sp. Immobilized cells from the short-time illumination and continuous darkness reactors show a smooth surface membrane. However, an uneven surface membrane and destruction of cell tissue were observed in the reactor with continuous illumination.

1. Introduction

Most conventional systems of anaerobic digestion are operated under conditions of continuous darkness. Use of the lighted upflow anaerobic sludge blanket (LUASB) method under mesophilic conditions has been studied [1]. Phototrophic bacteria that grew in the LUASB reactor removed ammonium and phosphate in the light; however, methane production was lower than that in continuous darkness. Phototrophic bacteria using electron donors competed with methanogens, leading to a decreased methane yield. In contrast, photoenhancement by incandescent lighting of the methane production process from thermophilic anaerobic digestion was reported by Tada and Sawayama [2]. After operation for 10 days, the volume of methane produced from reactors using continuous illumination was higher than that from unilluminated reactors. No bacteriochlorophyll was detected in the effluent of either the illuminated or unilluminated reactor. Phototrophic bacteria did not grow in the light thermophilic (55 °C) reactor, unlike in the mesophilic LUASB reactor [2]. They also compared the carbon balance between light and dark conditions and found that more methane was produced from hydrogen and dioxide in the illuminated reactors than that produced from the unilluminated reactors. However, similar 16S rRNA gene copy numbers for hydrogenotrophic *Methanothermobacter* sp. were detected under both the illuminated and unilluminated reactors [3]. Furthermore, with acetate feeding, methane production from the illuminated reactor was not accelerated and remained lower than that from the unilluminated reactor [3]. In an anaerobic digester, methane made from acetate usually accounts for 60–80% of the total [4]. There have been few studies of methane production from anaerobic digestion under illumination, especially regarding optimum light conditions. The performance of methane production was investigated at different illumination times using acetate as the sole carbon substrate. Therefore, a study of the effective and appropriate illumination time for anaerobic digestion would be of great interest for industrial applications as

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well as for academic research. Carbon felt has a high specific surface area and porosity. It appears to be an excellent colonization matrix and to have good endurance for anaerobic filter reactors [5]. In the present study, an anaerobic fluidized bed reactor packed with carbon felt was used in semi-continuous mode for acetate synthetic substrate removal under thermophilic anaerobic conditions.

This paper reports on an effective and appropriate illumination time for activating methanogens for the photoenhancement of methane production. Then thermophilic (55 °C) anaerobic digestion condition was used in the present research. Molecular and microscopic studies were used to understand the methanogenic community under different illumination conditions.

2. Materials and methods

2.1. Batch reactor operation

Ten tube-shaped glass bottles (500 ml) containing a stir bar were used as reactors. They were set in an incubator with the temperature on 55 °C. A 400 ml mixture of thermophilic methanogenic sludge (20%, w/w) from a cattle waste treatment plant (Kyoto, Japan) and a synthetic medium (80%, w/w) containing acetate (2 g/l), yeast extract (300 mg/l), NH_4Cl (200 mg/l), KH_2PO_4 (16 mg/l), and a trace mineral solution (200 ml/l) was first added to each reactor. The chemical composition of the trace mineral solution was that described in Yang's paper [6]. Nitrogen gas was added to exclude oxygen from the reactors. A gas sampling syringe was connected to the reactors for measuring the biogas yield. The reactors were operated in batch mode for 10 days and the temperature was maintained at 55 °C under constant stirring.

The reactors were regularly illuminated for 15, 30, 45, 60, 90, 150, 240, and 480 min/day and under continuous illumination. A control operation was performed on unilluminated reactors. The unilluminated reactors were wrapped entirely in aluminum foil to keep out all light. The reactors were illuminated by four 60-W incandescent lamps (LW110V60W, Mitsubishi Osram, Tokyo, Japan) which were evenly placed 7 cm from the incandescent lamps at a light intensity of 100 ± 10 μmol m^{-2} s^{-1}. The light intensity was measured with a light meter LI-250 (LI-COR, Lincoln, NE).

2.2. Semi-continuous operation

In this operation, three reactors were set up. Each contained carbon felt (Japan Carbon Company, Tokyo, Japan), which was used for microbial immobilization. The reactor was a 500-ml glass vessel containing 20 pieces of carbon felt (1 cm × 1 cm × 0.5 cm) with the same working volume (about 2.5%, v/v) as a fluidized bed reactor. A 400 ml mixture of thermophilic methanogenic sludge (20%, w/w) from a cattle waste treatment plant (Kyoto, Japan) and a synthetic medium (80%, w/w) was added to each reactor. The composition of the medium was similar to that of the previous batch test. One of the reactors was illuminated for 60 min/day and the other was illuminated continuously. The third reactor was used as a control and was not illuminated. After incubation for 20 days, the reactors were operated in semi-continuous mode for a hydraulic retention time of 12 days; the temperature was maintained at 55 °C until day 59.

2.3. Analyses

Portions of each of the reactor contents were sampled. The effluent was centrifuged at 10,000 rpm for 10 min to allow precipitation of the microbes. The supernatant was used to measure the dissolved organic carbon (DOC) with a TOC analyzer (TOC-5000A, Shimadzu, Kyoto, Japan). Biogas production and the reactor pH were measured. The composition of the biogas was determined using a gas chromatograph (GC-8A, Shimadzu) with a thermal conductivity detector equipped with a steel Porapak Q column (Shinwakakou, Kyoto, Japan) at 90 °C. All samples were measured in duplicate.

The microbes present in the carbon felt were observed through a scanning electron microscope (SEM) (DS-720, Topcon, Tokyo, Japan). Under no illumination, 60 min illumination per day, and continuous illumination, three parts of the attached bed material in each reactor were removed and the cells washed with buffer solution (pH 7.0). Samples were prepared for SEM according to Yang et al. [6].

2.4. Phylogenetic analysis of 16S rRNA gene

At the end of the fermentation, the samples were collected from three different pieces of the carbon felt from the three different reactors. All the samples were collected in triplicate. DNA was extracted by using a Fast DNA Spin Kit for Soil (Qbiogene, Carlsbad, CA, USA) and a Fast Prep FP120 instrument (Qbiogene) according to the manufacturer's instructions. The archaeal 16S rRNA gene sequences were amplified by polymerase chain reaction (PCR) with the primer set S-P-MArch-0348-S-a-17 (5′-GYGCAGCAGGCGCGAAA-3′) and S-D-Arch-0786-A-a-20 (5′-GGACTACVSGGGTATCTAAT-3′) [7,8]. The PCR reaction was performed with a program in which cycling consisted of 20 cycles each of 1 min at 95 °C, 1 min at 55 °C, and 2 min at 72 °C.

The PCR product was purified in a Microspin S-400 HR column (Amersham Biosciences, Piscataway, NJ, USA) and cloned with a TA Cloning Kit (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's instructions. The cloned DNA was sequenced using a dRhodamin Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and an automated sequence analyzer (Model 377; PerkinElmer Applied Biosystems). The sequences (417 bp) of 20 DNA clones were determined and the determined sequences were compared to known 16S rRNA sequences by a nucleotide–nucleotide BLAST search [9]. The best matching sequences were selected from the results.

3. Results and discussion

3.1. Batch operation

Fig. 1 shows the cumulative biogas production from the thermophilic anaerobic digestion of the acetate synthetic medium with various illumination times by incandescent lighting after 10 days of incubation. It was shown that the illumination times of 0 (dark),

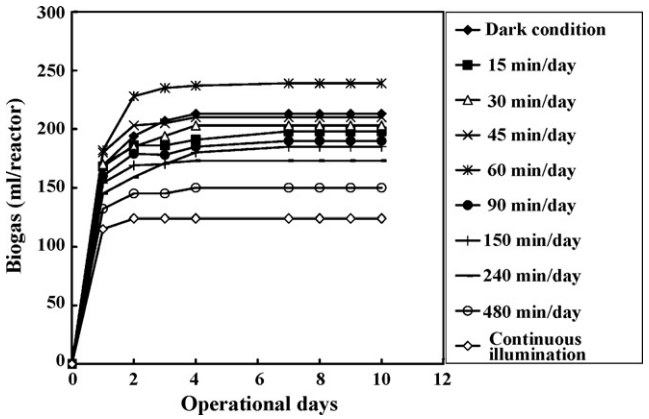

Fig. 1. Cumulative biogas production from the thermophilic anaerobic digestion of acetate synthetic medium with various illumination times of incandescent lighting after 10 days of incubation.