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Effect of adding carbon fiber textiles to methanogenic bioreactors used to treat an artificial garbage slurry

Kengo Sasaki,[1] Masahiko Morita,[1,*] Shin-ichi Hirano,[1] Naoya Ohmura,[1] and Yasuo Igarashi[2]

Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), 1646 Abiko, Abiko-shi, Chiba 270-1194, Japan[1] *and Department of Biotechnology, Graduate School of Agricultural and Life Science, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan*[2]

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To compare the performances and microbial populations of methanogenic reactors with and without carbon fiber textiles (CFT), we operated small-scale (200 ml) reactors using a slurry of artificial garbage. For both types of reactors, the organic loading rate (OLR) was stepwisely and rapidly increased in the same manner. Start-up period was shortened by adding CFT. Reactors with CFT showed greater efficiency for removal of suspended solid and volatile suspended solid than reactors without CFT at a long hydraulic retention time (HRT) between 8 and 13 days. The reactors with CFT maintained stable methane production at an OLR of 15.3 g dichromate chemical oxygen demand (CODcr)/l/day and DNAs from microorganisms were highly concentrated in adhering fractions on CFT. As shown by quantitative PCR analysis, the proportions of methanogenic archaea were conserved more than 25% in adhering fractions on CFT in reactors with CFT. By contrast, reactors without CFT showed accumulation of volatile fatty acid and deteriorated at an OLR of 2.4 gCODcr/l/day. Methanogenic proportions dropped to 17.1% in suspended fractions of reactors without CFT. Denaturing gradient gel electrophoresis (DGGE) analysis revealed that all archaeal DGGE bands in both types of reactors were related to methanogens, but more bands were observed in reactors with CFT. Thus the higher performance of reactors with CFT likely reflects the greater abundance of microorganisms and methanogenic diversity.

[**Key words:** Packed-bed reactor; Carbon fiber textiles; Organic solid waste; Methane fermentation; Methanogen]

We are currently disposing of the huge amounts of organic solid wastes by allowing it to decompose in landfills or by incinerating it (1, 2). Because both of these processes cause environmental pollution, alternative systems would be highly desirable. For example, anaerobic microbial digestion is an attractive strategy because it produces a useful energy source in the form of methane, it is cost-effective, and it has only a limited environmental impact (3–5). In fact, methane fermentation is already being widely used for the treatment of wastewater having no or low solid content. In addition, anaerobic process for the treatment of organic solid waste is increasingly being studied and applied for methane production (6).

The up-flow anaerobic sludge blanket (UASB) process is one of the most popular high performance reactors (3); however, UASB has not yet been used for waste with substantial solid content (7). On the other hand, fixed-bed or packed-bed systems using various types of supporting materials also have been used to treat wastewaters (8, 9). In these systems, packing support materials are added to facilitate retention of microorganisms (10). For instance, carbon fiber textiles (CFT) have proved advantageous for decomposition of artificial liquid medium and enabled successful operation at a high organic loading rate (OLR) (11).

To achieve larger OLRs and obtain larger biogas yields from the organic solid waste, two-stage (comprised of hydrolysis/acidogenesis and methanogenesis processes) or packed-bed systems (single methanogenesis process) have been exploited (7, 12–14). Although the microbial community of the packed-bed reactor with CFT and this degradation of organic solid waste have been characterized (7, 15), a direct performance comparison between reactors with and without CFT, taking into consideration such parameters as degradation rate, gas production, stability of the operation, and the microbial community, has not been carried out. Consequently, the merit of adding CFT was within the limit of the speculation. In addition, there has been little evaluation of the support materials used in the treatment of solid waste (16).

The aim of the present study, therefore, is to assess the effect of adding CFT to the methane fermentation process for degrading organic solid materials. To accomplish that aim, we constructed small-scale (200 ml) reactors and compared the performances and microbial populations in anaerobic bioreactors with and without CFT using an artificial garbage slurry (AGS) as a model of organic solid waste. OLRs of both types of reactors were stepwisely and rapidly raised in the same manner, under the same operational conditions.

MATERIALS AND METHODS

Reactor operation Seed cultures were collected from a thermophilic anaerobic digester (Kajima Corporation, Chofu-shi, Tokyo, Japan), in which stable gas production

* Corresponding author. Tel.: +81 (0)4 7182 1181; fax: +81 (0)4 7183 3347.
E-mail address: masahiko@criepi.denken.or.jp (M. Morita).

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from garbage slurry was being observed. The seed cultures were inoculated into eight media bottles (250 ml, Duran, Germany) that were then sealed with a plug and cap. Two types of reactors, with and without support material, were prepared. Three of eight bottles were packed with two sheets of CFT (type; pitch, porosity; about 98%, diameter; 30.0 mm, height; 70.0 mm, thickness; 2.4 mm) as support materials. The initial anaerobic conditions were established by replacing the gas phase with nitrogen gas. All reactors had a working volume of 200 ml and were cultivated at 55 °C.

The AGS contained (g/l in distilled water) commercial dog food (Vita-one, Nihon Pet Food, Tokyo, Japan), 20 or 40; KH_2PO_4, 1.1; and K_2HPO_4, 1.7. To enable methanogens to have high activity, $NiCl_2.6H_2O$ and $CoCl_2.6H_2O$ were added to give Ni^{2+} and Co^{2+} concentrations of 0.10 and 0.12 mg/l, respectively (17). The characteristics of the AGS containing 2% dog food were as follows: dichromate chemical oxygen demand (CODcr), 19.0 gCODcr/l; suspended solid (SS), 10.7 g/l; volatile suspended solid (VSS), 9.6 g/l; the ammonia + ammonium (NH_3–N) concentration, 9.0 mg-N/l. The pH of the AGS was adjusted to 7.5 with NaOH. The cultivation was carried out as follows: every 24 h, a predetermined amount of AGS in the reactor was discharged, and the same amount of fresh AGS was added. Inside the reactor, AGS was mixed using a magnetic stirrer, and the pH was maintained between 6.8 and 8.0. Figs. 1A and B summarize the time schedule for changes of OLR and the hydraulic retention time (HRT), respectively. OLR and HRT were defined as follows:

$$\text{OLR}\quad(\text{gCODcr}/1/\text{day}) = \text{daily loaded CODcr}$$

$$\text{HRT}\quad(\text{day}) = \text{working volume}\quad(1)/\text{replaced volume}\quad(1/\text{day})$$

OLR was gradually increased by reducing the HRT. The dog food concentration in the AGS was 2% until an OLR of 3.1 gCODcr/l/day was reached, after which the dog food concentration was increased to 4%. All reactors were initially operated at an OLR of 0.9 gCODcr/l/day until the rate of methane production had increased to more than 100 ml/l/day. The OLRs of three reactors packed with CFT were raised after fluctuation in the rate of gas production had declined to under ±8%. The same criterion was used for scheduling the increases in the OLRs of the five reactors without CFT until an OLR of 1.9 gCODcr/l/day was reached. However, due to deterioration of the reactors without CFT at an OLR of 2.4 gCODcr/l/day, the OLRs of two reactors were stopped at that point; and those of the remaining three reactors were stopped at 1.9 gCODcr/l/day.

Analyses Gas production was monitored using the water displacement method. The broth in the reactor was periodically sampled and analyzed. SS, VSS and CODcr in the samples were analyzed according to the Japanese Industrial Standard K-0102. The

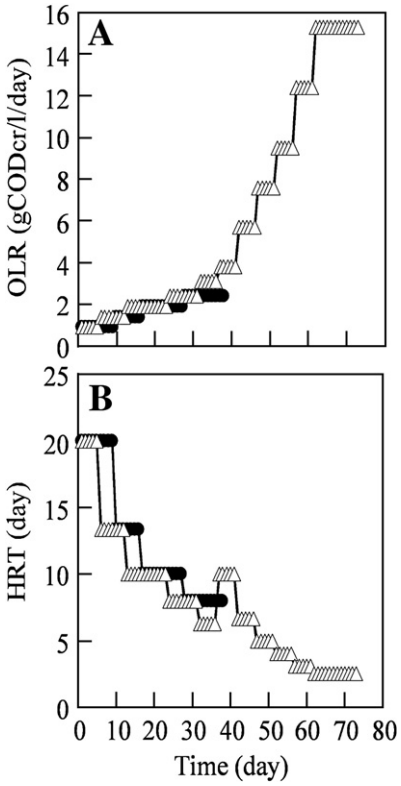

FIG. 1. Time-dependent changes in OLR (A) and HRT (B) in reactors with CFT (open triangles) and reactors without CFT (closed circles).

CODcr removal rate (RR_{COD}) was then calculated as the CODcr at time $[t+\Delta t]$ minus the CODcr at time $[t]$, divided by the elapsed time:

$$RR_{COD}\quad(\text{gCODcr}/1/\text{day}) = (\text{CODcr}_{t+\Delta t} - \text{CODcr}_t)/\Delta t$$

To calculate the CODcr removal efficiency, RR_{COD} was normalized to the OLR as follows:

$$\text{CODcr removal efficiency}\quad(\%) = RR_{COD}\quad(\text{gCODcr}/1/\text{day})/\text{OLR}\quad(\text{gCODcr}/1/\text{day})$$

The rates of SS removal (RR_{SS}) and VSS removal (RR_{VSS}), and the SS and VSS removal efficiencies were calculated in the same manner as the CODcr parameters:

$$RR_{SS}\quad(\text{g}/1/\text{day}) = (\text{SS}_{t+\Delta t} - \text{SS}_t)/\Delta t$$

$$RR_{VSS}\quad(\text{g}/1/\text{day}) = (\text{VSS}_{t+\Delta t} - \text{VSS}_t)/\Delta t$$

$$\text{SS removal efficiency}\quad(\%) = RR_{SS}\quad(\text{g}/1/\text{day})/\text{loaded SS}\quad(\text{g}/1/\text{day})$$

$$\text{VSS removal efficiency}\quad(\%) = RR_{VSS}\quad(\text{g}/1/\text{day})/\text{loaded VSS}\quad(\text{g}/1/\text{day})$$

The volatile fatty acids (VFAs) were determined using a liquid chromatograph (L-2000; HITACHI, Tokyo, Japan) fitted with an organic acid analysis system (TSK-GEL OApak-A,P; TOSOH, Tokyo, Japan). The methane, carbon dioxide, and hydrogen levels in the biogas were measured using a gas chromatograph equipped with a thermal conductivity detector (6890N; Agilent Technologies, Santa Clara, CA, USA) and a packed column of Active Carbon (GL Sciences, Tokyo, Japan). NH_3–N concentrations were measured colorimetrically as previously described (18).

DNA extraction and quantification For the microbial analyses, suspended fraction of the reactors without CFT were sampled on days 27 and 38 (end phase at an OLR of 1.9 and 2.4 gCODcr/l/day, respectively), and suspended and adhering fractions of the reactors with CFT were sampled on days 23 and 73 (end phase at an OLR of 1.9 and 15.3 gCODcr/l/day, respectively). CFTs were collected and vigorously shaken by vortex in PBS buffer, and any biomass still adhering biomass was then scraped off. The microorganisms in the collected effluents and adhering biomass were pelleted by centrifugation and suspended in 500 μl of Tris–EDTA buffer ([pH 8.0], 100 mM Tris–HCl, 40 mM EDTA), after which the DNA was extracted as described previously (19, 20) with slight modifications. The suspensions were collected in 2-ml plastic screw-cap vials containing 1 g of sterile 0.1-mm-diameter zirconium–silica beads with 50 μl of 20% sodium dodecyl sulfate. The mixture was then shaken for 45 s at 5.5 m/s in a FastPrep 100 A instrument (Qbiogene, Tokyo, Japan), and the supernatant was saved after centrifugation. The pellet was then extracted a second time using 500 μl of a phenol–chloroform–isoamyl alcohol (25:24:1) mixture and a second round of bead beating. The DNA was then isolated by phenol–chloroform treatment and RNA digestion as described previously (21). The DNA recovered from each sample was quantified spectrofluorometrically using a fluorescent DNA quantification kit containing the Hoechst 33258 dye (Bio-Rad, Hercules, CA, USA). Before PCR amplification, the extracted DNA was further purified using a QIAamp DNA micro kit (Qiagen, Hilden, Germany).

Quantitative PCR analysis Real-time PCR was performed with the LightCycler 1.5 and LightCycler TaqMan Master (Roche Diagnostics, Tokyo, Japan). The primer set of MArch348F (5′-GYGCAGCAGGCGCGAAA-3′) and March786R (5′-GGACTACVSGGGTATCTAAT-3′), and the double-dye probe of March515F (TGCCAGCMGCCGCGGTAAYACCGGC-3′), were used for measuring the 16S rRNA copy number of methanogenic archaea (22). The primer set of Uni340F (5′-CCTACGGGRBGCASCAG-3′) and Uni806R (5′-GGACTACNNGGGTATCTAAT-3′), and the double-dye probe of Uni516F (5′-TGYCAGCMGCCGCGGTAAHACVNRS-3′) were used for prokaryote (23). All double-dye probes were labeled with FAM at the 5′ end and TAMRA at the 3′ end. The concentrations of each primer and the TaqMan probe were 0.5 and 0.2 μM, respectively. The quantitative measurement by real-time PCR was conducted in triplicate. The real-time PCR amplification was as follows: initial 10 min denaturation (95 °C), followed by 40 cycles consisting of 10 s denaturation (95 °C), 30 s annealing (50 °C), and 1 s elongation (72 °C). The standard RNA genes for methanogenic archaea and prokaryote were plasmids prepared by using the pGEM-T Easy vector system (Promega, Tokyo, Japan) after PCR of *Methanosarcina barkeri* (JCM 10043) with the primer set A21F (5′-TCCGGTTGATCCYGSCRG-3′; *Escherichia coli* positions 8–25) (24) and U1492R (5′-GGYTACCTTGTTACGACTT-3′; *E. coli* positions 1492–1510), and *E. coli* (IAM 1264) with the primer set B8F (5′-AGAGTTTGATCCTGGCTCAG-3′; *E. coli* positions 8–27) and U1492R (25).

PCR and denaturing gradient gel electrophoresis (DGGE) PCR amplification was performed using Ampli Taq Gold (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions for DGGE analysis. The PCR primers used to amplify bacterial 16S rRNA genes were B357F-GC, 5′-*CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGG*CCTACGGGAGGCAGCAG-3′ (*E. coli* positions 341–357), and U517R 5′-ATTACCGCGGCTGCTGG-3′ (*E. coli* positions 517–534) (26). The sequence in italics is a GC clamp. The archaeal 16S rRNA genes were amplified by nested PCR. The primer sets were A21F plus A915R 5′-GTGCTCCCCCGCCAATTCCT-3′ (*E. coli* positions 915–934) (27) for the first PCR and A348IF 5′-GGIGCAICAGGCGCGAAA-3′ (*E. coli* positions 348–365) plus U806IR-GC 5′-*CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCC-GCCCCGGACTACCIGGGTITCTAA-3′* (*E. coli* positions 788–806) (28) for the second. DGGE