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Thermophilic anaerobic co-digestion of garbage, screened swine and dairy cattle manure

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Methane fermentation characteristics of garbage, swine manure (SM), dairy cattle manure (DCM) and mixtures of these wastes were studied. SM and DCM showed much lower volatile total solid (VTS) digestion efficiencies and methane yield than those of garbage. VTS digestion efficiency of SM was significantly increased when it was co-digested with garbage (Garbage: SM = 1:1). Co-digestion of garbage, SM and DCM with respect to the relative quantity of each waste discharged in the Kikuchi (1: 16: 27) and Aso (1: 19: 12) areas indicated that co-digestion with garbage would improve the digestion characteristic of SM and DCM as far as the ratio of DCM in the wastes was maintained below a certain level. When the mixed waste (Garbage: SM: DCM = 1:19:12) was treated using a thermophilic UAF reactor, methanogens responsible for the methane production were *Methanoculleus* and *Methanosarcina* species. Bacterial species in the phylum *Firmicutes* were dominant bacteria responsible for the digestion of these wastes. As the percentage of garbage in the mixed wastes used in this study was low (2–3%) and the digestion efficiency of DCM was obviously improved, the co-digestion of SM and DCM with limited garbage was a prospective method to treat the livestock waste effectively and was an attractive alternative technology for the construction of a sustainable environment and society in stock raising area.

[**Key words:** Anaerobic digestion; Methane fermentation; Swine manure; Dairy cattle manure; Microbial community]

In Japan, approximately 300 million tones of biomass wastes are generated annually. Livestock wastes and garbage account for about 20% and 5%, respectively. Several methods are used for the treatment of livestock waste, including field dispersal, composting, activated sludge and methane fermentation. Most of garbage is treated by incineration and composting, although methane fermentation is also employed in some areas. The use of methane fermentation for the treatment of these organic wastes has gained importance and increased in recent years (1,2), due to some new regulations for the disposal of these wastes and production of electricity from renewable energy sources.

Compared to the quantity of garbage generated, livestock waste is the dominant biomass generated in stock raising area such as the Kyusyu area in Japan. A proper treatment method of these wastes, particularly suited to stock raising areas, is important for sustainable development and environment protection. Methane fermentation seems to be a more suitable method compared to composting, since the demand on the amount of compost is very limited in these areas due to the huge quantity of manure generated. Garbage (3–5) and manure from livestock (6–9) for methane fermentation have been extensively studied. Due to the very large biodegradable organic content of garbage, anaerobic digestion of this waste is very rapid and usually high loading rates with high VTS digestion efficiencies are easily obtained even though treated in full scale (3,10). However, manure is known to have a poor methane yield and it is not common to use manure as a sole substrate for biogas production on a large scale. The biodegradability of cattle manure is low, typically in the range of 30–43% yielding 150–240 l CH_4/kg-volatile solid (VS) (6,7), while swine manure has a slightly higher biodegradability, in the range of 40–69%, potentially giving 280–360 l CH_4/kg-VS (6,9). One of the possibilities to increase the biogas production from manure is to co-digest it with other organic wastes such as garbage from which necessary or inadequate nutrients are provided. Co-digestion is a technology that is increasingly applied for simultaneous treatment of several solid and liquid wastes. The main advantages of this technology are improved methane yield because of the supply of additional nutrients from codigestates and more efficient use of equipment and cost-sharing by processing multiple waste streams in a single facility (11–13). However, the percentage of garbage added for co-digestion reported till now is relatively high, from 10% to nearly 100%. It is not possible and not realizable for most of stock raising areas since the actual quantity of garbage generated in those areas is very limited. The percentage of garbage generated in Kikuchi and Aso area, famous stock raising areas in Kyushu, is only about 2–3%. The effect of garbage on co-digestion should be studied in a relatively low percentage range.

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TABLE 1. Characteristics of wastes used in the study

Parameter	Garbage	SM	DCM	Garbage:SM	Garbage:SM:DCM	
				1:1	1:16:27	1:19:12
TS (g/l)	202.2	62.7	69.8	121.4	68.8	65.5
VTS (g/l)	195.2	49.4	52.7	110.1	54.4	50.0
SS (g/l)	158.4	38.6	47.4	74.2	46.5	37.1
VSS (g/l)	154.4	33.6	36.9	69.9	38.2	34.9
T-COD_{Cr} (mg/l)	N.M.	89,000	71,000	162,000	N.M.	81,000
S-TOC (mg/l)	N.M.	19,451	9726	7825	4505	16,910
S-IC (mg/l)	N.M.	849	1926	158	1700	977
T-VFA (mg/l)	1267	37,145	11,822	9560	8053	31,064
Succinic acid	N.D.	348	N.D.	237	N.D.	256
Lactic acid	N.D.	19,770	N.D.	3202	2087	16,980
Acetic acid	452	7050	9261	3000	3827	7830
Propionic acid	815	3525	1480	1257	1077	2282
Butyric acid	N.D.	3960	418	1869	490	2479
I-valeric acid	N.D.	1046	N.D.	N.D.	275	571
N-valeric acid	N.D.	1446	663	494	N.D.	666
PO_4^{3-} (mg/l)	120	625	50	N.M.	N.M.	463
NH_4^+ (mg/l)	245	1382	1412	N.M.	806	1432
pH (−)	5.3	5.5	7.1	6.1	7.1	6.1
Viscosity (cP)	N.M.	200	4900	N.M.	N.M.	280

SM: screened swine manure; DCM: screened dairy cattle manure; TS: total solid; VTS: volatile total solid; SS, suspended solid; VSS, volatile suspended solid: T-COD, total chemical oxygen demand; S-TOC, soluble total organic carbon; S-IC, soluble inorganic carbon; T-VFA, total volatile fatty acid; N.M., not measured; N.D., not detected. Ni^{2+} and Co^{2+} were added to each waste at final concentrations of 74 μg and 21 μg per g VTS, respectively.

In this study, the methane fermentation of garbage, swine manure (SM), dairy cattle manure (DCM), and mixed wastes of these three kinds of wastes, especially those reflecting the real relative quantity of wastes generated in two representation areas, Kikuchi and Aso, are studied. In addition, the microbial community responsible for the digestion of these mixed wastes is analyzed using 16S rRNA gene clone analysis.

Materials and methods

Garbage, screened swine manure (SM) and screened dairy cattle manure (DCM) Swine and dairy cattle manure were kindly provided by the Mashiki and Kojima farm (Kumamoto, Japan). Synthetic garbage, screened swine and dairy cattle manure were prepared as described (3,9). Synthetic garbage contained fruits, vegetables, meat and fish, and staple foods. These materials were mashed using a mixer and tap water was added to obtain a final total solid (TS) concentration of 20%. Swine manure and dairy cattle manure were screed using a vibrating sieve (opening 2.8 mm) liquid–solid separator. Table 1 shows the representative characteristics of the individual wastes and mixture of them. Ni^{2+} and Co^{2+} were added to the waste to give final concentrations of 74 μg and 21 μg per gram VTS to enhance methane fermentation rate (14,15).

Anaerobic digestion of screened dairy cattle manure (DCM) As the viscosity of DCM was high (4900 cP), we used a continuous stirred tank reactor (CSTR) with a working volume of 1.5 l to study the anaerobic digestion of DCM. Thermophilic anaerobic digestion sludge provided by Nishihara-Syouten Co. Ltd. (Kumamoto) was used as seeds for starting up the reactor. DCM was supplied once a day using the draw-and-fill method. The reactor was maintained at the temperature of 53 °C by circulation of thermostated water through a water jacket. pH in the reactor was kept at 7.5–7.8 by adding 1N HCl solution using a peristaltic pump with a pH controller. The effect of VTS volumetric loading rate was studied by changing the volume of DCM fed into the tank.

Anaerobic digestion of the mixed wastes The digestion characteristics of different mixed wastes were studied under different VTS loading rates using an upflow anaerobic filter (UAF) reactor (working volume, 4 l) as described (16) (Fig. 1). Mixed wastes were fed once a day by the draw-and-fill method. The temperature and pH were maintained at 53 °C and 7.5–7.8, respectively. Four sheets of nonwoven fabric material (each was 5 mm in thickness) were fixed in the reactor and served as the support for microorganisms. Microorganisms accumulated in the support, when the mixed waste with 1:19:12 (wet weight) of Garbage: SM: DCM was treated at a VTS loading rate of 6 g/l·d, were analyzed by 16S rRNA gene clone analysis.

16S rRNA gene clone analysis The nonwoven fabric support from the UFA reactor treating mixed waste GSD (Garbage:SM:DCM = 1:19:12) at the VTS loading rate of 6 g/l·d was sampled and the microbial community attached in the support was studied by 16S rRNA gene clone analysis (17,18). One arachaeal-16S rRNA gene library (GSDA library) and one bacterial-16S rRNA gene library (GSDB library) were constructed using extracted community DNA and 19 clones from GSDA library and 21 clones from GSDB library were sequenced with a CEQ8000 genetic analysis system (Beckman Coulter, Fullerton, CA, USA). The phylogenetic analyses of these clones were carried out as described (16). The operational taxonomic unit (OTU) were designated GSDA01 to GSDA08 for clones of the GSDA library, and GSDB01 to GSDB16 for clones of the GSDB library. The DDBJ/EMBL/GenBank accession numbers for the sequences of OTUs GSDA01 to GSDB16 are AB425245 to AB425268.

Fluorescence *in situ* hybridization (FISH) A small cube (approximately 5 by 5 by 5 mm) of the support on which microorganisms were attached, was cut from the surface of the support fixed in the UFA reactor operated at the total organic carbon (TOC) loading rate of 6 g/l·d when mixed waste GSD (Garbage:SM:DCM = 1:19:12) were treated. FISH was performed as described (16).

Other analytical methods All of the following parameters of the culture solution in reactors, except for TS, VTS, suspended solids (SS), and volatile suspended solids (VSS), were analyzed in supernatants obtained after centrifugation at 8000 ×g for 10 min. TS, VTS, SS, and VSS were analyzed in accordance with standard methods (19). Soluble TOC and inorganic carbon (IC) were analyzed using a TOC auto analyzer (TOC-500; Shimadzu, Kyoto), according to the testing methods for industrial wastewater, JISK0102-1986 (19). Volatile fatty acids (VFAs) were analyzed as previously described (20). PO_4^{3-}, NH_4^+ and total chemical oxygen demand (T-COD_{Cr}) were measured using the HACH method (HACH Co., Loveland, CO, USA). The methane content of the biogas was measured by gas chromatography using a thermal conductivity detector (TCD) (KOR-

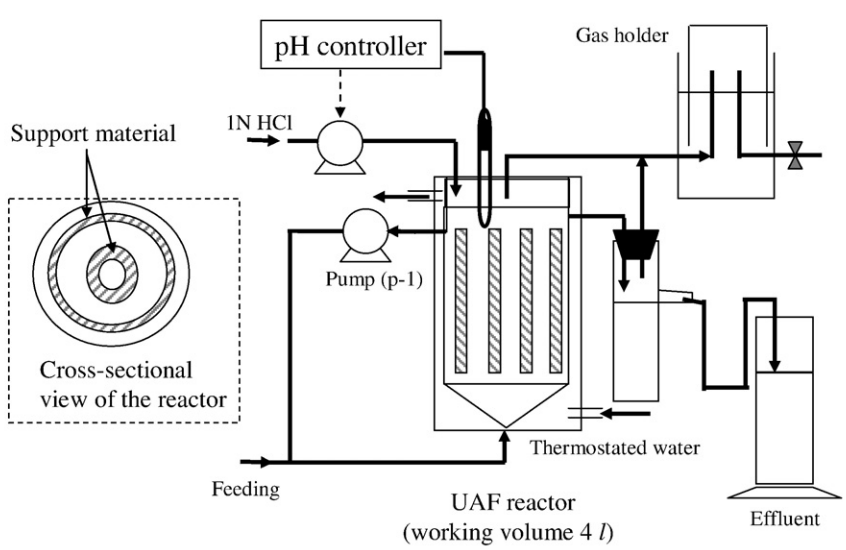

FIG. 1. Schematic diagram of an anaerobic digestion process using an upflow anaerobic filter (UAF) reactor.