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ORIGINAL ARTICLE

Solid-state anaerobic co-digestion of food waste, rice straw, and rice husk supplemented with cattle digesta under thermophilic conditions

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Abstract

Improving the solid-state digestion of food waste (FW) is important for recovering energy and utilizing the digested residue as fertilizer. Herein, solid-state anaerobic co-digestion was conducted using thermophilic digesters. The inhibitory effects of ammonium were controlled using seasonal rice straw (RS) and perennial rice husk (RH). Furthermore, to accelerate lignocellulosic biomass digestion, cattle rumen digesta was supplemented as an alternative microbial source. When only restaurant FW (average C/N ratio = 13) was used as feed, ammonium accumulation decreased methane production. Methane was successfully produced when RS and RH were mixed with FW, wherein the total solid (TS) ratio of RS and RH to FW was 2.0 (TS = 30–40%; volatile solids (VS) loading rate = 5.08 kg m^{-3}·day). The FW and RS methane yields were 0.573 m^3 kg^{-1} VS and 0.259 m^3 kg^{-1} VS, respectively, similar to the biomethane potential (BMP) of each biomass, whereas the methane yield of RH (0.115 m^3 kg^{-1} VS) was higher than the BMP. Microbial community analysis revealed that hydrogenotrophic methanogens *Methanoculleus* and *Methanothermobacter* were dominant in the digested sludge and cattle digest could be used as a supplement of rumen microorganisms.

Keywords Solid-state anaerobic digestion · Lignocellulosic biomass · Cattle digesta · Methane yield

Introduction

Excessive food waste (FW) is a worldwide issue. In Japan, the amount of FW generated in 2019 was 17,556,000 t [1]. Although more than 80% of manufactured FW is recycled, most FW generated by the food service industry, such as restaurants, is incinerated. Reportedly, the total amount of FW generated by the food service industry in Japan amounts to approximately 1,900,000 t per year. Anaerobic digestion (AD) represents an approach for the utilization of organic FW as a bioresource.

Although liquid-phase AD plants operate stably, they produce a substantial amount of digested sludge residue. This residue is a useful fertilizer, but controlling the distribution of such liquid fertilizer is particularly difficult near the city/town center. Therefore, coagulant is used to dewater the digested sludge. Moreover, since the supernatant contains relatively high concentrations of organic matter and nitrogen, it is necessary to treat this wastewater before discharging it into public water sources. In contrast, since the water contents of digested residues are low in the solid-phase AD of FW, wastewater treatment is unnecessary or low amounts of wastewater are produced.

Methane production is occasionally inhibited by ammonium production, owing to the high nitrogen content in sources, such as FW [2]. Although AD effectively increases the digestion rate under thermophilic conditions, the inhibitory effect of ammonium is more severe during thermophilic than methophilic digestion. Generally, the increase in ammonium concentration during thermophilic solid-state FW fails to foster methane production. Yirong et al. [3] reported that

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methane production ultimately fails at a total ammonia nitrogen (TAN) concentration of > 5.0 g N L^{-1}; however, the accumulation of propionic and other longer-chain volatile fatty acids (VFA) occurs at a TAN of ~3.5 g N L^{-1}.

To address this issue, the control of carbon to nitrogen (C/N) ratio by mixing FW with high-carbon-containing waste, such as paper waste [4, 5] or corrugated cardboard [6], was previously proposed. Additionally, lignocellulosic biomasses, such as forest biomass [7], rice straw (RS) [8], rice husk (RH) [9], giant reed [10], banana tree leaf [11] garden waste [12], lemon grass (*Cymbopogon citratus*) [13], and corn stover [14], were used as co-substrates for obtaining a high C/N ratio [15]. RS and RH are widely produced in Asian countries. In Japan, since RS is mostly used as a soil conditioner, rice fields contribute to extensive greenhouse gas (GHG) emissions [16]. Thus, the AD of RS would be useful for not only increasing the methane yield but also controlling GHG emissions from the rice field. The addition of RS and RH is reportedly useful in the digestion of sewage sludge [17, 18], kitchen waste, pig manure [19], and FW [20]. However, most reports describing the methophilic or liquid-state thermophilic conditions provided limited information on solid-state co-digestion of FW with lignocellulosic biomass under thermophilic conditions. RS is produced only in autumn, while RH is produced perennially. To maintain the process performance, a combination of RS and RH as co-digestion substrates would be useful. Moreover, degradation of lignocellulosic biomass is estimated to be a rate-limiting factor. Several pre-treatments, such as physical, chemical, mechanical, and thermal, have been proposed for lignocellulosic biomass [21]. Nakakihara et al. [17] conducted a methophilic co-digestion experiment on sewage sludge and RS pretreated by a mechanical softening machine, reporting that pre-treatment increased the methane yield of RS by 30%. As this machine is commonly used to soften RH for its use as livestock bedding, it would be useful for the AD pre-treatment of RH. In contrast, a pre-treatment method proposed for lignocellulosic biomass utilized rumen microorganisms [22]. However, the collection of rumen microorganisms represents a challenge, while rumen microorganism-containing cattle digesta can be easily procured from slaughter factories. Supplementing cattle digesta to the feed mixture is expected to stimulate the fermentation of RS and RH.

In this study, we performed a solid-state anaerobic co-digestion of restaurant FW combined with pre-treated RS and RH, using four pilot-scale thermophilic digesters (0.5 m^3), and evaluated the co-digestion capability of these biomasses. In addition, the effects of cattle digesta supplementation were investigated. Furthermore, the microbial community in the co-digestion mixture was evaluated.

Materials and methods

Biomass used in this study

FW was collected twice a week from a school cafeteria in Kanazawa University, Japan, and stored in a refrigerator (4 °C) until feeding. After undesirable materials, such as plastics and papers, were removed, the FW was minced in a food grinder before further use. The FW primarily consisted of unsold food items, such as chicken cutlets or hamburger steaks, and contained small amounts of cooking or vegetable waste, as shown in Table S-1.

RS was procured from a farm cultivating *Oryza sativa* L. in Ishikawa Prefecture, Japan, and RH was collected from a country elevator near the farm. The RS and RH were pre-treated using a mechanical softening machine (MSX-8, Meiwa Co., Ltd., Kanazawa, Japan), which pressurized these materials (water content: 30%) before ejecting them through a screw mechanism. Steam released from the ejected RS and RH suggested that temperature increased to over 100 °C by expansion. Pre-treatment of RS improves water absorbance and reportedly increases methane production potential by 30% [17] (Fig. S-1).

Cattle digesta containing rumen microorganisms was collected from a slaughter factory once every month and stored in a freezer (− 4 °C). Seed sludge was collected from a liquid-state thermophilic FW digester in Toyama City, Japan. Table 1 shows the characteristics of FW, RS, RH, cattle digesta, and seed sludge.

The volatile solids (VS)/total solid (TS) ratio and C/N ratio were within the value range reported for FW in the

Table 1 Characteristics of biomass used in this study

	TS (mg/kg)	VS (mg/kg)	VS/TS	COD$_{Cr}$ (mg/kg)	C/N
Food waste	315,000 ± 69,000	282,000 ± 57,000	0.89 ± 0.06	455,000 ± 20,000	13.2 ± 3.5
Rice straw (Pre-treated)	738,000 ± 58,000	635,000 ± 50,000	0.86 ± 0.05	–	75.9 ± 6.7
Rice husk (Pre-treated)	764,000 ± 40,000	604,000 ± 51,000	0.79 ± 0.05	–	106.0 ± 15.7
Digesta of cattle	218,000	–	–	–	34
Seed sludge	223,000	14,900	0.67	–	–

TS, total solid, *VS* volatile solid, *COD$_{Cr}$*, *C/N* carbon to nitrogen ratio

Japanese manual [23]. The C/N ratio was significantly higher than that of FW. TS and VS concentrations of seed sludge were relatively low, and ammonium concentration in the supernatant of the deeded sludge was 1,445 mg L^{-1}, which is a normal value for the liquid-state FW digester.

Batch experiment

To obtain the biomethane potential (BMP) of RS and RH, batch experiments were performed using a 100 mL disposable syringe [17]. Digested sludge (30 mL) and biomass (0.3 g TS) were placed in the syringe, incubated in a water bath, and subjected to shaking at 55 °C. Subsequent gas production was measured periodically. Gas composition was measured using the withdrawn gas. The batch experiment was conducted in triplicates. A control experiment without biomass was also conducted.

Experimental reactor and operating conditions

The experimental setup is shown in Fig. 1. Four horizontal cylindrical digesters (diameter, 0.6 m; length, 2.4 m; and total volume, 670 L) equipped with a paddle stirrer were used in this study. The active reactor volume was 500 L. The contents in the digester were maintained at 55 °C using an electric heater wrapped around the digester. The flow rate of the biogas produced after the removal of hydrogen sulfide was monitored using a gas meter.

Digested sludge (500 L) from the liquid-phase thermophilic digester was seeded in the digesters. The feeding of biomass mixture to the digester was started after the gas production from the seeded sludge was terminated. Feeding was conducted daily on weekdays. Immediately before feeding, the digester contents were collected. The collection volume was maintained at 70% of the feed volume to maintain the total content. An identical volume of digested sludge with feed was simultaneously collected from the digester and recycled to improve mixing. Feeding was achieved using an air-blocked inlet.

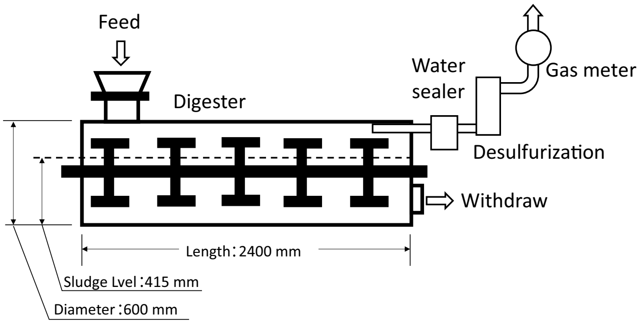


Fig. 1 Experimental setup

Table 2 presents the operating conditions of the digesters used in the experiments. Run 1 was planned as the control digester of FW. Run 2 was the co-digester of FW and RS, while Runs 3 and 4 were co-digesters of the FW and RS/RH mixture. In Run 1, only FW (without dilution) was added to the digester during Periods I and II. Since the concentration of the FW in Period II was considerably high, ammonium concentration increased immediately. Therefore, the input TS concentration was regulated to 25% in Period III and 12.5% in Period IV by adding water. After gas generation in the digester was stopped, a mixture of FW, RS, and RH was added to investigate the effect of mixing conditions in the digester after Period V. In Runs 2–4, only FW was directly added to the digesters during Periods I and II, similarly to Run 1. During Period III, RS mixed with FW was added to the digester in Run 2. The TS ratio of RS to FW was 0.5, and the water content of the mixture was maintained constant at 30%. During Period IV, by decreasing the input FW, the FW/RS ratio changed to 1:1, and total TS concentration decreased to 25%, which decreased the ammonium concentration. During Periods V−VII, the ratio of RS to FW increased to 2.0 under a TS concentration of 30%, and the TS loading rate gradually increased. In Period VIII, the FW to RS ratio was 1:1, owing to the increased input of FW, during which the VS loading rate increased to 7.0 kg m^{-3} day^{-1}. In Runs 3 and 4, along with RS, an equal amount of RH was mixed with the FW. The TS ratio of RS with RH to FW was identical to that used in Run 2, except for Period VIII in Run 4, wherein the input TS ratio and loading was set the same as that in Period VII, and the TS concentration was increased without dilution. In Runs 1–3, from Period VI onward, cattle digesta was used as a weekly supplement.

The generated gas volume was measured daily. The produced gas was collected once in a week in a gas pack, and its composition was analyzed. The water content of FW was analyzed daily. Chemical oxygen demand (COD$_{\mathrm{Cr}}$), was measured weekly, along with TS and VS concentrations of the collected feed and residue. The residue was filtered using a 0.2 μm membrane, and the concentrations of dissolved organic carbon (DOC), dissolved total nitrogen (DTN), ammonium, and VFAs (acetate, propionate, n-lactate iso-lactate, n-valerate, and iso-valerate) were analyzed.

Analytical method

Elemental composition of the FW, RS, and RH was determined using a CHN analyzer (CHN Corder MT-5, Yanaco, Kyoto, Japan). Daily water content was analyzed using a moisture analyzer (MOC-120H, Shimadzu, Kyoto, Japan). TS and VS concentrations were measured according to standard methods [24]. The COD$_{\mathrm{Cr}}$ was analyzed using the US EPA Reactor Digestion Method (DR2800 and DRB200 Reactor, HACH, Colorado, USA). Methane content of

Table 2 Operating conditions of the experimental digesters

	Period I (0–20)	Period II (21–49)	Period III (50–98)	Period IV (99–166)	Period V (167–202)	Period VI (203–230)	Period VII (231–271)	Period VIII (272–341)
Run 1								
Feed (kg-TS/day)								
Food waste	0.5	1	1	0.5	2	2	2	2
Rice straw	–	–	–	–	2	2	2	2
Rice husk	–	–	–	–	2	2	2	2
Feed TS concentration	30%	30%	25%	12.50%	30%	30%	30%	30%
Feed C/N ratio	13	13	13	13	61	61	61	61
DC (kg-TS/week)	–	–	–	–	–	0.1	0.1	0.1
SRT (day)	430	256	159	211	41	38	36	37
VS loading rate (kg-VS/m^3.day)	0.89	1.78	1.78	0.89	10.69	10.69	10.69	10.69
Run 2								
Feed (kg-TS/day)								
Food waste	0.5	1	1	0.5	0.5	0.8	1	2
Rice straw	–	–	0.5	0.5	1	1.5	2	2
Rice husk	–	–	–	–	–	–	–	–
Feed TS concentration	30%	30%	30%	25%	30%	30%	30%	30%
Feed C/N ratio	13	13	34	45	55	54	55	45
DC (kg-TS/week)	–	–	–	–		0.1	0.1	0.1
SRT (day)	431	256	146	176	164	100	71	56
VS loading rate (kg-VS/m^3.day)	0.89	1.78	2.64	1.75	2.69	4.01	5.22	7.00
Run 3								
Feed (kg-TS/day)								
Food waste	0.5	1	1	0.5	0.5	0.8	1	2
Rice straw	–	–	0.25	0.25	0.5	0.8	1	1
Rice husk	–	–	0.25	0.25	0.5	0.8	1	1
Feed TS concentration	30%	30%	30%	25%	30%	30%	30%	30%
Feed C/N ratio	13	13	39	52	65	65	65	52
DC (kg-TS/week)	–	–	–	–	–	0.1	0.1	0.1
SRT (day)	431	256	146	176	164	100	71	56
VS loading rate (kg-VS/m^3.day)	0.89	1.78	2.61	1.72	2.61	4.07	5.08	6.86
Run 4								
Feed (kg-TS/day)								
Food waste	0.5	1	1	0.5	0.5	0.75	1	1
Rice straw	–	–	0.25	0.25	0.5	0.75	1	1
Rice husk	–	–	0.25	0.25	0.5	0.75	1	1
Feed TS concentration	30%	30%	30%	25%	30%	30%	30%	40%
Feed C/N ratio	13	13	39	52	65	65	65	65
DC (kg-TS/week)	–	–	–	–	–	–	–	–
SRT (day)	431	256	146	176	164	100	71	56
VS loading rate (kg-VS/m^3.day)	0.89	1.78	2.61	1.72	2.54	3.81	5.08	5.08

TS total solid, *VS* volatile solid, *DC* digesta of cattle

the produced gas was measured via gas chromatography (GC-8A, Shimadzu) with a thermal conductivity detector (TCD) and a 2-m stainless column packed with a SHINCARBON ST 50/80 column. The operational temperatures at the injection port, column oven, and detector were 100, 70, and 150 °C, respectively. Argon was used as the carrier gas at a flow rate of 30 mL min^{-1}. DOC and DTN concentrations were measured using a TOC/TN analyzer (TOC-V, Shimadzu). Ammonium concentration was analyzed using an ion chromatograph (HIC-SP, Shimadzu) with Shim-pack IC-SA4 column using an aquatic solution of 1.7 mM sodium carbonate solution and 5.0 mM sodium bicarbonate solution as a mobile phase. Argon gas was used as the carrier gas, the column was packed, the injection temperature was 130 °C, and the column temperature was constant at 100 °C. VFA concentrations were measured using an ion chromatograph, following the post-column pH-buffered electroconductivity method (HPLC Organic Acid Analysis System, Shimadzu) with the Shim-pack SCR-102 H column linked to a guard column and an electroconductivity detector. An aqueous solution of 5 mM p-toluenesulfonic acid was used as the mobile phase, and an aqueous solution of 5 mM p-toluenesulfonic acid, 0.1 mM ethylenediaminetetraacetic acid disodium salt, and 20 mM bis(2-hydroxyethyl) iminotris(hydroxymethyl)methane was used as the post-column reaction phase.

Microbial community analysis

During Period VII, the microbial community in the digested sludge was analyzed using next-generation sequencing. DNA was extracted using a PowerSoil DNA Isolation Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. 16S rRNA was amplified via polymerase chain reaction (PCR) with an Applied Biosystems 2720 thermal cycler (Thermo Fisher Scientific, Waltham, USA) using the universal forward primer 515F/universal reverse primer 806R for bacteria [25] and 340F/806Rb for archaea [26, 27]. The PCR procedure consisted of 25 cycles of 10 s at 98 °C, 15 s at 55 °C, and 45 s at 68 °C for bacteria and 40 cycles of 30 s at 94 °C, 30 s at 55 °C, and 60 s at 72 °C for archaea. PCR products were subsequently sequenced using Illumina MiSeq (Illumina, San Diego, USA). The sequences used were aligned and clustered into operational taxonomic units (OTUs) in the UPARSE pipeline [28] and QIIME software [29].

Evaluation of the mixing conditions in the digester

To evaluate the mixing characteristics in the digesters, the systems were imaged using a video camera during Period VII of Run 1. A tracer experiment was conducted during Period VIII of Run 1. One hundred superballs (18 mm, 2.7 g), which were simulated solid biomass, were added into the reactor with feed once. The number of superballs in the withdrawal sludge was estimated every day.

Results and discussion

BMP of lignocellulosic biomass

Table 3 shows the BMP of each biomass. The BMP of RS was 0.209 N m^3 kg^{-1} VS, increasing by 30% after the pre-treatment. These values were consistent with the BMP of pretreated RS obtained in previously conducted batch experiments [8, 17, 30]. It was reported that polysaccharides (cellulose, xylene, and starch) accounted for over 50% of RS [31]. The BMP of glucose was 0.373 N m^3 kg^{-1} VS. When all polysaccharides in RS were converted to methane, BMP was estimated to be 0.23 N m^3 kg^{-1} VS, considering ash contents (18.2%). The value of obtained BMP was almost consistent with the calculated value, meaning that most of the polysaccharides could be converted to methane gas via pre-treatment. Although the BMP of RH was also increased by 30% after pre-treatment, it was much lower than that of RS. The cellulose and hemicellulose contents of PH were 24−39 and 17−26%, respectively, almost the same or greater than those of RS. It is known that the surface of RH is covered with SiO_2, which amounts to 13−29% [32]. Therefore, the biodegradability of RH was considered very low even after the pre-treatment used in this study.

Table 3 BMP of each biomass

	Biomethane potential (Nm^3/kg-VS)
Rice straw (RS)	0.209 ± 0.012
Pre-treated rice straw (pre-treated RS)	0.272 ± 0.015
Rice husk (RH)	0.023 ± 0.004
Pre-treated rice husk (pre-treated RH)	0.053 ± 0.007

Performance of the digesters

Figure 2 shows the cumulative gas production, changes in TS and VS concentrations, and the supernatant composition in each reactor. Table 4 shows the methane yield during each period. Because all reactors were operated under identical conditions during Periods I and II, the amount of gas generated was also identical, indicating that the variation in reactors was negligible. As the TS concentration of inoculated sludge was 2.3% and the hydraulic retention time (HRT) was relatively long, a gradual increase in the TS and VS concentrations was observed in the digested residue. In Run 1, during Periods I–VI, wherein only FW was fed to the digester, the amount of methane generated depended on the loading rate. A high methane gas yield (0.585–0.695 m^3 kg^{-1} VS) was obtained during Periods I–III. However, the ammonium concentration in the reactor increased immediately, reaching more than 5,200 mg L^{-1} by the end of Period III. During Period IV, a decrease in the TS concentration of the feed did not decrease the ammonium concentration. Acetate accumulation and biogas generation stopped after 120 days of operation, as shown in Fig. 2. These results were consistent with those of a previously reported study [2]. During Period V, RS and RH were mixed into the feed,

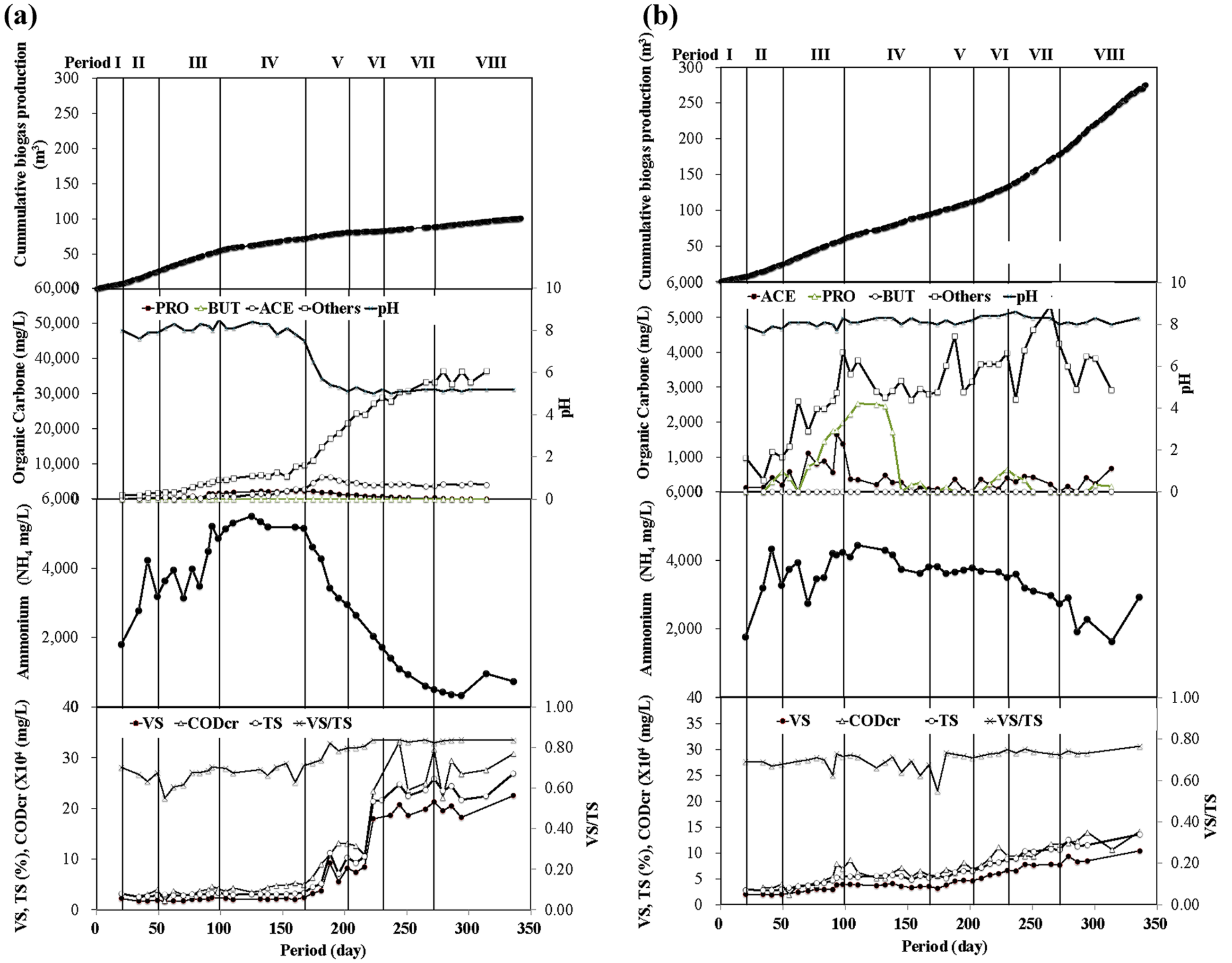


Fig. 2 Cumulative gas production, DOC and VFA concentrations, ammonium concentration, and biomass concentration in different experiments. (**a**) Run 1, (**b**) Run 2, (**c**) Run 3, and (**d**) Run 4

which increased the C/N ratio; however, this did not recover gas generated.

In Run 2, the C/N ratio increased to 31 by mixing of RS with FW (TS base 50% of FW), and the total TS concentration was maintained at 30% in Period III. Although the optimum C/N ratio was reported as 20−30 [33], the ammonium concentration increased to over 4,000 mg L^{-1}, causing the accumulation of organic acids. The methane conversion rate of pre-treated RS based on COD_{Cr} was 0.46−0.56 [30], which was lower than the value for FW (59.4−100) [34], suggesting that a C/N ratio of 20−40 is not sufficient to decrease ammonium concentration. Therefore, the TS ratio of RS to FW increased to 1, the C/N ratio to 40, and the input TS concentration decreased to 25% during Period IV. As a result, the ammonium concentration decreased to 3,800 mg L^{-1}, and the accumulated organic acids disappeared. These observations indicated that the added RS effectively decreased the ammonium concentration and the inhibition of methane production. During Periods V–VII, the input TS concentration returned to the target value (30%), and the TS ratio of RS to FW increased to 2 (C/N ratio 48). The organic loading rate then increased gradually. Biogas was generated stably until a loading rate of 5.2 kg VS m^{-3} day^{-1} was achieved during Period VII. Although FW volume increased and the RS/FW ratio decreased to 1 during Period VIII (VS loading rate of 7.0 kg VS m^{-3}), gas generation persisted stably. These results indicated that the RS/FW ratio of 1 was sufficient for solid state thermophilic co-digestion of FW and RS.

In Runs 3 and 4, RS and RH were combined and mixed with the FW. The ratio of lignocellulosic biomass to FW was similar to that in Run 2, except during Period VIII in Run 4. Since the carbon contents of RH were higher than those of RS, the C/N ratio was calculated as a higher value. However, the ammonium concentration at the end of Period III was approximately 4,000 mg L^{-1}, indicating that the mixing ratio

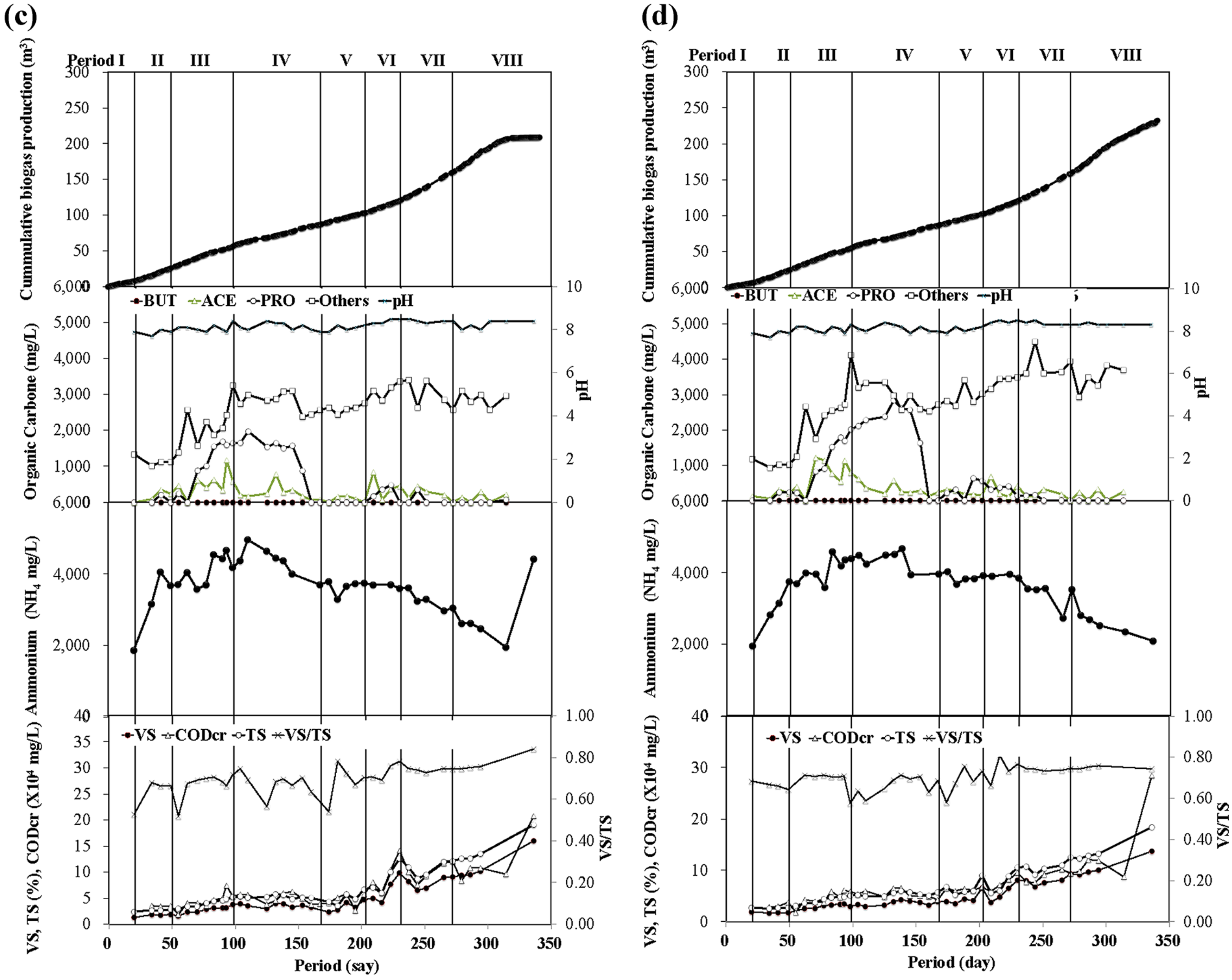


Fig. 2 (continued)

Table 4 Methane yield in each period

Period no	I	II	III	IV	V	VI	VII	VIII
Run 1								
Methane production (m^3/day)	0.197	0.389	0.382	0.163	0.151	0.020	0.012	0.001
Methane yield (m^3/kg-VS)	0.695	0.586	0.605	0.533	0.045	0.006	0.005	0.000
Run 2								
Methane production (m^3/day)	0.189	0.378	0.460	0.287	0.277	0.370	0.623	0.803
Methane yield (m^3/kg-VS)	0.633	0.531	0.409	0.361	0.261	0.219	0.249	0.136
Run 3								
Methane production (m$^{3/}$day)	0.202	0.396	0.371	0.265	0.255	0.337	0.579	0.382
Methane yield (m^3/kg-VS)	0.711	0.596	0.471	0.436	0.318	0.275	0.319	0.159
Run 4								
Methane production (m$^{3/}$day)	0.174	0.392	0.387	0.277	0.238	0.347	0.549	0.584
Methane yield (m^3/kg-VS)	0.612	0.592	0.460	0.455	0.297	0.283	0.302	0.291

VS volatile soil

of the RS/RH mixture to FW (1:1) was not sufficient due to the low biodegradability of RH. Therefore, the ratio of lignocellulosic biomass increased during Periods IV–VI. Stable operation was achieved at a VS loading rate of 2.6–5.1 kg VS m^{-3} day^{-1}. However, during Period VIII of Run 3, gas generation was suppressed by increasing the amount of FW. Although the apparent C/N ratio was approximately similar to that in Run 2, the ammonium concentration became higher than that in Run 2, owing to the lower biodegradability of RH, and ammonia inhibition was observed. In contrast, although the TS concentration input increased to 40% in Run 4, stable gas generation continued, which implied the successful co-digestion of lignocellulosic biomass and FW without dilution. However, when RH was used in addition to RS, a higher ratio of lignocellulosic biomass was required.

In Runs 1–3, cattle digesta from Period IV were used. Comparison of the parallel operation of Runs 3 (with cattle digesta) and 4 (without cattle digesta) during Period IV−VII revealed no remarkable difference nor any negative influence. An acceleration of cellulose degradation was expected upon the addition of cellulose-degrading bacteria obtained from cattle rumen. Since most of the polysaccharides in RS were converted to methane gas only by mechanical pre-treatment as mentioned before, cellulose degradation might not be considered as a rate-limiting factor. Moreover, biodegradability of RH was estimated to depend on SiO_2 decomposition.

The supernatant in all reactors contained a high concentration of organic carbon. Gu et al. [35] reported that the difference among the DOC and total organic-acid carbon contents was increased by the addition of RS, and a high concentration of humic substances was detected in the methophilic co-digesta of sewage sludge and RS. In this experiment, other organic carbon (difference among the DOC and VFA-carbon contents) was also estimated to be humic substances. Although the water content in digested sludge was considerably low and the supernatant water negligible, the color of the treated water should be paid close attention to. Humic substances are effective soil conditioners [36]. When the digested residue was applied to the agricultural field, humic substances in the supernatant are expected to be effective for plant cultivation.

The methane yield of FW was calculated using the data obtained during Periods I–III in Run 1 and Periods I and II in Runs 2–3, wherein only FW was used as the substrate, and stable methane production was observed. The overall methane yield of FW was 0.573 N m^3 kg^{-1} VS, which was higher than the reported biomethane potential of FW based on the composition of household garbage (0.507 N m^3 kg^{-1} VS [37]). Most FW used in this study was comprised of unsold entrees, such as chicken cutlets or hamburger steaks, in addition to a small amount of fried foods. The oil content of fried food was 12−27 g per 100 g of food, and that of a hamburger was 14 g per 100 g of food [38]. Moreover, oil-containing FW has a high methane potential. For example, the BMP of fried tofu is reportedly 0.79–1.08 N m^3 kg ^{1} VS [39]. Since FW components varied weekly, methane production also showed weekly variation. However, the yield was calculated for an average of 100 days. We observed negligible organic acid accumulation and stable operation during this period. Therefore, the methane yield of FW was considered as the biomethane potential of FW in the subsequent operation of Runs 2−4.

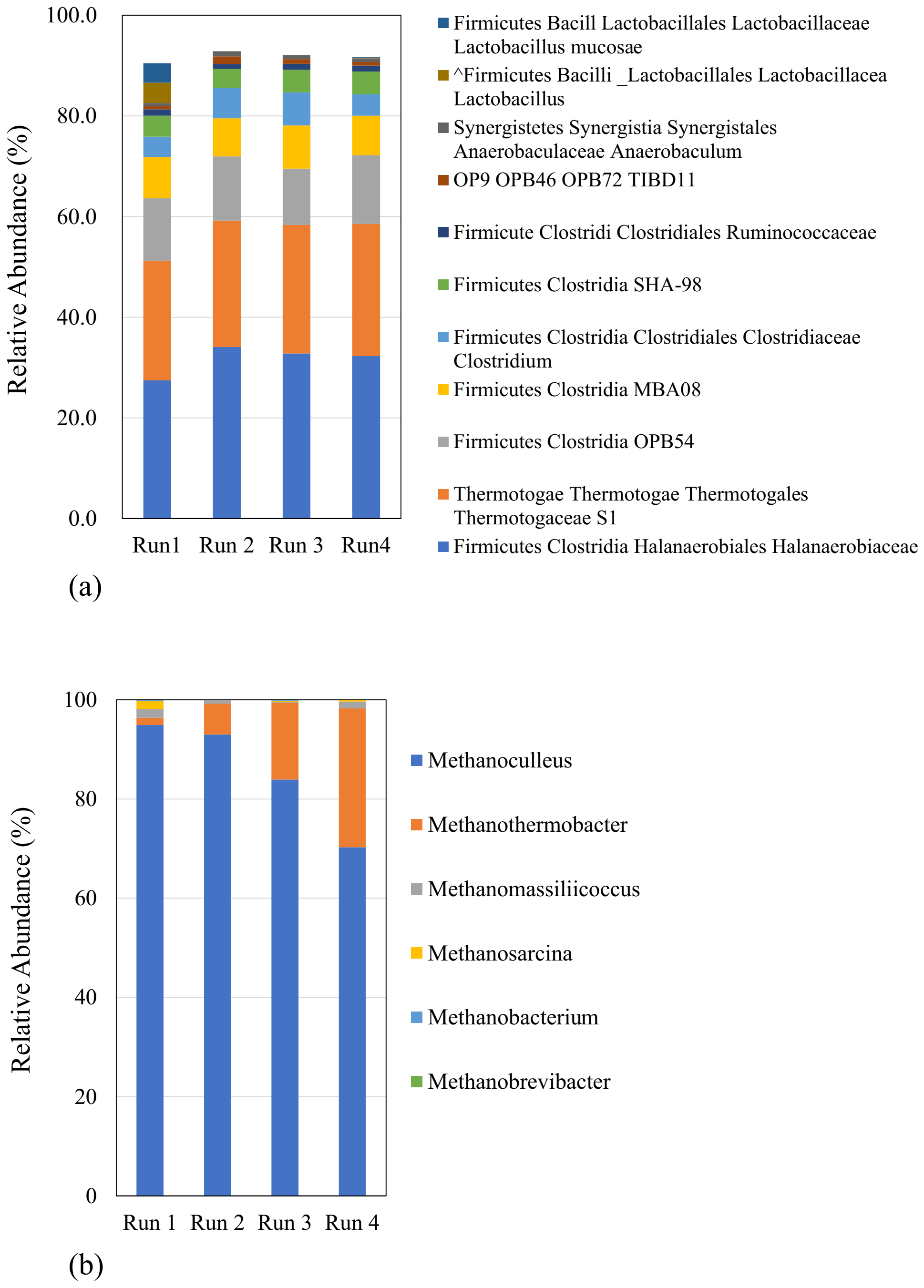
The methane yield of RS was calculated during Periods III–VII of Run 2 using the FW methane yield. The methane yield of RS was 0.259 N m^3 kg^{-1} VS, which was approximately identical to the BMP of pre-treated RS. These results indicated that co-digestion of FW and RS did not exhibit a synergistic effect; however, the addition of RS positively affected FW digestion by increasing the C/N ratio. The methane yield of RH was calculated using the data from Periods III–VII in Runs 3 and 4 as well as the BMP of FW and RS. Although the obtained methane yield of RH at 0.115 m^3 kg^{-1} VS was lower than that of the RS, the value was higher than its BMP (0.05 m^3 kg^{-1} VS) obtained in the batch experiment. These results suggested that acclimation improved the degradation of RH during thermophilic digestion.

Microbial community in the digester

The microbial community in digested sludge from each digester was analyzed during Period VII. The most abundant bacterial phylum was Firmicutes, accounting for 71.2, 68.8, 68.8, and 68.0% in Runs 1, 2, 3, and 4, respectively. Thermotogae were also abundant in all runs (23.8, 25.1, 25.3, and 26.3% in Runs 1, 2, 3, and 4, respectively). OP9 (0.7–1.9%), Bacteroidetes (0.6–0.8%), and Synergistetes (0.6–1.0%) were also detected. The most abundant Archaeal phylum was Euryarchaeota, accounting for 2.6–3.1% of the total microbial community. There were no remarkable differences in the phylum distribution between runs.

Figure 3 shows the genus-level distribution of bacteria using universal primers (Fig. 3a) and of archaea using archaeal primers (Fig. 3b). The most dominant bacteria were members of the *Halanaerobiaceae* family, which are halophilic obligate anaerobes, and a member of the family *Thermotogaceae*, which is a thermophilic hydrogenetic bacterium. Approximately 62–68% of the genera belonged to class *Clostridia*. As certain members of *Clostridia* reportedly degrade cellulose [40], this might be the key bacterial class for cellulose decomposition. *Ruminococcaceae,* a typical rumen microorganism decomposing cellulose, was detected in all Runs (1.0–1.2%), with no difference in its abundance between Runs 3 and 4. Microorganisms enriched in cattle digesta insignificantly contributed to the microbial community in the digester. *Lactobaccillus* (accounting for

Fig. 3 Genus-level distribution of (**a**) bacteria using universal primers and (**b**) archaea using archaeal primers



8%) was detected only in Run 1. Lactic acid fermentation was expected to occur and decrease the pH. However, we could not detect lactate concentration via the applied analytical method. As the organic carbon concentration in Run 1 was higher than that in Runs 2–4, lactate was possibly included.

In the archaeal community shown in Fig. 3b, the genus *Methanoculleus* was most abundant. *Methanothermobacter* was also detected in all reactors. In Run 1, *Methanoculleus* accounted for 95.0% of the archaeal community. In Run 1, methane production was inhibited during Period IV and did not recover even though RS and RH were mixed to FW from Period V, suggesting that most archaea in the inoculum had been inhibited. As the dominant archaea in cattle rumen [41], *Methanomassiliicoccus*, *Methanobrevibacter*, *Methanosphaera*, and *Methanoculleus* were expected to be detected in the digested sludge. The abundance of *Methanoculleus* was higher in Runs 2 and 3 than in Run 4, which did not include cattle digesta. Thus, cattle digesta supplementation might affect the archaeal community. Both *Methanoculleus* and *Methanothermobacter* are hydrogenotrophic methanogens, suggesting that methane production occurred via the hydrogen pathway.

Mixing conditions in the digester

The recorded footage showed that contents were mixed well by a paddle stirrer in the digester. Figure 4 shows the results of the tracer experiment. As the substrate was added to the reactor daily on weekdays, the withdrawal of superballs was also monitored on weekdays. The theoretical line was calculated assuming that the reactor mixture was withdrawn every day depending on the HRT and that the reactor was mixed completely. The difference between the actually collected and theoretically calculated number of superballs was within one. Despite the tracer method being suboptimal, these results suggested that the reactor feed was well mixed.

Application of the proposed system

The abundance of RS in Japan is reportedly 8,203,000 t, with 92% of it mixed with the soil [1]. This amount of RS is sufficient for dealing with the generated FW. The abundance of RH is approximately 20% that of RS [42]. While RS generation is seasonally limited, RH is generated in all seasons, as the husk is removed prior to shipping. Thus, the combination of RS and RH is practical. Further, the storage area of RS can be reduced by combining it with RH.

The present study demonstrated that solid-state thermophilic co-digestion of FW with the combination of RS and RH is useful. Methane yield was in the range of 0.261–0.319 N m^3 g^{-1} VS at a VS loading rate of 5.08 kg m^{-3} day^{-1} under solid-state conditions (input TS 30–40%). Water was only added before pre-treatment of RS and RH. As the water content of the residue was approximately 87%, it can be utilized in agriculture without dewatering. However, the supernatant had a high concentration of organic acid, necessitating the examination of the effect of the residue on plant cultivation.

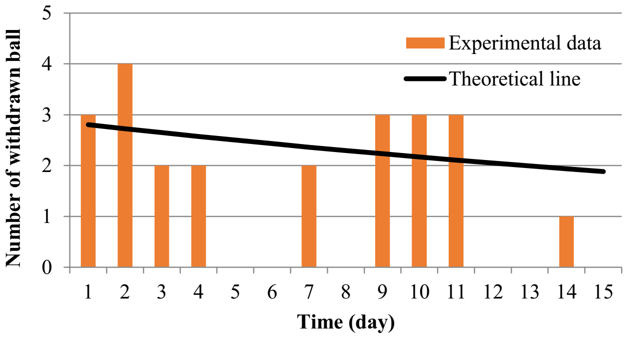


Fig. 4 Result of the tracer experiment

Although the biomethane potential of RH was low, methane yield increased to approximately 50% of RS in the digester, indicating that RH can be used as a bioresource. Moreover, SiO_2 contents of RS and RH were 0.03−13.4% [31] and 13−29% [32], respectively. Togari et al. [43] conducted a co-digestion experiment of sewage sludge and RS, reporting that SiO_2 content in the residue was increased by the addition of RS. The content of SiO_2 in the digested sludge is estimated to increase when RH is used as a co-substrate. High-Si-containing sludge represents a useful soil conditioner, as Si is an essential element for rice cultivation. Moreover, Yagi et al. [44] reported that application of rice straw to the paddy fields at a rate of 6−9 t ha^{-1} increased CH_4 emissions by 1.8- to 3.5-fold, and application of a compost only slightly increased these emissions. When the digested residue is used as a soil conditioner instead of directly mixing it with RS, a decrease in GHG emissions is to be expected.

In the present study, cattle digesta supplementation did not have remarkable effects on methane yield. Hence, the microbial community was successfully altered. This implies that cattle digesta from the slaughter factory could be used to supplement rumen microorganisms. Further, such supplementation might be useful for quick startup and stable operation.

In this study, the input TS concentration was regulated. In the actual plant operation, input concentration will vary, with RS and RH ratio exhibiting seasonal variation. Further studies are needed to verify the utility of the system. In addition, a scale-up of the reactor remains to be examined.

Conclusions

Solid-state thermophilic co-digestion of FW, RS, and RH was investigated using four 0.5-m^3 digesters. A combination of RS and RH mixed with FW was effective in controlling ammonium production inhibition during AD. A methane yield of 0.26–0.32 (m^3 kg^{-1} VS-added) was achieved at a VS loading rate of 5.08 kg VS m^{-3} day^{-1} under a 30–40% TS input and a total harvest ratio of 2.0 (TS base). Approximately 0.873, 0.259, and 0.115 m^3 of methane could be recovered from 1 kg TS of FW, RS, and RH, respectively. The hydrogen pathway was suggested to be the main pathway utilized for methane production, with cattle digesta supplementation affecting the microbial community. Mixing conditions in the digester were relatively satisfactory. Further, the abundances of RS and RH were sufficiently utilized in co-digestion. As RS and RH are also important in livestock cultivation, their utilization after livestock cultivation may be a possibility. This remains to be explored in different regions. In addition, as mixing conditions are critical in solid-state AD, a large-scale follow-up study is necessary.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10163-022-01484-9.

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