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Effect of Feeding Ratio on Anaerobic Co-Digestion of Rice Straw and Cow Manure

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ABSTRACT

Mesophilic (37°C) anaerobic co-digestions of rice straw and cow manure were carried out based on different VS ratios (0:1, 1:2, 1:1, 2:1 and 1:0). Results indicated that co-digestion has a better performance than mono-digestion of rice straw; both biogas and methane yield were significantly increased. Biogas yield was 363.33, 402.45, 390.25, 388.50 and 389.33 mL/gVS for RS: CM ratios of 1:0, 2:1, 1:1, 1:2 and 0:1, and the corresponding methane yield was 175.01, 194.91, 185.13, 194.59 and 189.09 mL/gVS, respectively. Average methane content was 48.17%, 48.43%, 47.44%, 50.09% and 48.57%, respectively. No volatile fatty acids and ammonia inhibition was observed in the experiment. The pH values were in the range of 6.8-7.5. By comparing the ratios of actual to theoretical biogas yield (Y_a/Y_m), it was found that the ratio increased when carbon to nitrogen (C/N) ratio was 15.10-28.85; the further C/N ratio increase led to Y_a/Y_m decrease. In actual application, the ratio of rice straw to cow manure of 2:1 and HRT of 18 days was recommended.

INTRODUCTION

Anaerobic digestion has been considered as an environmentally beneficial and energy-efficient waste disposal process, which converts organic waste into a clean and sustainable energy source-biogas (Holm-Nielsen et al. 2009, Weiland 2010). Biogas can be widely used for replacement and supplement of fossil fuel in power and heat production or as vehicle fuel, thus beneficial in GHG reduction (Holm-Nielsen 2009). Crop straw is one of the most abundant agriculture waste in China, the annual theoretical production is up to 820 million tons and keep on raising (12th Five-year Plan For Biomass Energy Development 2012). Currently, 31.3% of the straw is discarded or left to burn on the fields, not only a waste of resource, but also leads to environmental pollution. Lot of studies have demonstrated that it is possible to use crop straw for biogas production through anaerobic digestion (Khalid et al. 2011, Salminen & Rintala 2002). However, when using crop straw as single-substrate, the anaerobic fermentation system usually suffers with nutrient imbalance (high C/N ratio), which could lead to low efficiency and even process failure (Himmel et al. 2007). Co-digestion has been considered as an effective and cost efficient way for improving biogas yield of crop straw. Manure as a co-substrate overcomes the problems of the low C/N ratio and pH instable for crop straw digestion by supplementary high C/N ratio and high buffering capacity. On the other hand, adding crop straw in manure digesters could be beneficial for avoiding ammonia inhibition caused by low C/N ratio of the substrate (Hansen et al. 1998, Hashimoto 1983, Procházka et al. 2012, Wu et al. 2010), which showed that co-digesting swine manure without straw and wheat straw could enhance daily maximum biogas volume by 8.45-fold and 6.12-fold, respectively. Research by Wang et al. (2012) suggested that high methane potential could be reached when co-digesting dairy manure (DM), chicken manure (CM) and wheat straw in ratio of DM:CM as 40.3:59.7 and C:N as 27.2:1. Lehtomäki et al. (2007) conducted experiments on co-digestion of grass silage, sugar beet tops and oat straw with cow manure in CSTRs. Highest specific methane yields of 268, 229 and 213 mL CH_4/g VS were obtained with the ratio of crop to manure 3:7 for cow manure with grass, sugar beet tops and straw respectively, which increased the methane production by 16-65% compared to digesting manure alone.

Despite numerous studies done in the relevant areas, there are few studies using rice straw as co-substrate with one of the most abundant livestock manure, cow manure for co-digestion. Considering rice straw accounting more than 25% of the total straw production in China and the main straw species in south China, it is of significance to investigate the co-digestion characteristic of rice straw. In addition, there are also knowledge gaps regarding optimized feeding ratio of rice straw to cow manure. Therefore, the aim of this study was to evaluate the feeding ratio effects on biogas yield from rice straw-cow manure co-digestion, and investigate the degradation characteristics of the straw-manure mixture through important intermediate compounds in the process.

MATERIALS AND METHODS

Collection and preparation of substrate and inoculum: The air dried rice straw was collected from a rural area in Guangzhou, China, then chopped into small particles. Fresh cow manure was obtained from a farm in Guangzhou. Prior to use, the substrate was stored in refrigerator at 4°C. The inoculum was from a biogas plant on pig farm, and had been domesticated with cow manure for two weeks, then left with no feeding till no significant gas production before seeding. The characteristics of substrate and inoculum are given in Table 1.

Experimental design: The experiment was carried out in 2.5 L filter bottles with a working volume of 2 L. Each reactor was connected to a gas gathering system based on saturated brine displacement. Before fermentation started, the reactors were purged with N_2 gas for three minutes to ensure anaerobic environment. The reactors were then placed in the electricity-heated thermostatic water bath at 37 ±1°C. Mixing was done manually twice a day. All reactors started with initial substrate concentration of 30 gVS/L. The VS ratio of the rice straw and cow manure, inoculum, substrate and water were added according to Table 2. All reactors were operated in duplicate.

Table 1: Characteristics of substrates and inoculum.

Characteristic	Unit	Inoculum	Rice Straw	CowManure
pH	-	8.10		
C/N ratio	-	-	47.50	15.10
Total solids (TS)	%	2.80	92.85	13.69
Volatile solids (VS)	%TS	68.1	85.87	83.91
[C]	%TS	-	38.19	36.81
[N]	%TS	-	0.80	2.30
[O]	%TS	-	43.78	28.83
[H]	%TS	-	5.08	4.70

Table 2: Experimental design.

No.	RS:CM	RS (g)	CM (g)	Water (g)	Inoculum (g)	Total Weight (g)
R1	1:2	25.1	341.6	433.3	1200	2000
R2	2:1	50.2	170.8	579.0	1200	2000
R3	1:1	18.8	128.1	653.1	1200	2000
R4	1:0	75.3	0.0	724.7	1200	2000
R5	0:1	0.0	512.4	287.6	1200	2000

Analytical methods: TS and VS were determined using standard techniques (APHA 1998). pH was measured by pH meter (PHS-3C, Shanghai REX Instrument Factory). Elementary analysis was performed by Vario EL element analyser (Elementar Analysensysteme GmbH). Heat values were measured by a WGR-1 heat value analyser (Changsha Bente Instrument Corporation). Gas samples analysis was performed with Gas Chromatography Agilent 6890 (Agilent Technologies, USA). The settings were as follows: Porapak Q stainless steel column; thermal conductivity detector; Ar as carrier gas; the temperatures in injection port and detector were 100°C and 150°C respectively; the program for column temperature rise was 40°C for 2 min then rise with the speed of 10°C/min to 80°C, kept for 1 min. The injection was via a sampling loop. The liquid samples were centrifuged at 12000 rpm for 20 minutes and then filtered with a 0.22 µm membrane filter for VFA analysis. VFA including formic, lactic, acetic, propionic, butyric, isobutyric, valeric and isovaleric acid were measured by Gas Chromatography Agilent 6820 (Agilent Technologies, USA). The settings were as follows: 30 m × 0.25 mm × 0.25 µm capillary column (DB-FFAP); flame ionization detector (FID); N_2 as carrier gas; the temperatures in injection port and detector were 250°C and 300°C respectively; the program for column temperature rise was 50°C for 5 min then rise with the speed of 10°C/min to 250°C, maintained for 5 min.

Calculation of theoretical methane potential: Calculation of theoretical methane potential follows the Buswell equation (Buswell & Muller 1955):

$$C_nH_aO_bN_c + \left(\mathrm{n} - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)$$

$$CH_4 + cNH_3 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2$$

$$\text{Theoretical } CH_4 = \frac{1000 \times 24.5 \times (\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8})}{12n + a + 16b + 14c} \frac{mL\ CH_4}{gVS}$$

$$\text{Theoretical } CO_2 = \frac{1000 \times 24.5 \times (\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8})}{12n + a + 16b + 14c} \frac{mL\ CO_2}{gVS}$$

RESULTS AND DISCUSSION

Reactors performance: Fig. 1 shows the daily biogas production of all five reactors. It was found that three co-digestion reactors displayed similar trends in daily biogas production. High biogas production over 1900 mL/d was observed in the first fermentation day. For instance, when rice straw (RS) and cow manure (CM) were fermented at the ratio of 2:1, the reactor produced 2440 mL biogas on the first day, small hydrogen fraction of 1.7%, low methane concentration of 18.8% and high carbon dioxide concentration of 70% was obtained, which suggested that the reactor was in the acidogenesis stage (Kleybocker et al. 2012). When there was high acetic acid concentration in the substrate, it can be converted into methane within very short time. Then the gas production started to decrease rapidly till day 4, increasing gas production from day 5 reached a peak of 2000-2400 mL/d on day 7. The second gas production peak of 1950-2200 mL/d was found around day 10. Thereafter, the biogas production of co-digestion reactors decreased rapidly and maintained low production till the termination of the experiment. The second peak could be due to the propionate acid utilization which was considered as a slow-degraded intermediate. Mono-digestion of cow manure showed a similar trend with the co-digestion, whereas, rice straw exhibited different gas production trend of delayed peaks. Instead of high biogas production on the first day, rice straw reactor exhibited first peak on the second fermentation day, and the following gas peaks were one or two days later than the co-digestion reactors. Rice straws mainly composed of cellulose, hemicellulose and lignin, which could be quite difficult for bacteria to digest (Himmel et al. 2007), the start up process could be comparatively slow, therefore delayed peaks were observed.

Biogas yields for reactors with RS:CM ratio of 1:0, 2:1, 1:1, 1:2 and 0:1 were 363.33, 402.45, 390.25, 388.50 and 389.33 mL/gVS; the corresponding methane yields were 175.01, 194.91, 185.13, 194.59 and 189.09 mL/gVS, which were much higher than that reported by Somayaji & Khanna (1994) (Fig. 2 and Fig. 3). This could be due to the feedstock they used which was wheat straws, and it was found that as it contained more lignin, the recalcitrant structure lead to limited lignocellulose degradation. Compared to mono-digestion of rice straw, co-digestion was able to significantly

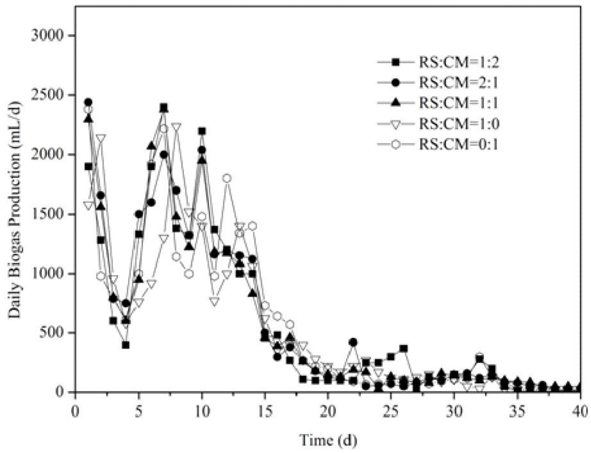

Fig. 1: Daily biogas production of all reactors.

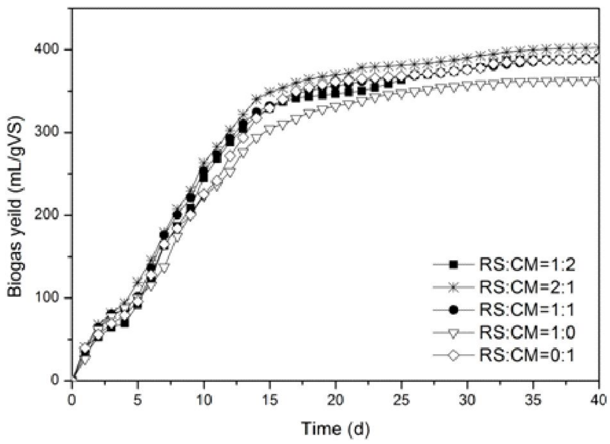

Fig. 3: Methane content of all reactors.

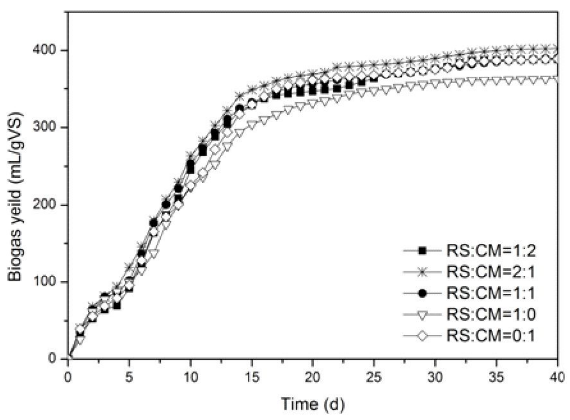

Fig. 2: Cumulative biogas yield of all reactors.

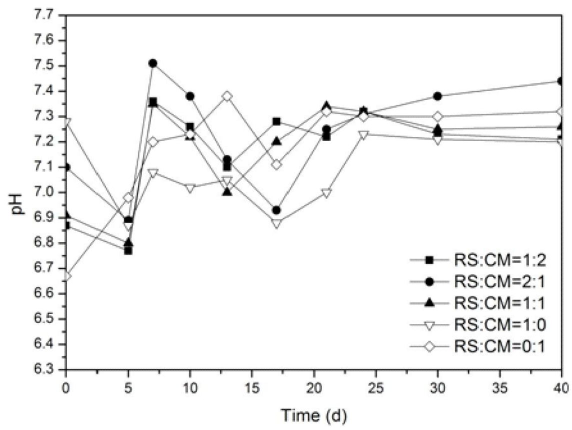

Fig. 4: Variation of pH.

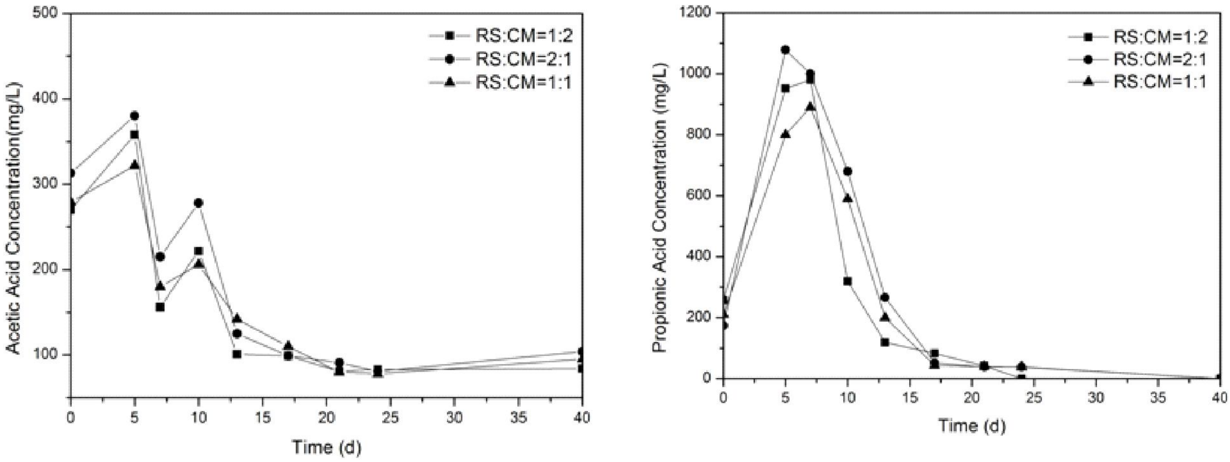

Fig. 5: Dominant VFA concentrations in co-digestion reactors.

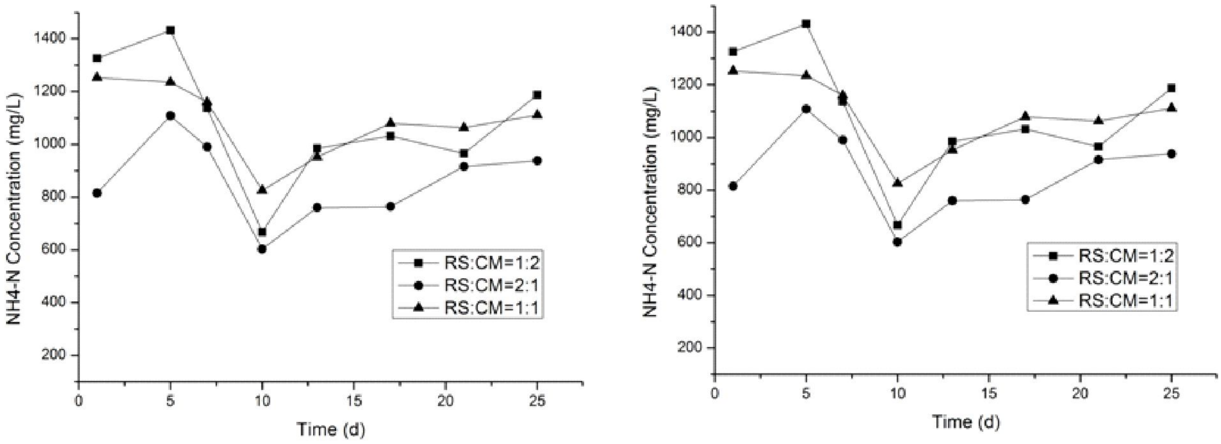

Fig. 6: Ammonia and free ammonia concentrations in co-digestion reactors.

increase the biogas yield; when RS:CM ratio was 2:1, comparable high biogas yield was obtained, which was slightly higher than the mono-digestion of cow manure. The results were similar to the study conducted by Jiang et al. (2014), which indicated that biogas yield from RS:CM ratios of 7:3 and 5:5 were significantly higher than that was found in reactor 9:1, and slightly higher than 3:7 and 1:9. In actual application, proper HRT should give full consideration to the degradation time of straws. In this experiment, 90% of the cumulative biogas production was reached on day 18, 18, 19, 22 and 18 for ratios of 1:0, 2:1, 1:1, 1:2 and 0:1, respectively, therefore the recommended HRT for co-digestion of rice straw and cow manure in actual application was 18 days.

Composition of liquid phase: pH is a preliminary indicator for anaerobic digester, the pH for a functioning reactor is 6.8-8.0. In this study, pH for all the reactors with different ratios were in the range, therefore, no inhibition was found for co-digestion of rice straw and cow manure (Fig. 4). pH of the liquid phase of anaerobic digester is controlled by the buffer system, alkalinity is normally used to evaluate the buffer capacity (Beneragama et al. 2016). Alkalinity is generally affected by VFAs, ammonia nitrogen and CO_2 partial pressure, since the CO_2 partial pressure in a stable anaerobic digester varies insignificantly, therefore, alkalinity is determined by concentrations of VFAs and ammonia.

VFAs mainly refer to acetic acid, propionic acid and butyric acid (including isobutyric acid), which are the primary products from hydrolysis of organics in anaerobic digestion, and also the substrate for methanogens, thus VFAs concentrations are considered as an important indicator for balance evaluation of hydrolysis and methanogenesis. Since growth and metabolism of acidogenic bacteria was faster than methanogens, the VFAs concentrations in this study showed a trend of first increase and then decrease (Fig. 5), with the mass multiplication of methanogens and balance

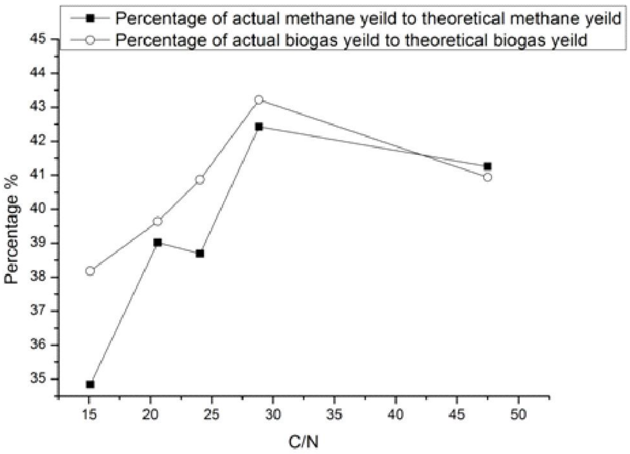

Fig. 7: Performance of anaerobic digestion at different ratios of carbon to nitrogen.

of acidogenesis and methanogenesis, VFAs maintained a low concentration. The highest concentrations of acetic acid, propionic acid and butyric acid were 380, 1079 and 277 mg/L for the co-digestion reactors. Butyric acid was only found on the first day. Compared to acetic acid and butyric acid, degradation rate of propionic acid was slower, with delayed decrease and a much higher concentration. This delayed decrease could be the reason for the second biogas production peak. With the growth of methanogens and methanogenesis process, VFAs showed no accumulation; in the 4th post-digestion stage, acetic acid was the dominant intermediate, trace amount of propionic acid was also found.

Ammonia mainly comes from hydrolysis of proteins, amino acids and urea; low concentration of ammonia can provide necessary nitrogen for microorganism growth and is also beneficial for stabilizing pH. However, high concentration of ammonia has adverse effects on methanogenesis process. In this experiment, ammonia concentration first increased because of protein hydrolysis, and with the consumption by rapidly growing methanogens, the concentration decreased (Fig. 6). When the methanogens growth reached a stable stage, the demand for nitrogen decreased, which led to the increase in ammonia concentration. Ammonia is composed of free ammonia (NH_3) and ammonium (NH_4), inhibition mostly comes from free ammonia (Chen et al. 2008). Inhibition of free ammonia is dependent mainly on methanogens, especially on acetic acid utilizing methanogens. The IC_{50} of free ammonia on methanogens without acclimation is 20 mg/L (Koster 1983); this concentration may also increase with acclimation. It was reported that, free ammonia tolerance could increase to 552 mg/L with acclimation (He 1998). In this experiment, the highest total ammonia and free ammonia concentrations were 1432 and 39.8 mg/L, no inhibition was found. However, it could be also observed that both ammonia and free ammonia in the reactors revealed trends of increasing; in actual application with continuous feeding, there could be accumulation when co-digesting rice straw and cow manure, inhibition was possible to be found after a period of operation.

Comparing biogas yield: Theoretical methane and biogas yield based on elementary analysis can be used to evaluate the biogas potential of different feedstocks. However, due to composition such as lignin being recalcitrant to biodegradation, feedstock has deficiency of trace elements, and the actual biogas yield under different experimental setup vary significantly, the actual biogas yield is usually much lower than theoretical yield. The actual and theoretical yield of biogas, methane and methane content are presented in Table 3. The highest biogas and methane yield were found in reactor with RS:CM ratio of 2:1. Other than mono-digestion of rice straw, all the actual average methane contents were lower than the theoretical values.

Ratios of actual to theoretical yield (Y_a/Y_m) were used to evaluate the reactors performance under different carbon to nitrogen values (C/N) (Fig. 7). When C/N was in the range of 15.10-28.85, with the increase of C/N, Y_a/Y_m also increased significantly, then with the further increase to 47.5, Y_a/Y_m decreased. The possible reasons for better yield found in co-digestion reactors could be:

1) Optimization of nutrients, including both C/N, P and trace elements: Both too low and high C/N could cause inhibition of microorganisms in the reactors (Wang et al. 2012); lack of P and trace elements in mono-digestion of rice straw would also lead to less biogas yield (Jiang et al. 2014).

2) Microbial community and spatial distribution: Microorganisms in cow's rumen took mainly lignocellulosic agricultural products as substrate, which was rich in lignin, cellulose and hemicellulose. Therefore, in RS-CM co-digestion reactors, microorganisms from cow manure could accelerate the hydrolysis of lignocellulosic substrates, and rice straws could be considered as biodegradable packing providing support for growth of microorganisms. They were also beneficial to formation of *Zoogloea* and enhance the synergy between different microbial populations (Svensson et al. 2007).

CONCLUSION

Compared to mono-digestion, better biogas yield could be obtained from co-digestion of rice straw and cow manure. Biogas yields were 363.33, 402.45, 390.25, 388.50 and 389.33 mL/gVS for RS:CM ratios of 1:0, 2:1, 1:1, 1:2 and 0:1, and the corresponding methane yields were 175.01, 194.91, 185.13, 194.59 and 189.09 mL/gVS. Average meth-

Table 3: Methane yields, biogas yields and average methane content in all reactors.

Reactor	C/N	Theoretical MethaneYield (mL/gVS)	Actual MethaneYield (mL/gVS)	Theoretical BiogasYield (mL/gVS)	Actual BiogasYield (mL/gVS)	Theoretical Methane Content (%)	Average Methane Content (%)
RS:CM=1:0	47.5	424.12	175.01	887.55	363.33	47.79	48.17
RS:CM=2:1	28.85	459.38	194.91	931.26	402.45	49.33	48.43
RS:CM=1:1	24.05	478.48	185.13	954.94	390.25	50.11	47.44
RS:CM=1:2	20.61	498.68	194.59	979.98	388.50	50.89	50.09
RS:CM=0:1	15.1	542.74	189.09	1034.61	389.33	52.46	48.57

ane contents were 48.17, 48.43%, 47.44%, 50.09% and 48.57% respectively. With the initial volatile solid (VS) concentration of 3%, no volatile fatty acid and ammonia inhibition was found. The pH values were in the range of 6.8-7.5. By comparing the ratios of actual to theoretical biogas yield (Y_a/Y_m), it was found that the ratio increased when carbon to nitrogen (C/N) ratio at 15.10-28.85, the further C/N increase led to Y_a/Y_m decrease. In actual application, the ratio of rice straw to cow manure of 2:1 and HRT of 18 days was recommended.

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