

Improved Biogas Production from Chicken Manure Anaerobic Digestion Using Cereal Residues as Co-substrates

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ABSTRACT: Because of the resource abundance and high total nitrogen content, chicken manure (CM) is very suitable for anaerobic digestion (AD). In this study, a set of comparative assays was performed on the AD of CM using three main cereal residues (CRs) as co-substrates under various mixing combinations, with a total solid concentration of 8%. Under mesophilic conditions at 35 ± 1 °C, all combinations of CM and CRs significantly improved biogas and methane yields. Co-digestion of CM and corn stalks (CS) showed higher cumulative biogas productions than that of CM/wheat straw (WS) and CM/rice straw (RS). After 60 days of fermentation, the highest methane contents were produced by CM/WS, CM/CS, and CM/RS at a total solid (TS) ratio of 50:50 of 345, 383, and 378 mL/g of removed volatile solids (VS_{removed}), respectively. The value between 15 and 25 has been suggested as the optimal range of the C/N ratio for the co-fermentation of CM with CRs. The results of the volatile fatty acid/alkalinity analysis clearly demonstrated that the co-digestion of CM and CRs was conducive for not only improving biogas production but also stabilizing the digestion system. Predicted optimum CM/CRs proportions, optimum C/N ratios, and maximum biogas productions were calculated according to the best fit regression models for co-digestion of CM with CRs.

■ INTRODUCTION

As the world's second largest economy, China is a major energy consumer in the world. To date, the rapidly increasing energy demand in China has led to an energy gap problem. Moreover, the burning of traditional fossil fuels causes serious environmental problems for water and air.¹ China produces about 7.55×10^8 tonnes of cereal straw (corn, rice, and wheat straws account for 40.36, 24.65, and 14.48%, respectively) and 3.97×10^9 tonnes of poultry and livestock manure every year.² However, most of these products are used for cooking, heating, or as a fertilizer on a household scale.³ Therefore, the development of clean and renewable energy using straw and manure has extraordinary significance for easing energy shortages.

Anaerobic digestion (AD) is a series of biological processes in which bacteria break down biodegradable wastes under the absence of oxygen conditions. As the major production of AD, biogas is helpful in dealing with the energy shortage and environmental pollution problems of excessive use of fossil energy consumption by converting agricultural wastes into clean and safe energy.^{4–6}

For the past few years, animal manure has been used to extract biogas by AD in developing countries, specifically China and India.⁷ With the pursuit of large-scale and industrialization of livestock sector development in China, a large number of poultry manure is produced,⁸ thus causing severe environment contamination without effective treatment.⁹ As a common poultry manure, chicken manure (CM) in China has a total output of 4.03×10^8 tonnes per year.² The total organic nitrogen content of fresh CM (1.03%) is significantly higher than that of other poultry manure.¹⁰ This finding indicates that the biogas production potential of CM is appealing to areas with energy shortages, particularly in rural areas. Rice, maize, and wheat

account for the majority of nutrition and calorie intake in the world.¹¹ These crops produce abundant straw that are suitable for renewable energy. Historically, cereal residues (CRs) have not been selected as substrates for energy production because of their typically lignocellulosic contents that make them difficult to degrade.¹² Therefore, co-digestion that consists of a CM and CR mixture can optimize the digestion process with complementary characteristics. Co-digestion is one of the most effective approaches for increasing the efficiency of biotransformation.^{3,13} Proper mixing is beneficial to avoid suspended solid accumulation and scum formation in the digester.¹⁴

Although considerable research has reported that the development of co-fermentation with various raw materials in rural areas,^{14–23} most of them have used livestock manure co-digestion with fruit and vegetable wastes. Callaghan et al. co-digested CM with cattle manure but caused a steady deterioration because of the ammonia inhibition.²⁴ Gelegenis et al. studied the biogas production by co-digesting poultry manure with whey.²⁵ Wang et al. investigated the methane yields from co-digestion using a mixture of CM, cattle manure, and wheat straw (WS), but their co-digestion mixed only CM and WS.³ However, the optimal mixing combinations of different substrates between CM and CRs are not clear. In this study, we analyzed the biogas production and methane contents from anaerobic fermentation using CM and CRs as co-substrates. The best ratio in the different CM/CR mixtures was obtained by comparing the biogas production efficiency. Optimal co-

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digestion regression curves for methane production were conducted.

MATERIALS AND METHODS

Substrates and Inoculum. CM used in this experiment was obtained from a poultry farm located in Yangling, Shaanxi, China. WS, corn stalks (CS), and rice straw (RS) used in this experiment were obtained from the laboratory's experimental plots of Northwest Agriculture and Forestry (A&F) University and ground into segments with lengths of 2–3 cm. The anaerobic sludge used as inocula was collected from a normal operation anaerobic digester in a nearby village.

Experimental Digester and Setup. The fermentation was carried out in 1 L conical flasks.⁵ The co-fermentations of CM and three CRs were detected by batch digesters. All treatments had the same total solid content of 8%.⁵ A total of 140 g of inocula and various digesting material was added together into each digester with a determined working volume of 700 mL. To maintain the uniform reaction conditions, all digesters were tested at mesophilic conditions ($35 \pm 1^\circ\text{C}$) and gently mixed manually once a day.

Five different mixing combinations of CM and CRs were tested for obtain the best mixing ratio in 60 days of hydraulic retention time (see Table 1). Digestion of single CM and CR was also conducted as controls. All treatment was repeated in triplicate to determine the biogas production and methane yields.

Table 1. Experimental Design of Mix Component Batch Co-digestion Sets^a

treatment	mix composition in the reactor (% of TS ^b content)		
	CM/WS	CM/CS	CM/RS
set 1	100:0	0:100	100:0
set 2	83.3:16.7	83.3:16.7	83.3:16.7
set 3	75:25	75:25	75:25
set 4	50:50	50:50	50:50
set 5	25:75	25:75	25:75
set 6	16.7:83.3	16.7:83.3	16.7:83.3
set 7	0:100	0:100	0:100

^aAll proportions of all substrates in each mixing combination were summed up to 100%. ^bTS = total solid.

Analytical Techniques and Statistic Method. The total solid (TS), volatile solids (VS), and pH value of the material were determined on the basis of the American Public Health Association (APHA) standard methods.²⁶ The lignin and total organic carbon (TOC) contents were determined using the previously reported method.^{5,27} The TOC and total organic nitrogen (TON) values were used to calculate the carbon/nitrogen (C/N) ratio. To monitor the whole fermentation process, the drainage gas-collecting method was used to record daily biogas production and the methane producing rate was determined using a methane measurement device every day. Volatile fatty acid (VFA), TON content, and alkalinity were also determined using the APHA methods.²⁶

The significant differences between each mixing combinations were determined using analysis of variation (ANOVA). Fisher's least significant difference (LSD) multiple range tests were used for multiple comparison tests. The standard forms of the mixture models and regression equations were completely analyzed using ANOVA.

RESULTS AND DISCUSSION

Characterization of Feedstock. Table 2 showed the chemical characteristic comparison of CM and CRs. Significant

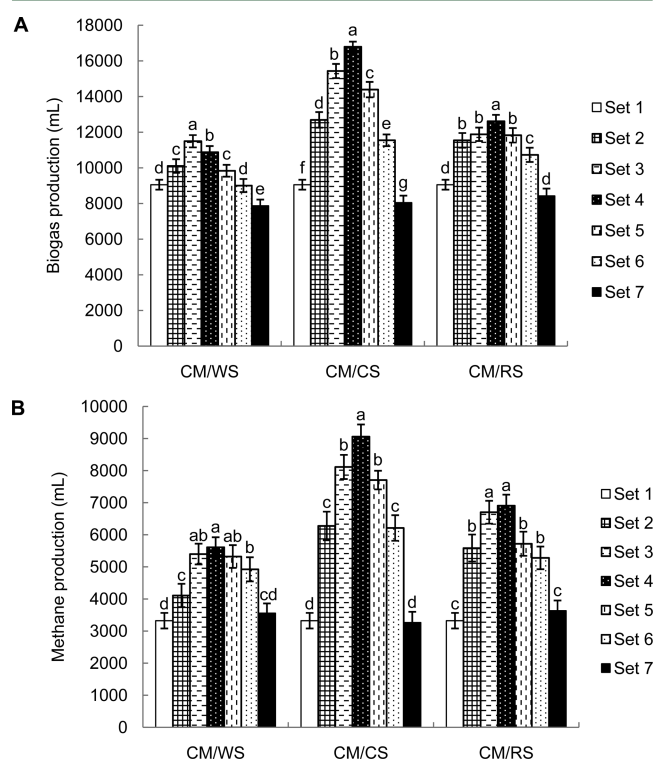


Figure 1. (A) Total biogas productions and (B) methane contents from co-fermentation of CM with three cereal residues at different mixing combinations. The mean values were calculated from three repeats, and standard deviations were represented by vertical bars. Different letters mean significant difference with a probability $p < 0.05$.

differences ($p < 0.01$) of TS, VS, TOC, TON, and C/N ratio were obtained between CM and CRs. The TS and VS of CM were below those of CRs, but the TOC and TON of CM significantly exceeded those of CRs ($p < 0.01$). The C/N ratios of different fermentation substrates positively influence biogas production in the AD process.^{28,29} Various experiments showed that the metabolic activity of methanogens were optimized under a C/N ratio range of approximately 9–30.³⁰ The C/N ratio of

Table 2. Basic Characteristics of Feedstock Used in Fermentation^a

	CM	WS	CS	RS	inoculum
pH	6.91	ND ^b	ND	ND	7.92
TS (%)	28.79 ± 1.21 b	81.08 ± 7.62 a	81.74 ± 7.43 a	77.92 ± 6.97 a	4.65 ± 0.31 c
VS (%)	65.24 ± 1.41 b	90.29 ± 9.25 a	91.42 ± 9.33 a	94.23 ± 9.42 a	67.3 ± 1.21 b
TOC (g of C/kg)	59.75 ± 1.35 a	358.3 ± 31.7 b	288.2 ± 20.3 c	319.6 ± 29.2 bc	ND
TON (g of N/kg)	5.36 ± 0.22 a	3.93 ± 0.2 b	3.27 ± 0.4 b	3.44 ± 0.2 b	ND
C/N	11.15 ± 0.21 b	91.17 ± 3.44 a	88.13 ± 4.65 a	92.91 ± 3.10 a	ND
lignin (%)	ND	24.34 ± 1.89 a	15.38 ± 1.21 b	9.49 ± 0.33 c	ND

^aThe mean value \pm standard deviation was obtained from triplicate measurements. Different letters mean significant difference with a probability $p < 0.01$. ^bND = not detected.

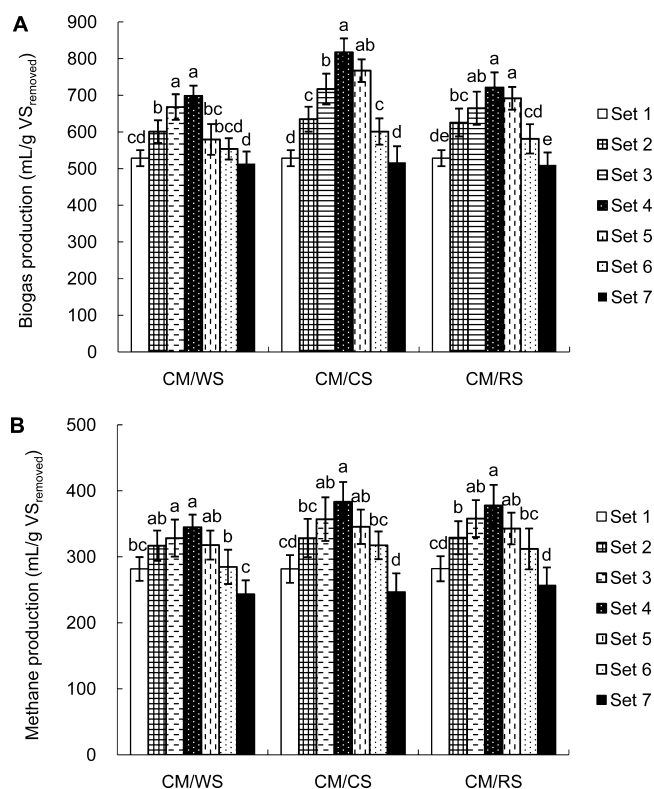


Figure 2. (A) Total biogas productions per $VS_{removed}$ and (B) methane contents per $VS_{removed}$ from co-fermentation of CM with three cereal residues at different mixing combinations. The mean values were calculated from three repeats, and standard deviations were represented by vertical bars. Different letters mean significant difference with a probability $p < 0.05$.

WS (91.17), CS (88.13), and RS (92.91) was much higher than that of CM (11.15). This finding indicated that the addition of CRs reduced the C/N ratio and increased biogas production when co-digested with CM.

Comparison of Biogas Production and Methane Content at Different CM/CR Combinations. For comparison of the difference of monosubstrate anaerobic fermentation and co-fermentation of CM with CRs, the final cumulative biogas productions and methane contents obtained in the monosubstrate digester and in the co-digester under mesophilic conditions are shown in Figure 1. During the same retention time (60 days), the biogas yield and methane production in the co-digester were significantly higher than those in the single-substrate digester ($p < 0.05$). The highest cumulative biogas productions were obtained at CM/WS set 3, CM/CS set 4, and CM/RS set 4 (see Figure 1A). Correspondingly, the highest methane yields were obtained at all set 4 of CM/CRs (see Figure 1B). Whether biogas production or methane yields, the mixture of CM/CS has

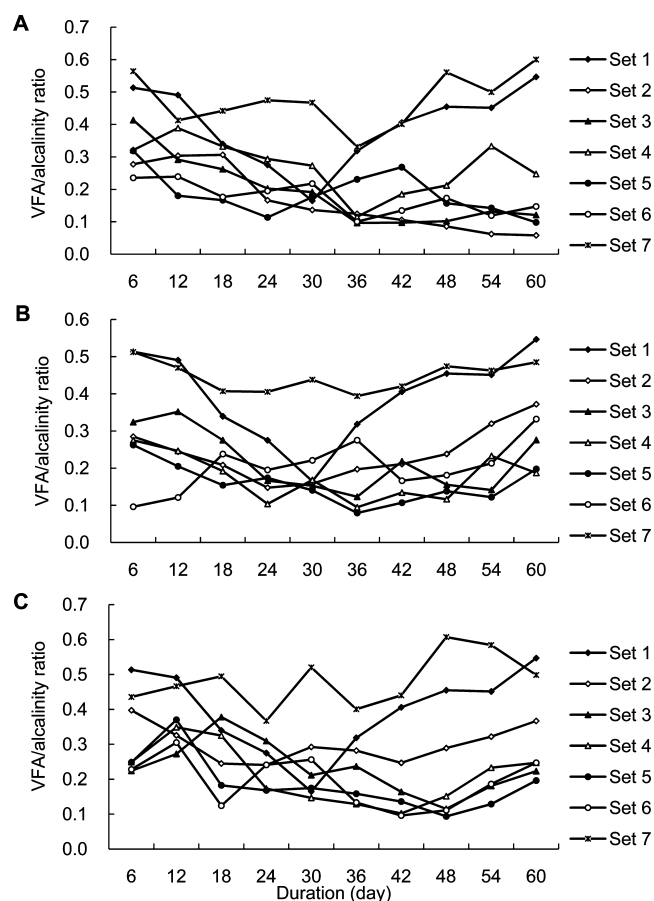


Figure 3. VFA/alkalinity ratio variation for the co-fermentation of CM and three cereal residues with different mixing combinations: (A) CM/WS, (B) CM/CS, and (C) CM/RS. VFA stands for volatile fatty acid.

more improving efficiency than that of CM/WS and CM/RS. These results have similar trends with our previous research on the co-fermentation of goat manure with CRs,³¹ indicating that the mixture of CM with CRs is beneficial to improve biogas production.

The biogas yields of CM/CS sets 2, 3, 4, 5, and 6 were calculated as 635, 717, 817, 768, and 601 mL/g of $VS_{removed}$, respectively (see Figure 2A). These statistics demonstrated an improvement of 20.0, 35.5, 54.4, 45.2, and 13.6% than single CM (529 mL/g of $VS_{removed}$) and an improvement of 22.8, 38.7, 58.0, 48.5, and 16.2% than single CS (517 mL/g of $VS_{removed}$). The similar tendency was noticed for the CM/WS and CM/RS, in which biogas production had considerably higher increases at different levels (see Figure 2B). The results of this study further validated the results of our previous work³¹ and Wu et al.,³² who revealed that co-fermentation goat manure or swine manure with CRs significantly increases biogas production at most C/N ratios.

Table 3. Average Values of C/N Ratios in the Co-fermentation of CM and CRs^a

treatments	co-fermentation mixing combinations						
	set 1	set 2	set 3	set 4	set 5	set 6	set 7
CM/WS	11.15 ± 0.21 g	13.38 ± 0.01 f	14.80 ± 0.09 e	21.20 ± 0.35 d	35.35 ± 0.75 c	44.60 ± 0.53 b	91.17 ± 1.34 a
CM/CS	11.15 ± 0.21 g	12.48 ± 0.15 f	13.35 ± 0.13 e	17.39 ± 0.09 d	27.26 ± 0.06 c	34.71 ± 0.45 b	88.13 ± 0.46 a
CM/RS	11.15 ± 0.21 g	13.84 ± 0.04 f	15.54 ± 0.04 e	23.05 ± 0.26 d	38.81 ± 0.62 c	48.77 ± 0.61 b	92.91 ± 1.03 a

^aThe mean value ± standard deviation was obtained from triplicate measurements. Different letters mean significant difference with a probability $p < 0.01$.

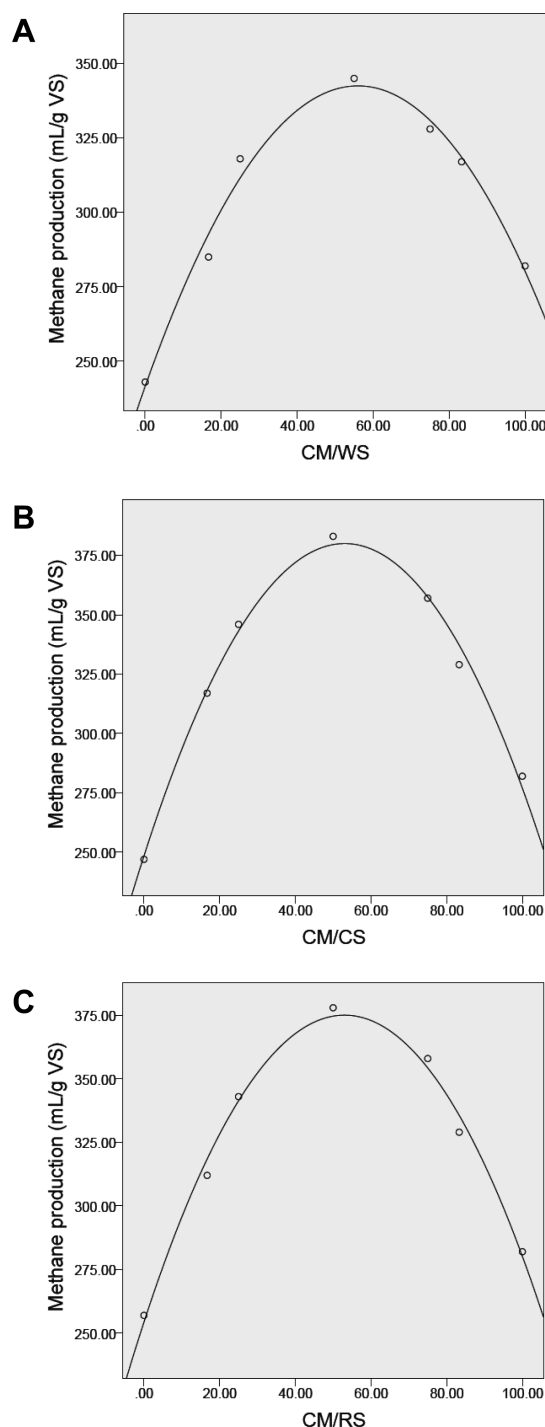


Figure 4. Regression curves of biogas potential in the co-fermentation of CM with three cereal residues: (A) CM/WS, (B) CM/CS, and (C) CM/RS.

The methane contents were higher in CM/WS set 4 (345 mL/g of VS_{removed}), CM/CS set 4 (383 mL/g of VS_{removed}), and CM/RS set 4 (378 mL/g of VS_{removed}) (see Figure 2B). These results agreed with some research on the AD of CM.^{24,33} However, these values were higher than the results by Abouelenien et al.³⁴ (less than 200 mL/g of VS_{removed}). This difference was probably caused by the differences in substrate composition, inoculums, and fermentation temperature.

The results of ANOVA indicated that the biogas yield and methane production of CM/WS were relatively lower than those of CM/CS and CM/RS (see Figures 1 and 2). This result was consistent with our previous study,³¹ which found that the higher TOC and lignin contents of WS inhibited methanogen growth and methane production because of low pH and lack of ammonium nitrogen.

Effect of the C/N Ratio on the Co-digestion Process.

The C/N ratio is one of the main indicators for the co-digestion process.²⁸ A relatively high C/N ratio means fast nitrogen degradation by microbials and results in low biogas yields and vice versa. A low C/N ratio is toxic to methanogens.³⁵ In this study, the range of C/N ratios for each co-fermentation and monosubstrate fermentation were between 11.15 and 92.91 (see Table 3). The C/N ratios of each CR were much higher than that of the co-digestions ($p < 0.01$; see Table 3), thus determining that co-digestion could be maintained a low level of the C/N ratios during the AD process. Cumulative biogas production results indicated that the co-digestion treatments had higher biogas yields than that of the corresponding single-substrate digestions (see Figure 2A). The highest methane contents (345, 383, and 378 mL/g of VS_{removed}) at CM/WS set 4 (C/N of 21.20), CM/CS set 4 (C/N of 17.39), and CM/RS set 4 (C/N of 23.05) increased 1.42, 1.55, and 1.47 times compared to that of CRs only, respectively (see Figure 2B). These results suggested that 15–25 was the ideal C/N ratio range for the co-fermentation of CM with CRs; this result was consistent with the results by Zhang et al.,³⁶ who found that the optimum C/N ratio in the AD process of cattle manure with food waste was 15.8. Wu et al.³² revealed that the optimal C/N ratio for the co-fermentation of swine manure with CRs was 20. Zhong et al.³⁷ revealed that the ideal C/N ratio for the fermentation of blue algae and CS was also 20. In contrast, previous studies from our laboratory revealed that the ideal C/N range for co-digesting goat manure with CRs (20–35) was higher than that of CM/CRs.³¹ The most likely explanations for this condition were that (i) the TON content of fresh CM (5.36%) is much higher than those of other manure of livestock and poultry and (ii) furthermore, a higher C/N ratio indicates fast nitrogen consumption by microbials and leads to less biogas production.³⁵

Effect of VFA and Alkalinity on the Co-digestion Performance. In the AD process, the VFA concentration was a very good indicator of the degradation status.³⁸ The measured data showed that the VFA level increased from 774 to 1168 mg/L, which was significantly influenced by different mixing ratios.

Table 4. Summary Statistics for Best Fit Regression Models for Co-digestion of CM with CRs^a

treatments	regression equation	R ² (%)	predicted optimum CM/CR proportion (%)	predicted optimum C/N ratio	predicted maximum methane production (mL/g of VS)
CM/WS	$y = 241.436 + 3.629x - 0.032x^2$	98.2	56.7:43.3	19.1	344.3
CM/CS	$y = 248.389 + 4.959x - 0.047x^2$	99.1	52.8:47.2	16.8	379.2
CM/RS	$y = 254.323 + 4.556x - 0.043x^2$	98.9	53.0:47.0	21.9	375.0

^ax represents the percentage of TS contents of CM, and y represents the methane yield of the AD process.

Table 5. Real and Coded Methane Yields for Different CM and CR Mixtures Using the Co-digestion Models

mixtures (CM % + CR %)	methane yields (mL/g of VS)					
	CM/WS		CM/CS		CM/RS	
	actual	predicted ^a	actual	predicted ^a	actual	predicted ^a
100 + 0	282	284	282	274	282	280
83.3 + 16.7	317	322	329	335	329	335
75 + 25	328	334	357	356	358	354
50 + 50	345	343	383	379	378	375
25 + 75	318	312	346	343	343	341
16.7 + 83.3	285	293	317	318	312	318
0 + 100	243	241	247	248	257	254

^aCalculated biogas potential of the individual regression model.

Throughout the co-digestion processes, the VFA levels at the steady state were low (less than 1000 mg/L). These values indicated that all treatments were highly stabilized after an initial transitory increase of VFA. This condition indicated the startup of an AD process when losing the balance of the hydrolytic microbes, fermentative microbes, and methanogens.³⁹ This stability was also confirmed by the pH values. The pH range of 6.3–7.2 improved the biogas productions, thus showing well buffer effectiveness in the co-digestion systems.

Alkalinity strongly influenced the ability of a solution to neutralize acids in the AD process.⁴⁰ The average alkalinity values ranged between 1815 and 2603 mg/L. Previous studies on mesophilic anaerobic digested organic wastes showed that the alkalinity range of 2000–4000 mg/L was appropriate for the fermentation process.^{12,41} Except the values obtained from several time points that fall within this range, all values were consistent with the reported values.

VFA/alkalinity ratio was a feasible criterion to estimate the stability of the fermentation system.^{15,42} The following critical values are used for this parameter: <0.4, digestion system should be relatively stable; 0.4–0.8, a sign of instability; and >0.8, significant instability.²⁴ When CM or CRs were digested separately, the VFA/alkalinity ratio was often in the range of 0.4–0.8 (see Figure 3). This range indicated that instability had the potential to inhibit the activity of methanogens.²¹ When CM and CRs were co-digesting, the ratio remained below the critical value of 0.4, even during the initial transitory period, which was characterized by a high VFA/alkalinity ratio (see Figure 3). These results suggested that the co-fermentation of CM and CRs was conducive for not only improving biogas production but also stabilizing the digestion system.

Optimization for Methane Production Potential. The standard forms of popular mixture models include linear, quadratic, full cubic, special cubic, and special quartic models.⁴³ For the present study, the regression models were conducted with a percentage of the TS contents of CM in each treatment as independent variables and biogas yields as response variables. The best models for co-digestion CM with CRs were found using the criteria with low standard error for regression and the high coefficient of determination in the SPSS software (see Figure 4 and Table 4). The performances of these models could be used to predict the co-digestion systems in accordance with the parameters of corresponding single-substrate digestion systems. The values of R^2 , which is a measurement of fitness of regression equations, were all more than 98% (see Table 4). This percentage suggested that the majority of obtained results were described by these models. The predicted optimum CM/CR proportions, optimum C/N ratios, and maximum methane

productions were calculated on the basis of the presented models (see Table 4). Thereafter, these models were also used to calculate the methane content from seven mixing combinations of CM and CRs. The calculated results (see Table 5) were further confirmed by the consistency between the obtained and predicted data.

CONCLUSION

Co-digestion of CM with CRs can balance the C/N ratios and increase biogas production over a 60 day fermentation period. At a TS ratio of 50:50, the highest methane contents were obtained from different mixing combinations of CM/WS, CM/CS, and CM/RS (345, 383, and 378 mL/g of VS_{removed}, respectively) throughout the entire fermentation process. The value between 15 and 25 has been suggested as the optimum range of the C/N ratio for the co-fermentation of CM with CRs. Moreover, the results of the VFA/alkalinity ratios showed that all co-digestion processes have more stable digestion systems than the monosubstrate of either CM or CRs. The optimum CM/CR proportions, C/N ratios, and maximum biogas productions were calculated using the optimization regression models.

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Notes

The authors declare no competing financial interest.

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