



Dry Anaerobic Co-Digestion of Citrus Wastes with Keratin and Lignocellulosic Wastes: Batch And Continuous Processes

Regina J. Patinvoh^{1,2} · Magnus Lundin¹ · Mohammad J. Taherzadeh¹ · Ilona Sárvári Horváth¹

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Abstract

Dry anaerobic co-digestion of citrus wastes (CW) with chicken feather (CF), wheat straw (WS) and manure bedded with straw (MS) was investigated in batch and continuous processes. Experiments were designed with different mixing ratios considering the inhibitory effect of CW, C/N ratio, and total solid content of individual feedstocks. Best mixing ratio (CF: CW: WS: MS) of 1:1:6:0, enhanced methane yield by 14% compared to the expected yield calculated according to the methane yields obtained from the individual fractions. The process performance of this mixture was then investigated in continuous plug flow reactors at different organic loading rates (OLR) with feedstock total solid contents of 21% TS (R_{TS21}) and 32% TS (R_{TS32}). At OLR of 2 gVS/L/d, a methane yield of 362 NmlCH₄/gVS_{added} was obtained from R_{TS21} , which is 13.5% higher than the yield obtained from R_{TS32} (319 NmlCH₄/gVS_{added}). However, it was not possible to achieve a stable process when the OLR was further increased to 3.8 gVS/L/d; there were increased total VFAs concentrations and a decline in the biogas production.

Keywords Solid wastes · Dry co-digestion · Batch process · Continuous process · Process performance

Statement of Novelty

Appropriate mixing ratio for dry codigestion of inhibitory and recalcitrant wastes was acquired for improved methane yield. This is to circumvent some problems associated with pretreatment of these wastes and also make the dry digestion process more economical.

Introduction

Global emissions from solid wastes are estimated to be between 20 and 40 million tons of CH₄ per year [1]. Biogas production from these waste streams through anaerobic digestion processes reduces greenhouse gas emissions and solve the problem of global energy challenge and environmental threat [2]. Solid waste streams like chicken feathers (CFs), manure bedded with straw (MS), wheat straw (WS) and other agricultural and forest residues are readily available; they account for the largest potential feedstock as sources of biomass energy. CF is a good potential source of nitrogen with over 90% of crude protein content [3, 4], and WS is mainly carbohydrate; a potential source of carbon. Additionally, fruit wastes contain a large amount of organic matter that are easily degraded with about 78.3% carbohydrates, 8.5% protein, and 6% fat [5], which makes them potential source of biomass for energy production. Large volume of fruit wastes are also generated globally; over 40 million tons of industrial citrus wastes (CW) are globally generated [6]. Converting these waste streams into energy through anaerobic digestion is a feasible option with low capital investment thereby ascribing value to these wastes.

Notably, because of the recalcitrant structure of most agricultural solid wastes such as CFs and WS, they degrade

✉ Regina J. Patinvoh
regina.patinvoh@hb.se

Magnus Lundin
magnus.lundin@hb.se

Mohammad J. Taherzadeh
mohammad.taherzadeh@hb.se

Ilona Sárvári Horváth
ilona.horvath@hb.se

¹ Swedish Centre for Resource Recovery, University of Borås, SE 501-90, Borås, Sweden

² Department of Chemical and Polymer Engineering, Faculty of Engineering, Lagos State University, Lagos, Nigeria

slowly, thus, resulting in low biogas yield. Other problems associated with the digestion of these waste streams are the high nitrogen or carbon content, leading to a nutrient imbalance when they are digested as the sole substrate. Several research studies have been conducted to improve the methane yield from keratin-rich and lignocellulosic solid wastes by chemical pretreatment [7–11], steam explosion pretreatment [12], and biological pretreatment [3, 13, 14]. Additionally, the presence of peel oil, with D-limonene as the main ingredient in CW, inhibits microbial growth [15], leading to a low biogas yield. This inhibition challenge in CW for biogas production has been addressed by leaching [16], steam explosion pretreatment [17], and membrane technology [18]. Although these methods have been reported to be effective in solving problems with low biogas yield, there are still problems of high energy consumption, expensive technology, harmful environmental by-products, and release of inhibitors during some of the pretreatment methods [19].

Considering the fact that easily degraded feedstocks are not readily available and the challenges associated with some pre-treatments methods, co-digestion is an alternative strategy for improving the biogas yield from these types of solid wastes. This concept favors synergisms, dilutes harmful compounds, optimizes the biogas production, and increases the digestate quality [20]. Although nitrogen is an essential nutrient for the microorganisms, its excess leads to the formation of ammonia during degradation, which inhibits microbial growth at higher concentrations [21, 22]. Additionally, when the amounts of easily degradable carbon are too high, the process tends to be susceptible to acid accumulation [23]. Presence of inhibitor in some feedstocks also affects the microbial growth. Hence, there is need for co-digestion to enhance buffering capacity and reduce inhibitory effect on biogas yield. A considerably wider C/N ratio, between 10:1 and 30:1, has been reported in the literature for a stable digestion process [22, 24]. Nevertheless, the composition of feedstocks, the availability of carbon and nitrogen to the microbial community [25], and different operation parameters during the digestion process are also significant for an effective anaerobic digestion.

There has been great concern over large volume of water used during anaerobic digestion of solids wastes and in the digestate residue. To address this challenge, recent studies have focused on dry anaerobic digestion of these waste streams [26–30]. However, dry digestion of these wastes requires further improvement for industrial applications; there is a challenge of longer retention time and low mass transfer at higher solid content [31, 32]. Co-digestion of multiple feedstocks is a feasible option for addressing these challenges [32]. Present study investigates co-digestion of CW with CFs, WS, and MS in a dry anaerobic digestion process. Experiments were designed with different mixing ratios and evaluated in batch process. In addition, the

process performance of the best mixture in batch was investigated in continuous plug flow reactors at different organic loading rates (OLR) with feedstock total solid contents of 21% TS and 32% TS.

Materials and Methods

Substrates and Inoculum

The different substrates used during this study were CFs, CW, WS, and cattle MS. The CF waste was collected from a slaughterhouse (Håkantorps Slakteri AB, Håkantorps, Sweden) and prepared according to Patinvoh et al. [3] prior to use; the particle size of the CF was between 1 and 10 mm. CW were obtained from Brämhults Juice AB (Borås, Sweden) and chopped manually. WS and cattle MS were obtained from a farm outside Borås (Rådde Gård, Sweden). The manure was shredded manually to reduce the particle size of straw in the manure to an average of 2 mm, and the WS was milled to a particle size between 0.5 and 2 mm. The CF wastes and WS were stored at room temperature, while the CW and MS were stored in plastic containers at $-20\text{ }^{\circ}\text{C}$ until further use. During the experiment, weighted frozen substrates were thawed at room temperature and thoroughly mixed to gain a homogenized feed before use.

Prior to analysis, the feedstocks being solids were prepared according to Zupančič and Roš [33] by blending 1 g of the sample with water (dilution factor of 50) to allow homogenization. Thereafter, the theoretical methane potential ($\text{BMP}_{\text{thCOD}}$) of the feedstocks used were calculated related to their chemical oxygen demand (COD) content as described previously [27], after determining the COD content for the individual feedstocks using Eq. (1) [34] assuming that the equation is valid for any substance or product [35].

$$\text{BMP}_{\text{thCOD}} = \frac{n_{\text{CH}_4} RT}{p \text{VS}_{\text{added}}} \quad (1)$$

where $\text{BMP}_{\text{thCOD}}$ is theoretical yield under laboratory condition, R is gas constant (0.082 atm L/mol °K), T is working temperature (310 °K), P is pressure (1 atm), VS_{added} is volatile solids (VS) of the substrate added (g), and n_{CH_4} is methane produced (mol) determined according to Eq. (2):

$$n_{\text{CH}_4} = \frac{\text{COD}}{64 \left(\frac{\text{g}}{\text{mol}} \right)} \quad (2)$$

However, the COD content of CFs was not detectable because the mixture was heterogeneous; therefore, the theoretical methane yield of 496 Nml methane produced per g

VS for proteins [36] was used for the calculations, which is applicable if all the insoluble protein (keratin) are converted to soluble protein.

Anaerobic sludge used as inoculum was obtained from a digester treating wastewater sludge and operating at mesophilic conditions (Vatten and Miljö i Väst AB, Varberg, Sweden). This inoculum was incubated at 37 °C prior to use; the inoculum with 3.8% TS and 2.7% VS was then centrifuged at 10,000×g for 10 min to increase its TS content to 9.43% and VS content to 6.44%. Table 1 shows the characteristics of the substrates and the anaerobic sludge (inoculum) used during the experiments.

Statistical Design

The experiment was designed with statistical software MINITAB® (version 17.1.0), using 3-factor simplex lattice design, consisting of pure substrates and mixtures of two, three, and four substrates at wet weight ratios. The CF fraction was kept constant to maintain the C/N ratio of the mixtures between 12 and 21. The experiments were replicated according to the same setups and methane yield was used as response variable. The linear mixing model was used to obtain the expected methane yield from all the mixtures without synergetic or antagonistic interactions; the methane yield is expected to correspond to the amount of VS fractions from individual substrates. The quadratic model was used to obtain the predicted methane yield from all the mixtures; this measures the synergetic or antagonistic interactions between VS fractions of individual substrates. Table 2 shows the mixture compositions following the simplex lattice design.

Batch Anaerobic Dry Digestion of the Individual Substrates and Mixtures

Anaerobic batch digestion tests on individual substrates and the co-digestion mixtures were performed according to the method described by Angelidaki, Alves [37]. The assays

were carried out under mesophilic conditions (37 ± 1 °C) using 118 ml serum glass bottles as reactors; each reactor contained 39 ml of inoculum and 1.25 gVS of individual substrates and mixtures, keeping a VS ratio ($VS_{\text{substrate}}$ to VS_{inoculum}) at 1:2 in all setups. The total solid content of the individual substrates and mixtures were adjusted to 20% TS, the mixture compositions used in the various set ups are shown in Table 2. Inoculum and water was used as blanks for the determination of methane production from the inoculum itself. The pH in all the reactors was adjusted to 7.0 ± 1 using 2M NaOH and HCl solution, then the reactors were sealed with rubber septa and aluminum caps, and the headspace was flushed with a gas mixture of 80% N₂ and 20% CO₂ for 2 min to create an anaerobic environment in each setup. The reactors were placed in an incubator at 37 ± 1 °C and were shaken manually once a day during the incubation period of 82 days. All experimental setups were performed in duplicates, and gas samples were taken twice a week during the process and once a week toward the end of the digestion period.

Co-Digestion of the Best Mixture in the Plug Flow Reactors

The best mixing ratio, i.e., the mixture with the highest methane yield, as determined from the batch process was applied in the continuous co-digestion process using plug flow reactors. The reactor has an impeller installed on a hexagonal shaft that runs through the inlet to the outlet. This impeller allowed mixing of the feedstock at the bottom part of the inlet, and transported the materials very slowly towards the outlet taking several rotations depending on the viscosity of feedstock and working volume of reactor. The plug flow reactors worked as described previously [27], with a working volume of 5 L.

The first part of the experiment was to carry out batch digestion in plug flow reactors with the same condition used in the serum bottles (118 ml); this was done to adapt the inoculum to the feedstock. The plug flow reactors

Table 1 Characteristics of the substrates and inoculum (mean values and standard deviations based on triplicate measurements)

Substrates	pH	Bulk density (g/L)	TS (%)	VS (%) [*]	Ash (%) [*]	Total carbon (%) [*]	TKN (%) [*]	C/N	COD gCOD/ gVS _{substrate}
Chicken feather	ND	ND	93.47 ± 0.06	99.26 ± 0.01	0.74 ± 0.01	55.14 ± 0.01	15.50 ± 0.30	3.6	ND
Citrus wastes	3.24	753.60 ± 24.32	23.42 ± 2.23	96.07 ± 0.74	3.93 ± 0.74	53.37 ± 0.41	0.99 ± 0.08	55.5	1.03
Wheat ^a straw	ND	190.47 ± 6.93	89.07 ± 0.08	94.96 ± 0.14	5.04 ± 0.14	52.76 ± 0.08	0.83 ± 0.08	69	0.89
Manure with straw	8.01	542.00 ± 26.87	25.84 ± 0.92	77.21 ± 2.74	22.79 ± 2.74	42.89 ± 1.52	2.26 ± 0.04	19	0.89
Anaerobic sludge	8.19	1006.7 ± 5.52	9.43 ± 0.09	68.29 ± 0.01	29.43 ± 0.35	39.20 ± 0.19	4.73 ± 0.18	8.3	ND

TKN total kjeldahl nitrogen, C/N carbon to nitrogen ratio, COD chemical oxygen demand, ND not determined

^aExtractives 8.27%, Total lignin 16.52%, Cellulose 42.74%, Hemicellulose 27.99%—*Dry basis

Table 2 Mixture compositions used in the batch anaerobic co-digestion process

Mixing ratios ^a	Mixtures	C/N	Mixing compositions							
			Chicken feather (CF)		Citrus wastes (CW)		Wheat straw (WS)		Manure with straw (MS)	
			% ^b g _{added}	% VS _{added}	% ^b g _{added}	% VS _{added}	% ^b g _{added}	% VS _{added}	% ^b g _{added}	% VS _{added}
1:1:1:5	M1	14	3.4	12.5	14.0	12.5	3.7	12.5	78.9	62.5
1:1:5:1	M2	19	6.6	12.5	27.0	12.5	35.9	62.5	30.5	12.5
1:1:0:6	M3	13	3.0	12.5	12.5	12.5	0	0	84.5	75
1:1:6:0	M4	21	8.5	12.5	35.2	12.5	56.2	75	0	0
1:0.5:2.2:4.3	M5	15	3.9	12.5	8.1	6.25	9.3	27.1	78.8	54.2
1:0.5:4.3:2.2	M6	17	5.6	12.5	11.5	6.25	26.6	54.2	56.3	27.1
1:0.5:1.1:5.4	M7	14	3.4	12.5	7.0	6.25	4.0	13.5	85.6	67.7
1:0.5:5.4:1.1	M8	19	7.1	12.5	14.7	6.25	42.3	67.7	35.9	13.5
1:0.5:0:6.5	M9	13	3.0	12.5	6.2	6.25	0	0	90.8	81.3
1:0.5:6.5:0	M10	21	9.8	12.5	20.2	6.25	70	81.3	0	0
1:0.25:2.25:4.5	M11	14	3.9	12.5	4.1	3.125	9.7	28.1	82.3	56.3
1:0.25:4.5:2.25	M12	17	5.7	12.5	5.9	3.125	28.3	56.3	60	28.1
1:0.25:0:6.75	M13	12	3.0	12.5	3.1	3.125	0	0	93.9	84.4
1:0.25:6.75:0	M14	21	10.6	12.5	10.9	3.125	78.5	84.4	0	0
1:0:1.2:5.8	M15	13	3.4	12.5	0	0	4.3	14.6	92.3	72.9
1:0:5.8:1.2	M16	19	7.8	12.5	0	0	49.9	72.9	42.3	14.6
1:0:0:7	M17	12	3.0	12.5	0	0	0	0	97	87.5
1:0:7:0	M18	21	11.5	12.5	0	0	88.5	87.5	0	0

^aRatios of CF: CW: WS: MS, respectively; the mixing ratios are based on VS_{added}

^bWet basis

were loaded with the feedstock at 20% TS and inoculated with anaerobic sludge; VS ratio (VS_{substrate} to VS_{inoculum}) was kept at 1:2, thereby, having a TS of 11% in the reactors. The process was operated in batch mode for 28 days while the TS in the bioreactors was reduced to around 4% TS. Since the co-digestion process was studied under dry digestion, the TS in the reactors were increased to 15% by adding fresh feedstock to the acclimatized inoculum, achieving a VS ratio (VS_{substrate} to VS_{inoculum}) of 2.5:1. Thereafter, the continuous feeding operation began after a startup phase of 32 days. Reactors were fed every day; reactor 1 (R_{TS21}) was fed with a feedstock of 21% TS and reactor 2 (R_{TS32}) was fed with a feedstock of 32% TS. The process was investigated at OLR of 2.0 gVS/L/d (OLR 1) and 3.8 gVS/L/d (OLR 2) with corresponding retention times of 50 and 30 days, respectively. During the feeding, equivalent amounts of the digestate residue were withdrawn from the reactors every day; the digestate was mixed with fresh feedstock in a ratio of 1:2 (feedstock: digestate; wet basis) prior to the feeding. The impeller has described above was rotated manually (once daily) to allow flow of reactor content to the inlet and back to the outlet in order to minimize stratification in the reactor. Process parameters such as the volume of biogas produced, methane content of the produced biogas, pH, TS,

VS, VFA/Alkalinity ratio, total VFA, and total ammonia concentration were monitored regularly during the digestion process.

Analytical Methods

Total solids (TS), VS, pH, and ash content were determined according to the biomass analytical procedures [38]. Total nitrogen contents (TKN) were measured using the Kjeldahl method. The total carbon was obtained by correcting the total dry weight carbon value for the ash content [39], and the bulk density was determined according to Zhang et al. [40]. COD concentrations were analyzed using a COD test kit (Nanocolor, MACHEREY–NAGEL GmbH & Co. KG. Germany), and the concentrations were then measured using a Nanocolor 500D Photometer (MACHEREY–NAGEL GmbH & Co. KG. Germany). Extractives in the WS samples were determined according to the NREL protocol [41], using the Soxhlet method with successive water and ethanol extraction for 24 h. The total carbohydrate and total lignin content of the extractive free straw samples were then determined according to the NREL protocols [42]. The monomeric sugars obtained during the hydrolysis were determined by HPLC; a UV-absorbance detector (Walters 2487) operating at 210 nm

wavelength was used in series with a refractive index (RI) detector (Walters 2414) operating at 60 °C. Mannose, glucose, galactose, xylose, and arabinose were analyzed using an Aminex HPX-87P column (Bio-Rad) at 85 °C and 0.6 mL/min ultrapure water as an eluent. Acid soluble lignin (ASL) was determined using a UV spectrophotometer (Biochrom Ltd., Cambridge, England) at 320 nm. Acid insoluble lignin (AIL) was gravimetrically determined as residual solid after hydrolysis was corrected with the ash content. The ash content was determined as the remaining residue after keeping the samples in the muffle furnace at 575 °C for 24 h.

Samples of the digestates obtained from the reactors in the continuous experiment were centrifuged (5000×g for 10 min); thereafter, the supernatant diluted 50 times was analyzed for total ammonia concentration using the Ammonia rapid test kits (Megazyme, Megazyme International Ireland, Ireland), and the concentrations were measured at 340 nm wavelength using a spectrophotometer (Biochrom Ltd., Cambridge, England). Alkalinity was measured as the total inorganic carbonate and was determined by the Nordmann titration method, according to Lossie and Pütz [43]. Digestate samples were centrifuged at 5000×g for 10 min, and then 5 ml of the supernatant was titrated with 0.1 N sulfuric acid to pH 5; the titration was then continued until pH 4.4 was reached in order to determine the VFA concentration measured as the acetic acid equivalent. Total VFAs in the digestate filtrates were measured using a high-performance liquid chromatograph (HPLC, water 2695, Waters Corporation, Milford, MA, U.S.A.) equipped with an RI detector (Waters 2414, Waters Corporation, Milford, MA, U.S.A.) and a biohydrogen-ion exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, U.S.A.) operating at 60 °C. A UV-absorbance detector (Walters 2487) operating at 210 nm wavelength was used in series with a RI detector (Walters 2414) operating at 60 °C.

Gas samples, taken from the headspace of each batch reactor using a 250 µl pressure-lock gas syringe (VICI, precious sampling Inc., U.S.A.), were analyzed with a gas chromatograph (Perkin-Elmer, U.S.A.) equipped with a packed column (6'×1.8" OD, 80/100, Mesh, Perkin Elmer, U.S.A.) and a thermal conductivity detector (Perkin-Elmer, U.S.A.) with an inject temperature of 150 °C. The carrier gas was nitrogen operated with a flow rate of 20 ml/min at 60 °C. Gas measurement and analysis were carried out as described previously [44]. The biogas volume from the continuous plug flow reactors was measured with a drum-type gas meter (TG 05 Model 5, Ritter, Germany) and was corrected for reporting at standard temperature and pressure (0 °C, and 1 atm) using the ideal gas law. The methane content of the biogas produced was determined with gas chromatograph as described above.

Results and Discussion

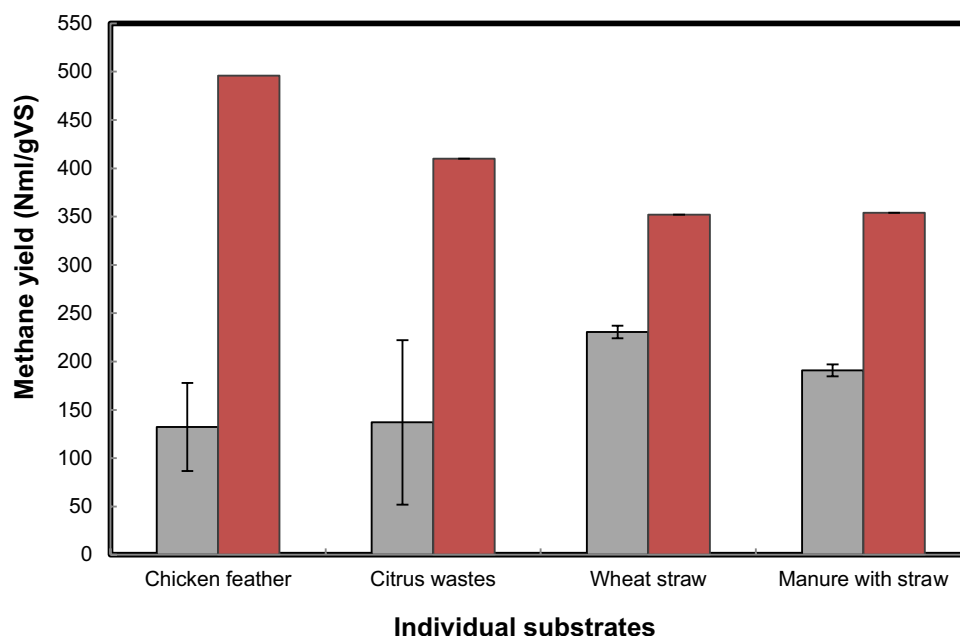
Substrates and Inoculum Composition

Characteristics of the feedstocks examined in this study are presented in Table 1; all the substrates had a solid content of between 23% and 93%, which makes them suitable for dry anaerobic digestion. CF had the lowest C/N ratio (3.6) due its high nitrogen content, while WS had the highest C/N ratio (69) as a result of its high carbon content. CW also contained high carbon, but very low nitrogen, while MS had higher nitrogen content compared to that in citrus waste (CW) and WS. Additionally, CW were the only acidic waste of all the substrates investigated, with its pH of 3.24; moreover, it had previously been reported to contain 3.78% of limonene [45] inhibiting methanogens [15]. All the different mixing ratios examined in this study had a C/N ratio between 12 and 21 which is within the optimal C/N range required for a stable anaerobic digestion process [22, 24].

Methane Yield from Individual Substrates

The theoretical maximum methane potential of the individual feedstocks calculated based on the COD content, together with the experimental results of methane yields obtained during the batch anaerobic digestion are presented in Fig. 1. Mono-digestion of CW resulted in a methane yield of 137 NmlCH₄/gVS, which is 33% of the theoretical maximum yield. This is in accordance with the methane yields of 102 NmlCH₄/gVS [17] and 131 NmlCH₄/gVS (homogenized) [16] for similar substrates obtained earlier under thermophilic wet digestion conditions. CW degrade easily, but the methane yield is low due to the presence of D-limonene which is inhibitory to the microbial community, as reported previously by several researchers [15, 18]. Furthermore, the methane yield from CFs was 132 NmlCH₄/gVS, corresponding to 27% of the theoretical yield. Previously, Patinvoh et al. [3] and Forgács et al. [13] found methane yields of 105 (mesophilic condition) and 180 (thermophilic condition), respectively. These yields were low compared to the theoretical methane yield; this is due to the slow degradation of CFs as a result of its recalcitrant structure. An experimental methane yield of 191 NmlCH₄/gVS was obtained from the cattle MS, which is 54% of the theoretical maximum yield, while 65% of the theoretical methane yield was obtained from the WS, as shown in Fig. 1. Other studies reported methane yields between 100 and 300 NmlCH₄/gVS for the WS and MS obtained under different conditions [5, 9, 27, 28, 46].

Fig. 1 Cumulative methane yield during batch anaerobic digestion and theoretical methane potential obtained from COD compositions of individual substrates. Bars represent: grey (bar) experimental yield and red (bar) theoretical yield. (Color figure online)



Methane Yield from Mixtures—Synergetic and Antagonistic Interactions

Figure 2 shows the cumulative methane production curves obtained for the anaerobic dry co-digestion of CF, CW, WS, and MS during 82 days of digestion period. The compositions of the different mixtures are presented in Table 2. The co-digestion of the various substrate mixtures resulted in methane yields ranging from 165 to 238 NmlCH₄/gVS, with the mixing ratio (CF:CW:WS:MS) of 1:1:6:0 (M4), giving the highest methane yield. Figure 3 shows the comparison between the experimental values and the response variables (methane yield) obtained from the linear and quadratic mixing models.

In this work, the expected and predicted methane yields from all the mixtures were modeled using the linear mixing model and the quadratic mixing model respectively. Corresponding methane yields are presented in Fig. 3. Of the mixing models, mixing ratios (CF:CW:WS:MS) of 1:1:5:1 (M2) and 1:1:6:0 (M4) corresponding to C/N ratios of 19 and 21, respectively, gave the best performance, i.e., the methane yield was increased by 12% and 14%, respectively, compared to values calculated with the linear model. On the other hand, mixing ratios of 1:0:5.8:1.2 (M16) and 1:0:7:0 (M18) having the same C/N ratios of 19 and 21 showed antagonistic effects, since the measured methane yields and the calculated methane yields using the quadratic model were 9% and 11% lower compared to the values calculated according to the linear model, as shown in Fig. 3.

The experimental results showed synergistic or antagonistic effects, but these were not statistically significant (p -value > 0.05). The results from the mixing ratios showed

that the higher the amount of CW and WS in the mixture, the better the methane yield. CF and MS are the ones contributing least to the methane yield; however, the addition of CF and manure may have contributed to digestion buffering capacity through the release of ammonium cations. Co-digestion of CW with CF and WS with a mixing ratio of 1:1:6:0 (M4) gave the highest methane yield; it was therefore chosen for further investigations in continuous process.

Best Mixing Ratio in Continuous Process

Adaptation Phase

This phase was carried out in batch mode using the best mixing ratio; this allows for microbial adaptation and conditioning of the feedstock prior to the continuous process. This phase lasted for 28 days, and the daily as well as the cumulative biogas production are presented in Fig. 4. The anaerobic digestion of the best mixing ratio of 1:1:6:0 (M4) in the plug flow reactors were stable. The cumulative methane yield was 270 and 282 NmlCH₄/gVS in reactor 1 (R₁) and reactor 2 (R₂) respectively. During the 28 day of digestion, 57 l and 60 l of biogas was produced in R₁ and R₂, resulting in a TS reduction of 64% and 73%, respectively. The methane content was 63% ± 2.5 in R₁ and 62% ± 2.9 in R₂. The methane yield obtained during this adaptation phase in the plug flow reactors was between 13% and 18% higher than that obtained from this mixing ratio (M4) during the batch digestion test. Minimal mixing in the plug flow reactor provides good contact between the microorganisms and the feedstock, thereby improving the degradation rate and the methane yield. After this adaptation period, and since the

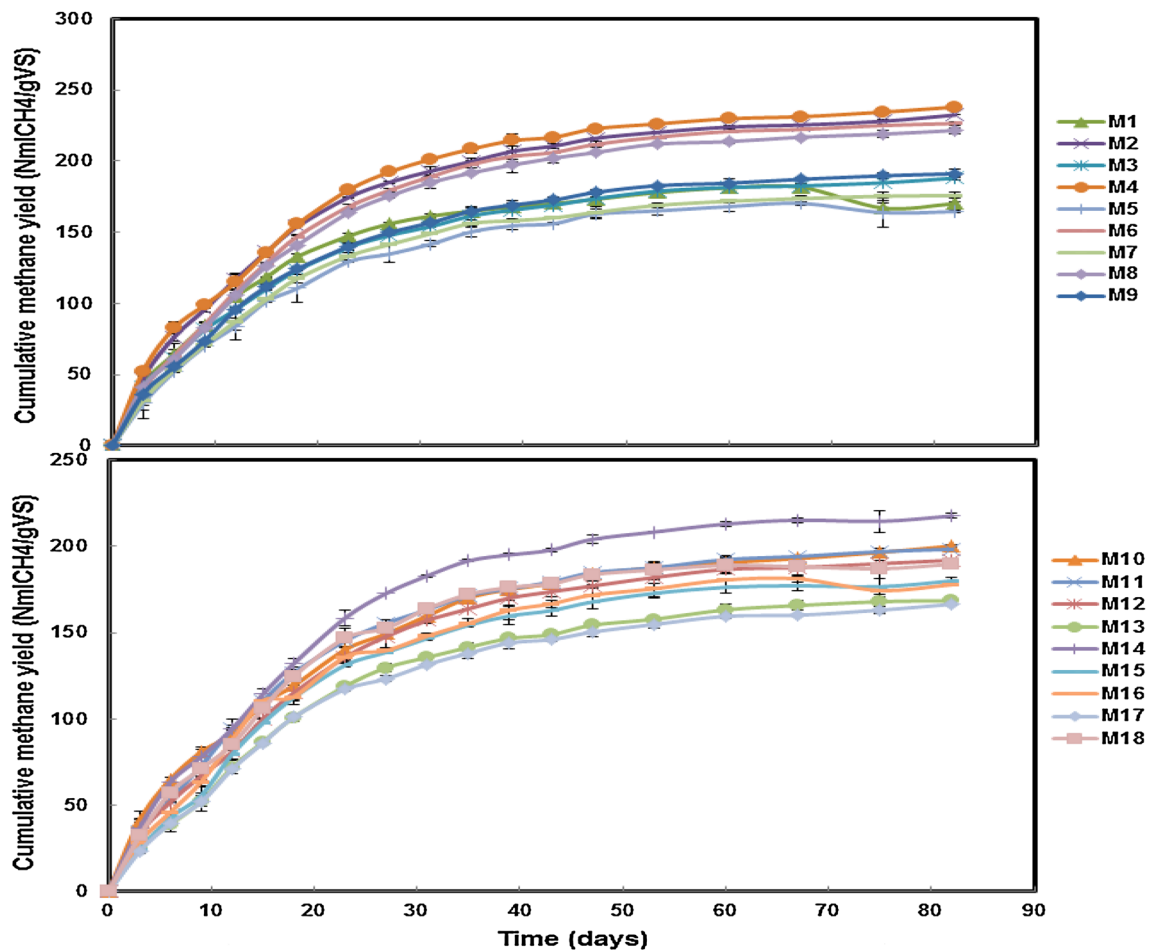


Fig. 2 Cumulative methane yield obtained during the dry co-digestion of the various mixing ratios in batch experiments. The composition of the different mixtures (i.e., M1...M18) can be found in Table 2

co-digestion process was aimed to be studied at dry digestion conditions, the TS in the reactors was increased to 15% by adding fresh feedstock to the acclimatized inoculum, keeping a VS ratio ($VS_{\text{substrate}}$ to VS_{inoculum}) at 2.5:1.

Process Performance at Different Loading Rates

The continuous digestion process started after the startup period; continuous operations were carried out at OLR of 2.0 gVS/L/d (OLR 1) and thereafter at 3.8 gVS/L/d (OLR 2) in both reactors. The daily biogas production and the methane content in the produced biogas during the continuous operation at different OLR are presented in Fig. 5. During OLR 1, a biogas production of about 4 L/d was obtained from both the reactors at the beginning, which increased slightly and became stable toward the end of the digestion period at OLR1, showing an average of 6.1 L/d in R_{TS21} and 5.7 L/d in R_{TS32} . The methane proportion in the biogas was 60% in R_{TS21} and 57% in R_{TS32} . The methane yield in R_{TS21} was 362 NmlCH₄/gVS_{added}, which is 13.5% higher than the

yield obtained from R_{TS32} (319 NmlCH₄/gVS_{added}). Chen et al. [47] also reported a decrease in the biogas yield during the continuous dry digestion of swine manure as the TS in the feedstock was increased from 20% to 35%. However, when the organic loading rate was increased to 3.8 gVS/L/d (OLR 2), the daily biogas production increased slightly at the beginning, but this could not be sustained and the biogas production dropped as feeding continued. The methane content also dropped slightly, to an average of 58% in R_{TS21} and 56% in R_{TS32} .

The pH was stable, between 7.4 and 7.8 for both reactors, during the digestion period at OLR1 (2.0 gVS/L/d), as shown in Fig. 6, in both reactors. Nevertheless, the pH decreased slightly as the OLR was increased to 3.8 gVS/L/d but did not drop below 7.0; hence, it was within the favorable pH range of 6.8–8 for the anaerobic digestion processes [48, 49]. At OLR of 2.0 gVS/L/d (OLR 1), the VFA/alkalinity ratio was below 0.3 for both reactors, which is also within the optimum range required for a stable operation [48]. Furthermore, the total VFA was below 1 g/l during this organic loading rate

Fig. 3 Experimental values and response variables obtained from the various mixtures investigated. Bars represent: grey (bar) methane yield from the quadratic mixing model (predicted yield), red (bar) methane yield from the linear mixing model (expected yield) and green (bar) obtained experimental yield. The composition of the different mixtures (i.e., M1...M18) can be found in Table 2. (Color figure online)

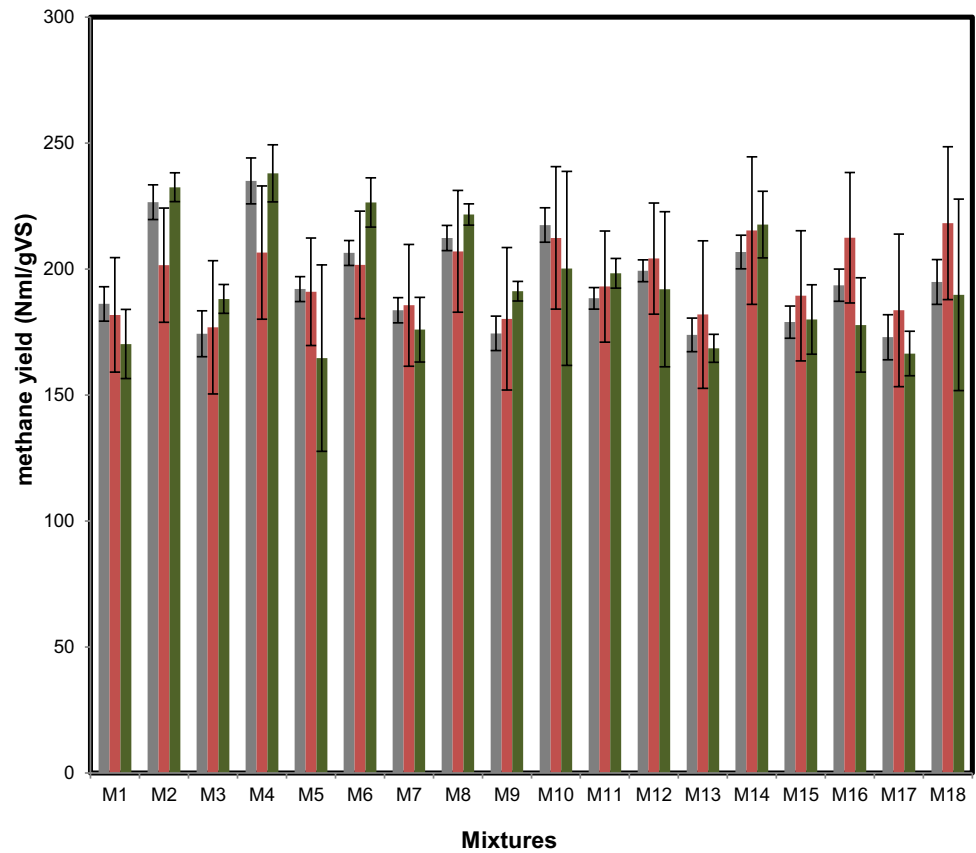
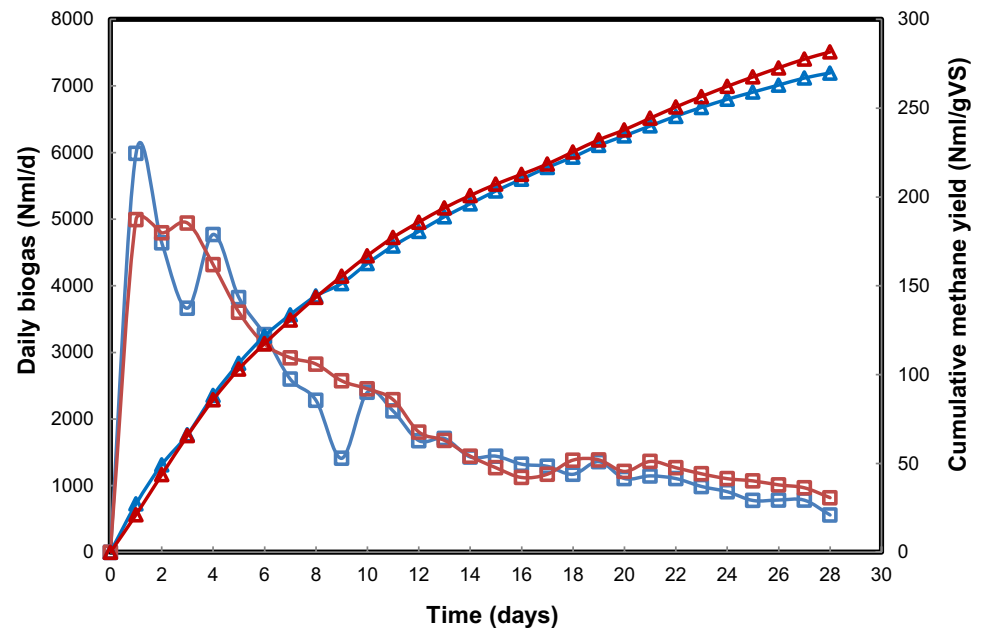


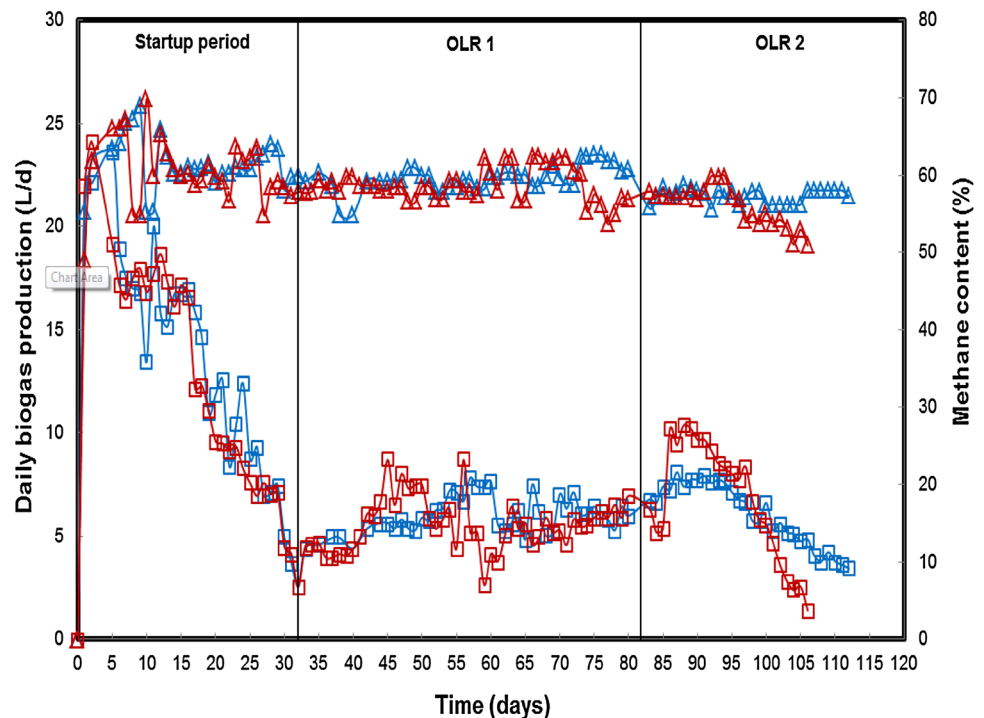
Fig. 4 Daily biogas production and cumulative methane yield obtained during the adaptation phase carried out in batch mode for digestion of the best mixture (M4) in plug flow reactors. Colors represent: blue for reactor 1 (R1) and red for reactor 2 (R2). Symbols represent: square for daily biogas production and triangle for cumulative methane yield. (Color figure online)



signifying a stable process [48]. However, the stability of the process declined as the OLR increased to 3.8 gVS/L/d, with a corresponding retention time of 30 days. The total VFA concentration increased to 8 g/l in R1 and to 4.4 g/l in R2 by

the end of the digestion period together with the VFA/alkalinity ratio, which increased sharply, indicating problems in both the reactors (Fig. 6). This resulted in a decline in the biogas production, as shown in Fig. 5. In this study, process

Fig. 5 Daily biogas production and the methane content of the produced biogas in reactor 1 (R_{TS21}) and reactor 2 (R_{TS32}) during continuous operation; OLR 1 (2 gVS/L/d for 50 days) and OLR 2 (3.8 gVS/L/d for 30 days). Colors represent: blue for reactor 1 (R_{TS21}) and red for reactor 2 (R_{TS32}). Symbols represent: square for daily biogas production and triangle for methane content of biogas produced. (Color figure online)



instability was observed at a lower organic loading rate of 3.8 gVS/L/d compared to an OLR of six gVS/L/d reported previously for instability while treating MS at 22% TS using the continuous plug flow reactor [27]. This can be attributed to the presence of CW in the co-digestion mixture in this study. For rapidly biodegradable substrates, such as CW, the acidogenic reactions can occur quickly, resulting in VFA accumulation [50]. Co-digestion of fruit, vegetable, and food wastes have been reported to be less stable at an $OLR > 2.0$ gVS/L/d [51] because of the accumulation of propionate. In addition, the total ammonia concentration was slightly higher in R_{TS32} than in R_{TS21} , as shown in Fig. 6; this could be the result of the moisture content reduction in R_{TS32} . Chen et al. [47] also reported high ammonia nitrogen concentration when the TS concentration of feedstock was increased from 20% to 35% in continuous dry fermentation of swine manure.

At OLR1 (2.0 gVS/L/d), the TS content of digestate was $9.8\% \pm 0.34$ in R_{TS21} and 11.12 ± 0.69 in R_{TS32} . As the OLR increased to 3.8 gVS/L/d, the TS content of the digestate

increased to $10.02 \pm 0.55\%$ in R_{TS21} and to $12.1 \pm 0.45\%$ in R_{TS32} . Additionally, reactor 2 (R_{TS32}), which was fed with a feedstock of 32% TS, was blocked toward the end of feeding with OLR of 3.8 gVS/L/d; there were problems both with the feeding and with the discharge of the digestate residue.

Conclusions

Co-digestion of CW, CF and WS with a mixing ratio of 1:1:6 gave the best performance in batch dry digestion process, with a methane yield of 238 Nml/gVS. The methane yields obtained during the adaptation phase in the plug flow reactors were between 13% and 18% higher than the obtained yield from the best mixture (M4 i.e., 1:1:6) during the batch anaerobic digestion process. Continuous dry digestion of the best mixture at 21% TS and 32% TS in the feed was successful with OLR of 2 gVS/L/d and corresponding retention time of 50 days. OLR of 3.8 gVS/L/d with corresponding retention time of 30 days favoured process instability at both total solid content of feed considered (21% TS and 32% TS). This instability in the process performance resulted into volatile fatty acids accumulation and subsequent reduction in biogas production.

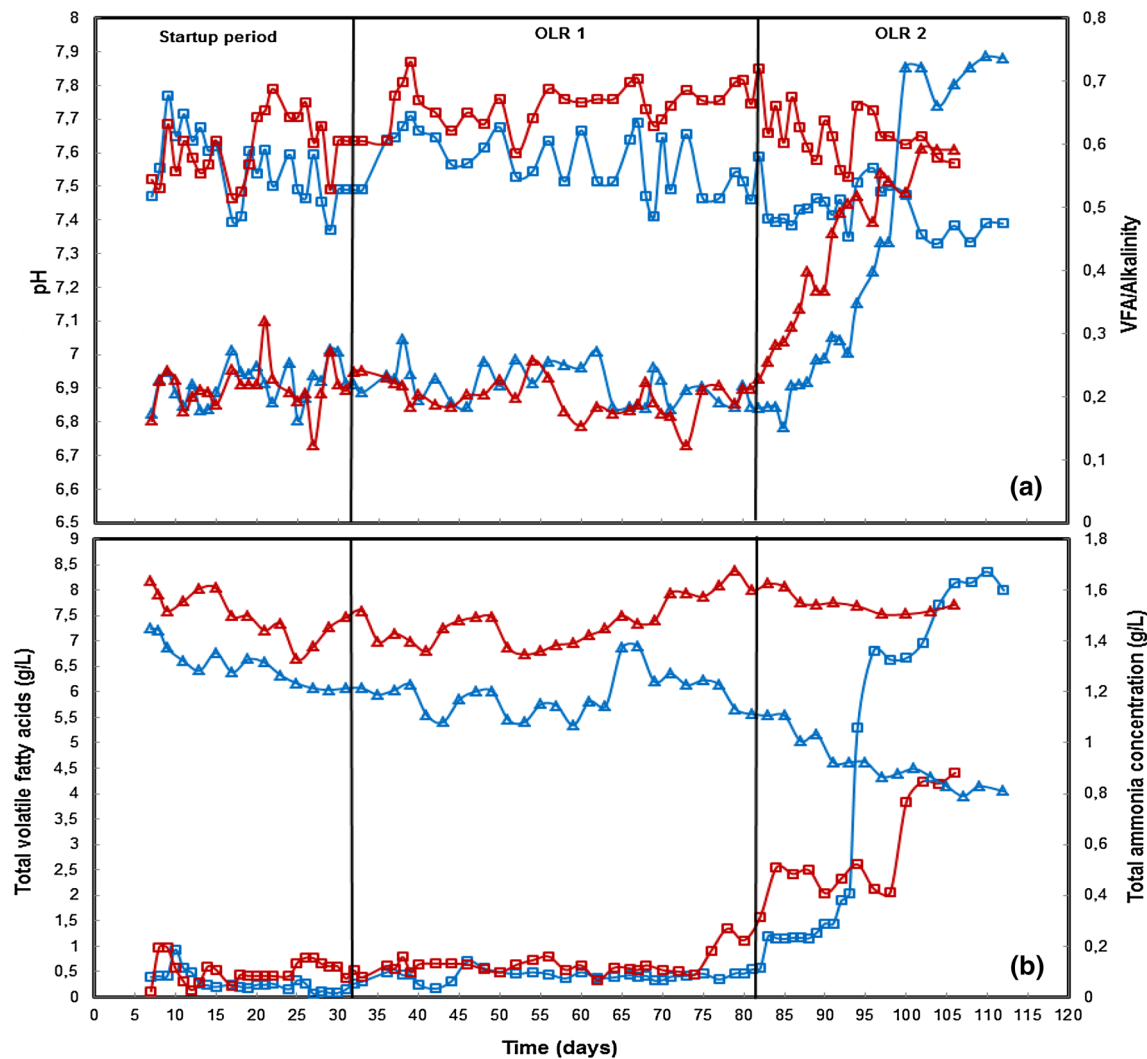


Fig. 6 Variation in pH, VFA/alkalinity ratio, total VFA, and total ammonia concentration in reactor 1 (R_{TS21}) and reactor 2 (R_{TS32}) during continuous operation; OLR 1 (2 gVS/L/d for 50 days) and OLR 2 (3.8 gVS/L/d for 30 days). Colors represent: blue for reactor 1 (R_{TS21})

and red for reactor 2 (R_{TS32}). Symbols represent: **a** Square and triangle for pH and VFA/alkalinity ratio respectively **b** square and triangle for total VFAs concentration and total ammonia concentration respectively. (Color figure online)

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