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Bio-pretreatment Enhances Biogas Production from Co-digestion of Rice Straw and Pig Manure

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ARTICLE INFO

Article history:
Received 16 July 2021
Received in revised form
11 October 2021
Accepted 18 October 2021

Keywords:
Anaerobic co-digestion
Biogas production
Biological pretreatment
Pig manure
Rice straw

ABSTRACT

Co-digestion between biomass and livestock waste increases methane production by providing an optimal C/N ratio. Also, bio-pretreatment has received more attention due to its effectiveness in biomass-derived material hydrolysis into biodegradable carbohydrates. Rice straw (RS) is abundant in the Vietnamese Mekong Delta (VMD), which potentially enhances biogas production from co-digestion with pig manure (PM) in case of shortage of livestock waste for biogas digesters. However, the high solid content of RS and its higher C/N ratio generally results in the low productivity of biogas when used as sole substrate. Therefore, we assessed the efficiency of biological pretreatment of RS on biogas production through single-stage batch anaerobic digestion under mesophilic conditions. The substrate ratio-based on volatile-solid (VS) rate was used at a 1:1 mixture (RS:PM) with a total concentration supplemented at 45 g-VS/L over a 60-day batch digestion. The bio-solutions included de-chlorinated tap water (TW), digester effluent (DE), ditch water (DW), and anoxic sediment (AS). The findings demonstrated that the pretreatment of RS enhanced biogas production by 78-84% compared with PM digestion without RS or bio-pretreatment. Likewise, AS and DE bio-solutions achieved the highest methane yield, which increased between 51% and 58%. Overall, the methane content (v/v) ranged from 50% to 55% during the stable phase, with VS removal efficiencies ranging from 39 to 46%. This study shows that DE and AS inoculums are feasible approaches for obtaining significant increases in biogas yields in co-digestion of RS and PM. Bio-solution pretreatment experimentation on distinctive biomass-derived materials under a series of C/N ratios is suggested.

1. INTRODUCTION

An increase in the standard of living is usually accompanied by increased consumption of fossil fuels and undesirable CO_2 emissions. Biogas, a renewable and eco-friendly energy source, is produced from agricultural residues, animal manures, or biodegradable wastes. Bioenergy can potentially substitute conventional sources of energy and, as such, provide a basis for sustainable economic development [1]. A further advantage of biogas over other renewable energy sources is its affordability as low-tech process that promote its wide use in most households [2]. In recent years, anaerobic digestion has been increasingly applied in Vietnam. It is considered as a sustainable long-term solution to tackle environmental pollution caused by ineffective livestock waste management [3]. At the farm scale, biogas is used for cooking and heating (heat lamps), saving the routine LPG and firewood usage. The use of biogas not only provides cheap CO_2-neutral energy but also upgrades the women's working environment, reduces unpleasant smells, pathogens, flies, and decreases the heavy workload for farmers, who spend a considerable amount of time collecting firewood [1]. Although biogas consumption offers socio-economic and environmental advantages, the fundamental limitation for expanding biogas systems is the frequent shortage of livestock wastes at most local farms.

In the VMD, many family farms have a small standing stock of pigs (permanently or momentarily), and small-scale application of biogas digester has long been applied in the rural areas. However, producing biogas at small-scale pig farms encounters a deficiency of pig manure (PM), leading to the scarcity of biogas for household use [4]. Moreover, PM is a poor substrate for biogas production owing to its low C/N content. Also, PM is characterized by high alkalinity and its high N content may result in toxic ammonia levels and possibly inhibiting methanogenesis in the biogas reactor [5], [6]. Therefore, co-digestion of mixed substrates for biogas production has recently attracted more interest.

Rice straw (RS) is one of the most abundant agricultural residue materials in the world [7].

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Approximately 650–975 Mt/year of RS is produced based on global rice production [8]. In the VMD, the RS is estimated at 26 Mt/year [9]. Thus, RS has a considerable potential for production of sustainable renewable energy. However, in most cases, the open burning of residue RS is the common practice for most ricegrowers in intensive rice production systems. The combustion of RS not only cause significant air pollution and public health risks but also squanders a renewable energy resource [8], [10]. Co-digestion of RS and PM provides a more flexibly digestion process in small livestock household digesters and potentially enhances biogas production via adjusting the C/N ratio to a more favorable range [1]. Several studies show that co-digestion of agricultural residues and PM have a colossal potential to generate biogas [5], [11]–[14]. However, RS contains 44.3% cellulose, 20.4% lignin, and 35.5% hemicellulose [15], which considerably restricts the hydrolysis efficiency resulting in low methane production. Therefore, the "breaking up" of recalcitrant structures is expected to accelerate anaerobic microbial activities and biogas production.

Recently various pretreatment producers have gained more attention due to its efficacy in accelerating hydrolysis of biomass-derived material. Pretreatment enables recalcitrant structural parts in RS to become more biodegradable, which are more appropriate for anaerobic digestion [14], [16]. There are several distinctive pretreatment methods, such as physical, chemical, biological, and combined approaches. Among the above-mentioned techniques, the bio-pretreatment approach shows a great promise as a safe and eco-friendly processes for increasing the biodigestability of lignocellulosic material with little generation of metabolic inhibitors. Whilst, physio-chemical pretreatments often require high production costs, complex production technologies, high energy consumption, use of harsh and toxic chemicals often resulting in generation of inhibitors of the anaerobic digestion process [17]. The effectiveness of bio-pretreatment is known as the lignin eliminations from lignocellulose via degradation by fungi, enzymes and microbial consortia [15], [18]. Accordingly, the bio-pretreatment method is not discharging toxic substances to the environment and focuses on the reutilization of chemicals.

Bio-pretreatment is effected by soaking materials in different inoculums such as digester effluent (DE) discharged from operating biogas digesters; anoxic sediment (AS) collected from drains; contaminated surface waters taken (DW) from stagnant ditches/ponds; or even in the tap water (TW). These bio-pretreatment processes are a waste-to-waste approach that take advantage of complex microbial populations available in these inoculums for pretreatment without additional microbial supplementation. In this way, several studies have used agro-residues fermented in industrial effluents or sewage sludge as an effective measure to enhance the biogas yield [15], [19]. Thus, the remarkable highlight of the present study is to demonstrate the utility of bio-pretreatment, especially, in relation to small-scale farms which often lack livestock wastes and expecting to use RS as input feedstocks for biogas production. Thereby, the research approach has considerable transferable potential. To date, there has been no reported field study comparing the effectiveness of the bio-solutions in enhancing biogas yield from co-digestion of RS and PM. Hence, we examined the effects of RS bio-pretreatment and co-digestion of bio-pretreated RS and PM on biogas production using different bio-solutions (inocula) in order to determine the applicability of this approach for household biogas digesters of the VMD. We, therefore, conducted a 60-day lab-scale batch reactor experiment. RS was soaked in 4 inoculums, including TW, DW, DE, and AS, for a 5-day pretreatment period.

2. METHODOLOGY

2.1 Collection and Preparation of Substrates and Inocula

RS was obtained from paddy fields in Can Tho city, Vietnam. The collected RS was then sundried at ambient temperature and chopped into small particles of around 1.39±0.14 cm (n=100). Fresh PM was collected from a pig farm in Can Tho city. The PM was also dried in a cool place and then grinded into fine particles (approximately <1 mm).

The pretreatment bio-solutions were prepared as follows: (i) TW: residual chlorine was removed by aerating for 24 hours continuously, (ii) DE was taken from a household polyethylene biodigester operated on the PM feedstock, (iii) DW: was obtained from a non-drained pond that directly received wastewater from a canteen in Can Tho University; the pond was used for breeding of *Pangasius*, and (iv) AS: was collected from a domestic wastewater drainage ditch in Can Tho University; it was then diluted by adding 1-L AS to 9-L de-chlorinated TW. The characteristics of the substrates and the four-pretreatment bio-solutions are presented in Table 1 and Figure 1.

2.2 Experimental Design

Reactors consisted of 21-L plastic containers with a 17-L fermenting volume. A 4-L free space volume was left at the top of the reactor for collecting the biogas generated. All reactors were started with an initial loading rate of 45 g-VS/L. Each digester thus contained 765 g-VS at a mixing ratio of RS:PM, 50:50 based on VS. For bio-pretreatments, a equal amount of RS (382.5 g-VS) was subjected to 10-L of each bio-solution (TW, DE, DW, and AS) and incubated for 5 days. RS bio-treatment solutions were mixed daily to ensure homogeneity among treatments. On day 5, air-dried PM (382.5 g-VS) was added to all reactors and filled with 7-L de-chlorinated TW to reach a 17-L mixture volume. Meanwhile, the control treatment was comprised of 765 g-VS of PM in 17-L de-chlorinated TW. After that, 200 ml of fresh DE (inoculum) was inoculated into all reactors to initiate biomethanation. All experimental treatments were simultaneously performed in 5 replicates over a 60-day digestion period. Each reactor was equipped with a sampling outlet and a gas sampling port. The lid was sealed with a 3 mm thick rubber disc pierced by a gas outlet pipe connected to a 20-L aluminum foil gas bag. The reactors were placed in a screenhouse at mesophile conditions. Batch reactors were wrapped in thick black plastic to avoid direct sunlight and photosynthesis that produce oxygen from cyanobacteria during the incubation period. Once a day, just prior to gas collection, the content of each reactor was manually shaken to achieve consistency in anaerobic digestion. Detailed information on the amount of RS and PM added into each reactor and control treatment is summarized in Table 2.

Table 1. Main characteristic of substrates and pretreatment inoculums.

Characteristic	Unit	RS	PM	TW	DE	DW	AS	Inoculum
Moisture content	%	12.3	7.1	ND	99.58	99.95	98.33	99.60
Total solids (TS)	% (w)	87.7	92.9	ND	0.42	0.05	1.67	0.40
Volatile solids (VS)	% (w)	73.6	66.1	ND	0.23	0.02	0.36	0.21
VS/TS	%	83.9	71.2	ND	54.2	41.8	21.4	53.6
TKN	%TS	0.92	1.99	ND	250†	15†	52†	236†
TOC	%TS	48.7	41.3	ND	ND	ND	ND	ND
C/N	-	52.9	20.8	ND	ND	ND	ND	ND
pH	-	ND	ND	7.28	7.96	7.22	7.37	7.96
Alkalinity	mg$CaCO_3$ L^{-1}	ND	ND	65	938	138	350	1,093

Note: "†" - mg L^{-1}, ND: - not determined

Table 2. Experimental design.

Treatment	RS:PM	C/N	RS (g)	PM (g)	TW (L)	DE (L)	DW (L)	AS (L)	Inoculums (L)	Total volume (L)
A	0:100	20.8	NA	765.0	17.0	NA	NA	NA	0.2	17.2
B	50:50	37.0	382.5	382.5	17.0	NA	NA	NA	0.2	17.2
C	50:50	37.0	382.5	382.5	7.0	10.0	NA	NA	0.2	17.2
D	50:50	37.0	382.5	382.5	7.0	NA	10.0	NA	0.2	17.2
E	50:50	37.0	382.5	382.5	7.0	NA	NA	10.0	0.2	17.2

Note: "NA" - not applicable

Fig. 1. Material preparation and experimental setup.

2.3 *Analytical Method*

The biogas collected in the aluminum foil bags and measured daily using a gas flow meter (TG 02, Ritter, Germany). The methane concentration of the biogas was measured by using a Shimadzu GC (2014AT, Shimadzu, Japan) gas chromatograph with a thermal conductivity detector (TCD) and a 60/80 Carboxen-1000 column (L x O.D x I.D: 4.57m x 3.1 mm x 2.1 mm). The operational temperatures of the injection port, column oven, and detector were 240°C, 180°C, and 240°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 10 mL/min. A standard gas mixture (Air Liquids Ltd., Singapore) composed of 49.95% methane, 30.05% carbon dioxide in nitrogen was used for calibration. A 2.5 mL gas-tight Samplelock® syringe (Hamilton, USA) was used for gas sampling.

The pH of digester liquids was measured directly in the reactors through the sampling outlet using a digital pH meter (pH 6+, ±0.01 pH accuracy, EUTECH

Instrument, Singapore). Parameters of substrates and bio-solutions were analyzed in accordance with Standard Methods for the Examination of Water and Wastewater (SMEWW) of the American Public Health Association (APHA) [20] - total solids (TS) and volatile solids (VS) were detected by drying to constant weight in oven at 105°C (SMEWW 2540 B), and igniting in muffle furnace at 550°C for 2 h (SMEWW 2540 E), respectively. Total organic carbon (TOC) was detected by the High-Temperature Combustion Method (SMEWW 5310B), total nitrogen (TKN) was detected by semi-Micro-Kjeldahl Method (SMEWW, 4500-N_{org} C) for bio-solutions, total alkalinity was detected by the Titration Method (SMEWW, 2320B).

2.4 Data Analysis

All data were checked and transformed as appropriate to meet variance homogeneity requirements. One-way ANOVA and Duncan's post-hoc test were used for multiple comparisons of cumulative biogas production (based on VS added) and specific methane yield (based on VS degraded). An alpha (α) level of 0.05 was used to determine the statistically significant difference. The analysis was performed using IBM SPSS 22.0. Graphs were plotted by using Sigma Plot software version 12.0.

3. RESULTS AND DISCUSSION

3.1 Biogas production

Figure 2 illustrates the daily and cumulative biogas production in reactors during 60 days of operation. The results obviously indicated that RS treated by bio-solutions (B, C, D and E) generated biogas earlier than the control treatment (A). Particularly, the control treatment produced unmeasurable amounts of biogas until day 6, while biogas production started from the first day in treatments of RS pretreated in TW (reactor B), DE (reactor C), and AS (reactor E), and on the second day in reactor pretreated with DW (reactor D). During the experiment, one of the key findings is that reactors C and E displayed a similar trend in biogas production. Initially, the volume of biogas production recorded from reactors of C and E varied between 3.79 and 2.47 L/kg-VS_{added}, respectively, whereas reactor B produced 0.68 L/kg-VS_{added}; the biogas generation in reactor D measured 0.69 L/kg-VS_{added} on the second day. The control treatment reactor produced 2.57 L/kg-VS_{added} after 6 days. These findings indicate that pretreatment of RS using bio-solutions reduced the lag phase of biogas generation by 6 days for the reactors of B, C and E, and 5 days in reactor D compared to the control treatment (A). The above observation revealed that bio-solutions reliably influenced the breakup of recalcitrant structures during the hydrolysis step and accelerated the earlier biogas production phase in reactors. It was observed that RS pretreated in DE (reactor C) showed the highest potential in increasing the stimulation of the biogas production phase amidst examined bio-solutions.

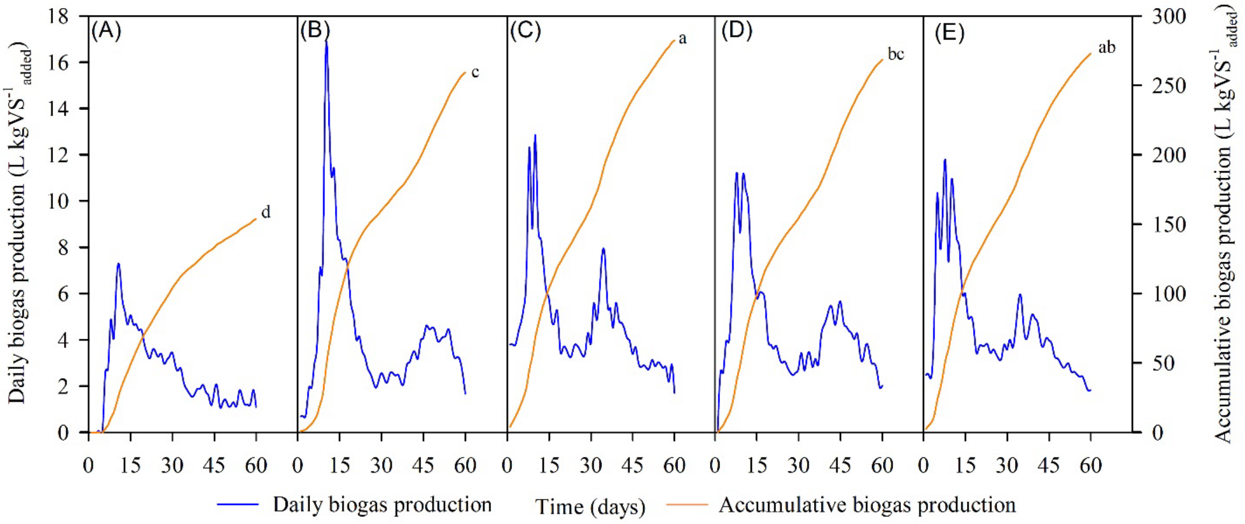

Fig. 2. Daily and cumulative biogas production from reactors. (A), reactor A (control treatment); (B), reactor B (tap water); (C), reactor C (digester effluent); (D), reactor D (ditch water); (E), reactor E (anoxic sediment). Lowercase letters indicate significant differences within the group of value of accumulative biogas production rates. At least a similar letter shows insignificant at *P* = 0.05 by one-way ANOVA multiple comparisons.

Figure 2 shows that two peaks were observed on the graphs depicting daily biogas production in all reactors over the 60-day digestion experiment, except for the control treatment (A). The first peak occurred on the 10th day in reactors B, C, D and E, while the second peak for these treatments was observed on the 46th, 34th, 45th and 35th day, respectively. The second peaks of daily biogas production were all lower than that of the first. The different peak magnitudes of reactors B, C, D and E were 11.67, 5.21, 5.40, and 4.84 L/kg-VS_{added}, respectively. The gap between the first and second peaks was small, indicating the effectiveness of bio-solutions in progressing biogas production due to the acceleration of biogas production. In addition, 2 peaks are representative for the co-digestion of RS and PM. It is suggested that the appearance of the first peak is largely due to methane production by PM, as it corresponds to the peak of reactor A. Whilst the second peak is mainly contributed from RS decomposition because lignocellulose structure of the straw retards degradation. The data, therefore suggest that co-digestion and bio-pretreatment process not only increased the efficiency of biogas production, but also provided a long-term stability when compared to mono-digestion (rector A).

As can be seen from Figure 2, daily biogas production had significantly decreased at the termination day, which indicates that the 60-day operation for a co-digestion batch of RS and PM or mono-digestion (PM) was suitable. On the other hand, if the operation time was prolonged, biogas production would decrease further. Figure 2 shows that the mean accumulative biogas production in reactors B, C, D and E was always higher than that of reactor A ($p > 0.05$) after 60 days. The difference was 106, 129, 115, and 119 L/kg-VS$_{\text{added}}$, respectively. This large disparity strongly indicates the effectiveness of bio-pretreatment applying different bio-solutions. Consequently, the efficacy of bio-solutions is ranked according to the priority order of reactors as following (i) DE (reactor C) > (ii) AS (reactor E)> (iii) DW (reactor D)> (iv) TW (reactor B). However, according to statistical analysis, the cumulative biogas production between rector C and reactor E treatment was not significantly different ($p>0.05$). Both show significantly higher cumulative production than treatments using DW (reactor A) and TW (reactor D). The five-day pretreated RS in both DE (reactor C) and AS (reactor E), enhanced the proportion of biogas production between 78 and 84%, respectively as compared to the control treatment digestion (100% PM without pretreatment), while TW and DW were 69-75% higher compared to that of the control treatment.

In general, the variation of biogas production in reactors is mainly effected by hydrolysis, acidogenesis [14], and digestible substrates are biologically converted by a diverse microbial group acting synergistically in anaerobic environments [21], [22]. The efficacy of pretreatment by bio-solutions could be partly attributed to (i) pretreatment by bio-solutions promote breakdown of recalcitrant straw such as cellulose, and hemicellulose, into more digestible matters and accumulates essential nutrients in reactors that are rapidly used by methanogenic microbial communities after commencing anaerobic digestion [14]; (ii) co-digestion maintains a C/N ratio balance that is favorable for microbial activities and methanogenesis. Thus, the combination of bio-pretreatment and co-digestion would be a feasible technology for promoting the usage of agricultural residues and livestock waste.

Overall, the results suggest that the soaking of dried biomass in bio-solutions could bring advantages when up-scaling to semi-continuous digestion in farm-scale biogas systems. This is because it reduces the absorption of water from the digester liquid and introduces water into air-filled intercellular spaces of the biomass materials. Therefore, it possibly lessens problems of floating materials often associated with the usage of biomass feedstock [23]. In general, all pretreatments of RS in combination PM were seen to boost biogas generation by 80% compared to reactors digesting PM solely. In the VMD, RS is currently the dominant substrate used for biogas fermentation in rural households. Hence, the results demonstrate that RS is a fully promising supplementary substrate for biogas digesters when RS and PM are mixed in a 1:1 ratio based on VS.

3.2 Methane Concentration

The methane content of biogas reflects the efficacy of anaerobic digestion. Figure 3 shows that the methane concentration (v/v) varied in reactors during the 60-day operation. The results illustrate that methane concentration was produced after a 3-day startup period in bio-solution treatments, whilst after 5-days in the control treatment. The absence of methane content in the earlier stage indicates that acidogenesis processes were dominant [24]. Methane was generated earlier in reactors with bio-pretreatment treatments, indicating that bio-solutions actually stimulated the acceleration of methane generation, compared to control treatment. The methane concentration in the 4 reactors fluctuated between 10.34 and 37.65% at the beginning of the digestion period, in which reactor C and reactor E showed higher methane concentrations compared to reactors A, B, and D. As can be seen from Figure 4, it is apparent that the methane concentration was greater than 50% after 7 days of digestion (reactor D and E), while the control treatment and reactors of B and C reached this level after 8 days of operation.

It is indicated that stabilization of methane formation started after 8 days digestion which was faster than reported in the study of Lee *et al.* [25] using RS digested in anaerobic sludge with the phosphate supplementation (30 days) and lower than that of Ye *et al.* [24] utilizing a co-digestion of RS, PM and kitchen wastes (3 days). Comparing reactors (B, C, D and E) and reactor A, although treatment of RS with bio-solutions could promote initiation of methane concentrations between reactors, the difference was also insignificantly in the stable period (after 8 days). Particularly, average methane concentration among reactors of B, C, D and E was 54.7, 54.8, 54.2 and 55.0%, respectively, while reactor A (control treatment) was 53.7% which were similar to the findings reported in previous studies [24], [26]–[28]. The maximum methane content in reactors was recorded between 60.1% and 66.8%. The highest methane concentration was obtained using DE bio-solutions (66.8%). However, methane production and its compositions depend on the substrate characteristics and liquid phases [24], [26].

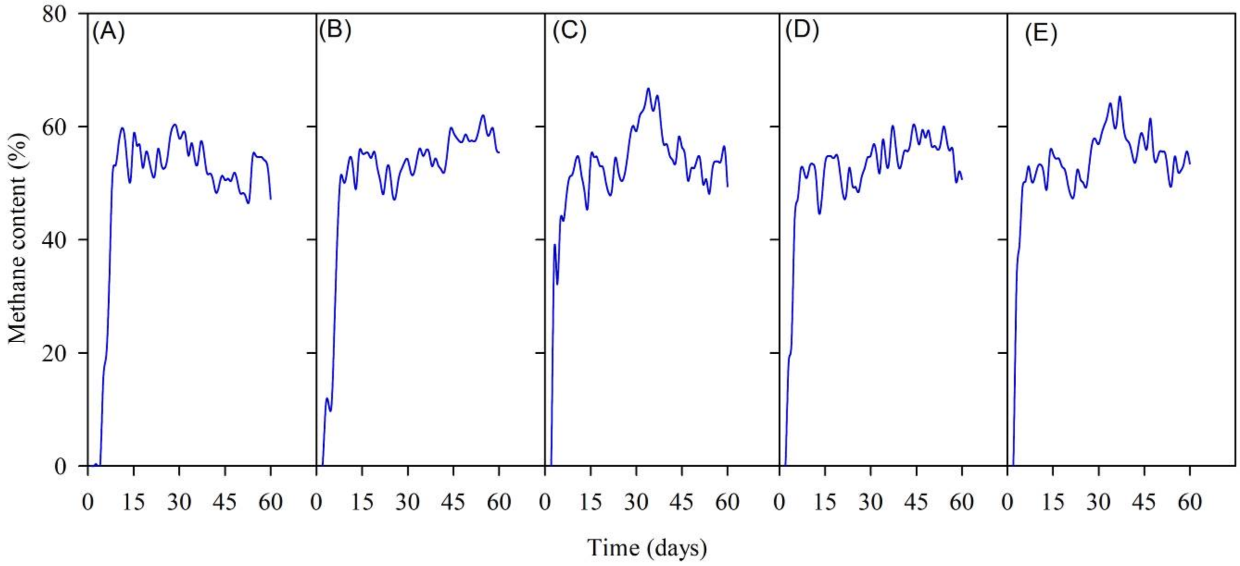

Fig. 3. Methane content of biogas from reactors A, B, C, D and E. A), reactor A (control treatment); (B), reactor B (tap water); (C) reactor C, (digester effluent); (D), reactor D (ditch water); (E), reactor E (anoxic sediment).

3.3 Specific Methane Yield

Figure 4 depicts specific methane yields for all reactors over 60 days of digestion. The methane yields have been calculated based on the mass of VS input in the liquid samples (Table 2). The methane yields in reactors containing RS bio-treated biologically was higher than that of the control treatment. AS showed the highest methane yield among reactors with RS pretreated by the four bio-solutions (370 L/kg-$VS_{degraded}$). Bio-solutions increased methane yield between 35.3% and 58.5% compared to the control treatment. Our results show that there was no significant difference in the methane yield between DE (reactor C) and AS (rector E) ($p<0.05$). Although the methane yield DW (reactor D) was lower than that of AS (rector E) ($p<0.05$), there was no significant difference to that of DE (reactor C). RS pretreated by TW (reactor B) and 100% PM (reactor A) digested in TW showed a lower methane yield compared to bio-solutions. The yield results demonstrate that it is effective that increasing biomethanation and promoting the activeness of methanogens in bio-pretretment and co-digestion treatments irrespective of the type of pretreatment used.

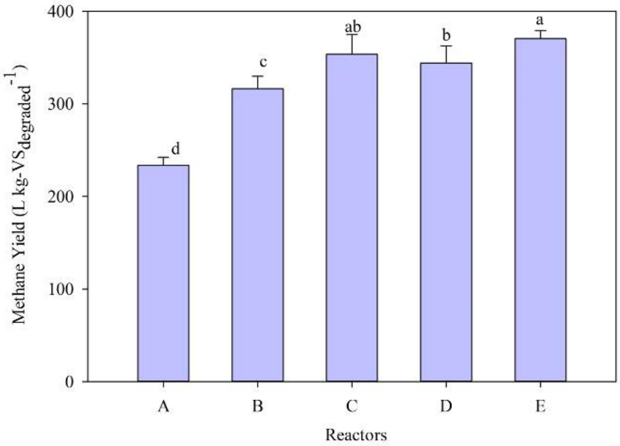

Fig. 4. Methane yield in different reactors. (A), reactor A (control treatment); (B), reactor B (tap water); (C) reactor C, (digester effluent); (D), reactor D (ditch water); (E), reactor E (anoxic sediment). Lowercase letters indicate significant differences among treatments. At least a similar letter shows insignificant at *P* = 0.05 by one-way ANOVA multiple comparisons.

Many researchers have reported biogas yields from anaerobic digestion of various kinds of lignocellulose wastes, although results are generally, presented in units of liters of CH_4 VS_{added} [29], [30] and may vary considerably due to the differences in digestion times. For example, Lei *et al.* [25] reported that methane yields of RS co-digested with PM and phosphate supplementation ranged from 400 to 440 L/kg-$VS_{degraded}$ for 120 days of digestion. Similarly, Zhong *et al.* [14] revealed that co-digestion of RS and PM biologically pretreated by a cellulolytic microflora yielded 342 L/kg-VS_{added}. Zhong *et al.* [31] reported that bio-pretreatment significantly enhances final anaerobic efficacy, as well as effective biological conversion. As observed, methane yield detected in bio-pretreatment treatments is consistent with previous studies. This suggests the research approach using bio-solutions is completely feasible for the improvement of methane productions.

3.4 pH

The pH reflects the effectiveness of anaerobic digestion, as well as the growth conditions of microbes in liquid phase. However, the activities of methanogenic and acidogenic microorganisms differ with regards to the optimal nutritional requirements, the C/N ratio balance and pH tolerance. Figure 5 shows the change of pH in the reactors during anaerobic digestion of RS and PM. All reactors with pretreated RS initiated at low pH, compared with the 100% PM reactor. The difference of pH was between 0.34 and 0.53, in which the initial pH gap of DE (reactor C) and AS (reactor E) was largest compared to the control treatment (reactor A). This indicates that these bio-solutions robustly increased the effectiveness of the hydrolysis process. It can be seen from Figure 5, that pH showed a sharp decrease in all reactors during the startup period (within 8 days). The lowest pH was 5.9 (reached on day 6 in the DE (reactor C), while the DW (reactor D) and AS (reactor E) showed similar drops on day 5. The pH values in the other reactors, remained higher than pH 6.0 throughout the digestion period. The sharp decline in pH observed during the early anaerobic stage is indicative of hydrolysis and active acidogenesis activities. In anaerobic digestion, a neutral pH (7.0) is optimal for methanogenesis bacteria, as well as methanation, while the hydrolysis and acidogenesis period necessitate a pH of between 5.5 and 6.5 [24]. However, we found that a gradual pH reduction in the early anaerobic stage increased the volumetric biogas production, yet methane concentration was low. This indicates that during the bio-pretreatment period, methanogens adapted in the digester liquids to stimulate biogas production. Nevertheless, after dropping to the lowest point, pH values in all reactors progressively increased and remained stable until the end of the experiment. They ranged between 6.6 and 7.2 which closely mirrors the reported favorable pH range of 6.8-7.2 for anaerobic digestion [1] and that of 6.6-7.8 for dynamic methanogens [32]. These tendencies clearly suggests the stability of biogas production as based on Figure 2 and Figure 5.

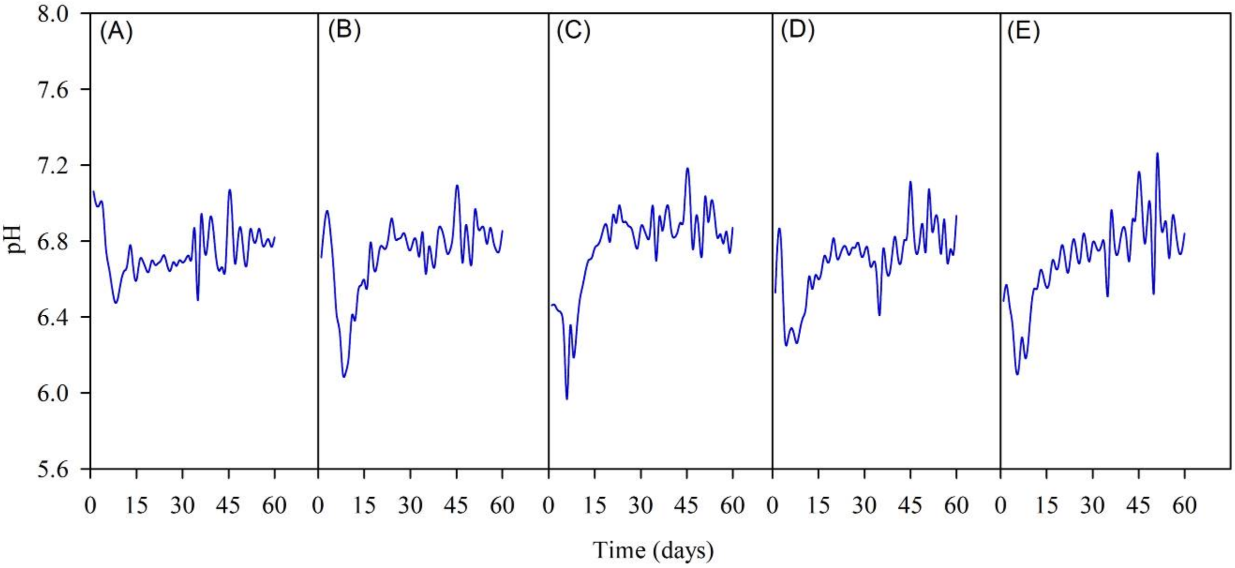

Fig. 5. pH variation in reactors A, B, C, D and E. A), reactor A (control treatment); (B), reactor B (tap water); (C) reactor C, (digester effluent); (D), reactor D (ditch water); (E), reactor E (anoxic sediment).

3.5 Volatile Solid Reduction

The VS reduction in reactors reflects the efficiency of anaerobic digestion and methane-producing capability. In this study, VS removal was analyzed at the end of the experiment period. After 60 days of digestion, the lowest VS reduction was seen in the control treatment (reactor A) with 36% of VS removal, whilst DW (reactor D) showed the highest VS reduction (45.8%). VS reduction of the other bio-solution treatments was in the range of 38.7 to 45.8%.

Table 3 shows that the VS removal in RS co-digested with PM was 2.7-9.8% higher than that of the control group. This disparity is small but indicates that bio-solutions supported the decomposition of lignocellulose components via hydrolyzing microbes. It is noted that the VS reduction was less than half of the initial VS input, which indicates biogas yields could be improved further if the time of anaerobic digestion process is prolonged. However, although Figure 2 obviously shows that daily biogas production was still ongoing at day 60, yields were at very low levels compared to mean values. Thus, prolongation would not increase the efficacy of biogas production considerably due to the heterogeneous and complex structures of remaining lignocellulose resulting in a low conversion rate. It would be more advantageous to increase biogas production by addition of new substrate at the end of the 60-day digestion period.

Methane production from the co-digestion of RS with acclimated anaerobic sludge displayed VS removal from 63.5 to 66.9% after 120 days [25]. In another study, VS removal rate of PM and dewatered sewage sludge in batch anaerobic digestion ranged from 34.7 to 62.6% for 85 days [33]. Zhong *et al.* [31] reported that VS reductions of corn straw varied from 40 to 56%. Zhang *et al.* [33] reported that co-digestion of PM with dewatered sewage sludge removed from 48 to 53% VS compared by mono-dewatered sewage sludge with 38%. Meyer *et al.* [34] reported that VS reductions of non-pretreated biosludge varied from 21 to 55%. Likewise, He *et al.* [35] obtained VS reductions of about 66% for mixtures of RS and acclimated anaerobic sludge incubated for a period of 200 days. Synthesizing from previous research studies, we found that the prolongation of anaerobic digestion processes of the RS material would not significantly increase the VS removal and biogas yield in reactors. Thus, the 60-day operation or perhaps shorter can be seen as feasible in the case of using these bio-solutions for a biological pretreatment process of locally biomass-derived materials.

Table 3. Volatile solid removal efficiencies in reactors.

Pretreatment	Initial VS concentration (%)	Final VS concentration (%)	VS removal efficiency (%)
A	4.5	2.88	36.0
B	4.5	2.67	40.7
C	4.5	2.76	38.7
D	4.5	2.44	45.8
E	4.5	2.67	40.7

3.6 Feasibility of Biogas Production from Co-Digestion of Bio-pretreated Rice Straw and Pig Manure

The results gained in this study demonstrate that RS is a superior substrate for solving the problems related to the deficiency of livestock feeding material sources in household-scale biogas digesters. This scenario is valid for all the pretreatment types tested (and all mixtures of RS and PM tested) and most certainly is due to the overall chemical composition of RS, which has a higher biogas potential than PM.

RS has a comparatively low lignin content at 7 – 12% [35]–[37] compared with other lignocellulose waste materials, such as wheat straw at 15-20% [38], and wood at 18-35% [36] on a dry matter basis. The low lignin content of RS may greatly facilitate pretreatment, enzymatic hydrolysis, and biogas production from this cheap and abundant waste material as a sole substrate or in co-digestion with animal manures. Apparently, the high silica content of RS is less an obstacle to biodegradation than lignin.

Chemical pretreatment such as NaOH (4%) enhanced biogas production by 87.5%, compared to untreated RS, whereas hydrothermal pretreatment alone increased the biogas yield by only 9.2% [29]. He *et al.* [35] obtained 27 – 65% biogas yield improvements using 6% NaOH pretreatment of RS at about 20°C for three weeks. Harsh and energy-intensive pretreatment methods are necessary to increase biogas yields from biomass materials substantially [28], [31], [39], [40]. Such methods are not likely to gain widespread practice for small household biogas digesters in rural areas – both for safety and economic reasons.

This study demonstrates that simple co-digestion combined with ecologically safe pretreatment methods of RS by soaking for 5 days in both AS and DE enhanced considerably biogas production by between 78 - 84%, respectively, as compared to mono-digestion of PM substrate only. RS is an abundant, cheap and available waste material that has been typically burnt directly in the field after harvest. Therefore, the use of DE as a bio-solution pretreatment should be promoted as it not only improves the performance of biogas production but also recirculates wastewater effectively. In a different perspective, AS could also be a potential candidate for accelerating biogas production velocity and maintaining a good environment for biogas production.

Maybe a short note that other experiments have shown that water hyacinth is a good substitute for RS in co-digestion with PM [4].

4. CONCLUSION

This study examined the effectiveness of biogas production of single-batch reactor bio-pretreatment and co-digestion of RS and PM at a mixing ratio of 50% RS/50% PM. The results suggest that the combination of bio-pretreatment and co-digestion is a promising approach for improving biogas production. RS undergoes a five-day pretreatment, which effectively stimulates biogas generation, enhances biogas yield, and improves VS removal efficiency. The biogas production in co-digestion scenarios increased by 78% and 84% compared to that of the control treatment (100% PM) when using bio-solutions AS and DE, respectively. Simultaneously, the highest methane yield was AS and DE bio-treatment, 354 and 370 L/kg-$VS_{degraded}$ ($p<0.05$), respectively. Pretreatment and co-digestion maintained almost stable biogas production during the experiment, while the mono-digestion reactor (PM) was more likely to decrease gradually and remain at a low yield level until the end. For VS removal efficiencies, all pretreated RS and PM mixture reactors digested faster than mono-digestion reactors (100% PM). However, the proportion of digestion is still low (less than 50% VS removal), compared to VS input. Our study recommended that bio-solution pretreatment experimentation on distinctive biomass-derived materials under a series of C/N ratios is suggested in future work.

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