Evaluating Rumen Fluid-Inoculated Lignocellulosic Substrates for Biogas Production

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Abstract

Background: The continuous decline in reserves of fossil and petroleum-based fuel and its undesirable environmental effects is a major problem faced by humanity. Biofuel production from lignocellulosic biomass such as agricultural, forest and crop residues, food waste and energy grasses is cost effective and could provide a sustainable solution to the problem. However, since a lignocellulosic substrate is recalcitrant, the choice of more effective digestion inoculums is crucial to achieve an improvement in the efficiency of digesting the substrate. Microbial community present in rumen is efficient in hydrolysis of complex organics at mesophilic temperature.

Objective: The objective of this study was to evaluate and compare biogas production potential of watercrown, potato peel, wheat and sorghum straw and sugarcane bagasse separately inoculated with rumen fluid of cow, goat and sheep.

Material and Methods: Total solid, volatile solid, C/N ratio and pH of the slurry before and after anaerobic digestion were determined following standard procedures. The volume of biogas produced was measured using water displacement method for 32 days and energy was estimated from a general methane composition of biogas and 1 m^3 of methane contains 34 MJ of energy.

Results: The initial C/N ratio of slurries of potato peel ranged from 15.33 to 16.33. The highest cumulative biogas yield of 1318.83 mL g^{-1} VS_{added}^{-1} was obtained from potato peel inoculated with cattle rumen fluid. The cumulative biogas yield of watercrown inoculated with cattle and sheep rumen fluid was higher than that of all the slurries of sugarcane bagasse and wheat straw.

Conclusions and implications: Slurries of potato peel and watercrown grass produced the highest amount of biogas and could be used potentially for biogas production at the pilot scale.

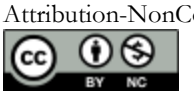
Keywords: Anaerobic digestion; Biomass; Cumulative biogas; Watercrown

1. Introduction

The increasing energy requirements along with the consequences of climate change, and depletion of unsustainable and polluting fossil fuels have driven the search for renewable energy sources (Cesaro and Belgiorno, 2015; Zhanga *et al*., 2019). Biomass is a renewable energy source that has potential for conversion into bioenergy via fermentation and anaerobic digestion (Cesaro and Belgiorno, 2015). Biofuel production from abundant and readily accessible resources such as lignocellulosic biomass (agricultural residues, forest residues, and crop residues) is cost effective and is a sustainable solution to the continuous decline in reserves of existing fossil and petroleum-based fuel, undesirable environmental effects, and associated unfavorable prices (Saratale and Oh, 2012). In biofuel production, a priority research area is looking for alternatives to conventional energy crops (Zhang *et al*., 2019). A promising alternative source is the agricultural sector which generates natural wastes such as manure and arable by-products that could be used for renewable energy generation by anaerobic digestion (Spence *et al*., 2019).

Grasses are potential alternative source of renewable energy due to their fast growing rate even in infertile land, low cultivation costs, high accessibility, consumption of whole plants and lower environmental impacts when compared to other plants (Pantawong *et al*., 2015). Perennial grasses, especially C4 grasses, are excellent substrate for renewable energy production because of a high potential of dry matter yields and fast growth compared to annual crops (Karp and Shield, 2008). Watercrown (PASPALIDIUM GEMINATUM) is aquatic, herbaceous and perennial grass. It can become locally abundant under extended warm season flooding (CABI, 2014). Growing tips and stem fragments of watercrown for lake restoration is an environmentally and economically useful way of obtaining biomass for biogas production (Mallison *et al*., 2006). Harvesting stem fragments of watercrown has a minimal, short term effect on offshore populations and within three weeks, emergent regrowth of the source plants can advance to the status of pre-harvest densities (Mallison and Thompson, 2010).

The choice of more effective digestion inoculums is crucial to achieve the improvement in the anaerobic fermentation efficiency of lignocellulosic substrate (He *et al*., 2018). Ruminants can digest a wide range of cellulosic substrates, including many bioenergy-relevant substrates such as corn, stover, switchgrass and miscanthus (Patra and Saxena, 2009) because the rumen, as part of the digestive tract in herbivores, houses a



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microbial community that naturally and efficiently hydrolyzes cellulose (Christopherson and Suen, 2013).

Microbial community present in rumen is efficient in hydrolysis of complex organics at mesophilic temperature. Especially, the community present in the fluid of rumen is stable (in terms of production) over time and it is resistant to contamination (Weimer, 1996). Rumen community of microbes together hydrolyzes and ferment chitin which is the second most abundant organic polymer after cellulose (Weimer *et al.*, 2009). The major fermentation products produced by most rumen microorganisms are methane, hydrogen and a diverse set of short-chain fatty acids. These products have potential for use as bioenergy-relevant alternative fuels (Weimer, 1996; Weimer *et al.*, 2009).

Several studies were conducted on biogas production from anaerobic digestion of potato peel (Liang and McDonald, 2015; Mu *et al.*, 2017; Achinas *et al.*, 2019), sugarcane bagasse (Janke *et al.*, 2015; Adarme *et al.*, 2017; Eshore *et al.*, 2017; Lima *et al.*, 2018; Nostratpour *et al.*, 2018; Hashemi *et al.*, 2019; Vats *et al.*, 2019), wheat straw (Ferraro *et al.*, 2018; He *et al.*, 2018; Kong *et al.*, 2018; Mancini *et al.*, 2018; Moset *et al.*, 2018; Kumer *et al.*, 2019; Liu *et al.*, 2019), sorghum straw (Garcia *et al.*, 2019) and different grass species (Mähnert, 2005; Uzodinma and Ofoefule, 2009). Sludge/digestate of anaerobic digestions was used as inoculums in most of these studies. In some, bacteria isolated from bovine rumen (Eshore *et al.*, 2017), anaerobic digestate mixed with fresh bovine manure and/or rumen (Uzodinma and Ofoefule, 2009; Lima *et al.*, 2018), cattle rumen fluid (He *et al.*, 2018) and anaerobic fungi isolate (Ferraro *et al.*, 2018) were used as inoculums.

Comparative studies have not yet been done to investigate the potential of inoculums of rumen fluid of cow, goat and sheep for biogas production. Furthermore, there is no study conducted on biogas production potential of watercrown. Given the effect of feeding pattern of rumen animal on biogas production (Uzodinma and Ofoefule, 2009) and the synergy of rumen microbe in carrying out food digestion (Christopherson and Suez, 2013), the effect of agriculturally important rumen animal such as cattle, sheep and goat rumen fluid on biogas production needs to be comparatively investigated. Therefore, the overall objective of this study was to evaluate and compare biogas production potential from watercrown with that from well investigated potato peel, sugarcane bagasse, wheat straw and sorghum straw separately inoculated with rumen fluids of cow, goat and sheep.

2. Materials and Methods

2.1. Collection Lignocellulosic Substrates and Inoculum

Wheat and sorghum straw were collected from Haramaya University research farm and sugarcane bagasse was collected from Wonji-Shewa Sugar Factory. Watercrown (*P. geminatum)* was collected from marshland around Lake Denbal (Ziway). Potato peels were collected from the student cafeteria of Haramaya University. The collected lignocellulosic substrates were dried in oven at 60 °C (Bozym *et al.,* 2015) until constant weight was obtained. The dried substrates were ground in an electric grinder and the powders were stored in air tight plastic bags at 28 °C (Kumer *et al.*, 2019). Fresh rumen fluid of cattle, goat, and sheep were collected from Haramaya town abattoir under aseptic conditions (Begum *et al.*, 2013). The rumen fluids were stored at 6 °C to avoid undesirable fermentation processes (Achinas *et al.*, 2019).

2.2. Characterization of the Slurry

In many studies, it is customary to investigate total solid, volatile solid and C/N ratio of the inoculums and substrate, separately. In this study, the slurries were formulated by mixing lignocellulosic substrates with the rumen fluids at 0.1:1 (w/v) ratio that based on study set up for biogas production by Achinas *et al.* (2019). Then, the slurries were characterized in terms of their total solid (TS in g), volatile solid (VS in g), C/N ratio and pH. These parameters were measured before and after anaerobic digestion. The amount of TS and VS was determined by modifying methods described by Eshore *et al.* (2017). Briefly, the powders of each substrate (5 g) and each inoculum (50 mL) were mixed separately in flask. The slurries were transferred and wrapped up in pre-weighted aluminum foil, and their fresh matter weights (FMW) were measured separately by incubating at 105 °C in a hot air oven till constant weights (DMW) were obtained. These constant dry matter weights (DMW) were TS weight of the slurries and the percentage of the total solid (%TS) was determined as follows:

$$\% \text{ TS} = \frac{\text{TS(g)}}{\text{FMW(g)}} \text{ x } 100$$

The obtained total solids were separately transferred to crucible, heated at 500 °C for 2 h in a muffle furnace and their weights were measured. These weights were volatile solid (VS) weight of the slurries and the percentage of the volatile solid (%VS) was determined as follows:

$$\% \text{ VS} = \frac{\text{VS(g)}}{\text{TS(g)}} \text{ x } 100$$

C/N ratio was determined by analysis of carbon and nitrogen composition of slurries. The percentage of carbon (%C) found in the slurry samples was determined as described by (Shaw, 1959). The percentage of nitrogen (%N) found in the slurry samples was determined as described by (SD, 2003). The measurement of TS, VS and C/N ratio after anaerobic digestions was conducted after decanting overlaying liquid solution found in the anaerobic digesters.

The pH of the slurries before and after the anaerobic digestion was measured using a pH meter (3310, Jenway). Measurement was conducted following operating procedures of the manufacturer meter manual. The pH meter was warmed up for 15 minutes and its electrode was calibrated before each pH measurements. After calibration, pH was measured by rinsing electrodes with distilled water and submerging it into the slurries.

2.3. Anaerobic Digestion

Prior to the anaerobic digestion experiments, each rumen fluid was incubated anaerobically at 37 °C for 5 days (Kumer *et al*., 2019). Biogas production was carried out in triplicate using 500 mL plastic bottles as digester. The digestion experiments were carried out in batch mode following the method of Achinas *et al.* (2019). Briefly, 10 g of lignocellulosic substrate powder was separately weighed into the digesters. Anaerobic digestion was initiated by adding 100 mL rumen fluid in to the digesters. For all experiments, 100 mL of distilled water was added, and no additional nutrients/ trace elements were added to the digesters as it was assumed that they were provided by the inoculums. Thereafter, the digesters were sealed using glue and they were incubated at 37 °C for 32 days. The method used to estimate biochemical biogas potential was a volumetric test which considers the amount of displaced liquid as the measurement of biogas produced. For this purpose, 500 mL of plastic bottles were filled with acidified brine solution and were connected to anaerobic digesters. The volume of displaced brine solution was measured every day.

2.4. Biogas Conversion to Energy

Energy was estimated from the produced cumulative biogas from a general %65 methane composition of biogas and 1 m^3 of methane contains 34 MJ of energy (IRENA, 2016).

1 m^3 of biogas = 22 MJ of energy

2.5. Statistical Data Analysis

Statistics Package for Social Sciences (SPSS) version 20 was used to carry out the analysis of total solid (%), volatile solid (%), C/N ratio (%), pH (change) and cumulative biogas yield. Microsoft excels 2007 used to carry out analysis of daily biogas yield and rate. During SPSS operation, paired sample t-test was used to compare percentage of total solid, volatile solid and C/N ratio at the beginning and at the end of anaerobic digestion and single-factor analysis of variances (ANOVA) was used to compare cumulative biogas yield. The means were separated using Duncan. Statistical significance was established at p-value < 0.05 level.

3. Results and Discussion

3.1. Characterization of the Slurry

For potato peel inoculated with cattle rumen fluid (PPCRF), the volatile solid (%) after anaerobic digestion was significantly less than volatile solid (%) before anaerobic digestion (Table 1). This might be one of the factors that contributed to the highest cumulative biogas production from PPCRF as compared to other slurries. In line with this, a previous study showed as volatile solid (%) removal was increased, cumulative biogas yield was also increased (Achinas *et al*., 2019). In fact, volatile solid content is the proportion of the solid material that can be digested by the micro-organisms and turned into biogas in the digester (IRENA, 2016).

Another important factor that affects biogas production is C/N ratio. Initial C/N rations of potato peel slurries were lower than that of other slurries. For potato peel slurries, values found (15.33 ± 0.48, 16.73 ± 0.97 and 14.26 ± 0.76 for PPCRF, PPGRF and PPSRF, respectively) were lower than the optimum value recommended for biogas production (20–30: 1) (Pontoni *et al*., 2015; Ajeej *et al*., 2016; Perazzolo *et al*., 2016; Achinas *et al*., 2019). Too high or low ratios as compared to the recommended one lead to process inhibition through surplus nitrogen conversion into free ammonia (BRC, 2014). As a remedy to inhibition caused by toxic levels of ammonia, stripping off ammonia as a measure to overcome too high levels and adjusting pH by addition acid shifting the NH_3/NH_4^+ balance in the system towards less free ammonia were suggested (BRC, 2014). For sugarcane bagasse, watercrown, and wheat straw slurries, the initial C/N ratios were beyond the optimum level. From the C/N ratios of these slurries, higher carbon content is clearly noted. However, these lignocellulosic substrates relatively produced lower biogas as compared to potato peel slurries that might occur due to bioavailability of not all of the carbon content in the substrate for anaerobic digestion.

A previous study showed a C/N ratio close to 15–30 for almost all of the investigated lignocellulosic substrate (Garcia *et al*., 2019). Eshore *et al.* (2017) reported 90.8 ± 0.60% TS, 80.48 ± 0.78% VS for sugarcane bagasse. Janke *et al.* (2015) reported extremely higher C:N ratio (116:1) than that of sugarcane bagasse slurries found in this study (44.78 ± 1.53, 39.11 ± 0.25 and 32.55 ± 1.51 for SbCRF, SbGRF and SbSRF, respectively). The difference was that the measured C/N ratios in this study were for slurries (blend of sugarcane bagasse and rumen fluid). The added rumen fluid might have a reduced C/N value. Uzodinma and Ofoefule (2009) reported 89.80% TS, 75.85% VS and 28.29 C/N ratio for *Panicum maximum* blended with cow manure. The related substrate watercrown (*P. geminatum*) blended with rumen fluid in this study showed lower %TS and %VS, and higher C:N ratio.

Table 1. Characteristics of slurry used in batch anaerobic digestion.

Slurry	Total solid (%FM)		Volatile solid (%TS)		C/N	
	Before	After	Before	After	Before	After
PPCRF	45.40±2.40[a]	43.02±1.92[b]	43.95±2.40[a]	41.57±1.92[b]	15.33±0.48[a]	11.69±1.07[b]
PPGRF	44.27±4.16[a]	43.52±4.85[a]	42.82±4.16[a]	42.07±4.85[a]	16.73±0.97[a]	11.49±2.18[b]
PPSRF	46.33±1.22[a]	44.47±4.79[a]	44.88±1.22[a]	43.02±4.79[a]	14.26±0.76[a]	9.807±0.71[b]
SbCRF	94.36±1.30[a]	85.24±1.51[b]	92.86±1.30[a]	83.74±1.51[b]	44.78±1.53[a]	42.00±8.28[a]
SbGRF	94.28±1.22[a]	87.38±1.56[b]	92.28±1.22[a]	85.38±1.56[b]	39.11±0.25[a]	30.95±1.65[b]
SbSRF	95.70±1.86[a]	87.05±0.81[b]	93.20±1.86[a]	84.55±0.81[a]	32.55±1.51[a]	32.19±0.60[a]
WcCRF	66.44±1.81[a]	62.38±1.04[a]	62.60±1.81[a]	58.54±1.04[b]	46.22±1.13[a]	37.59±2.19[b]
WcGRF	65.33±0.88[a]	61.15±1.24[b]	61.49±0.88[a]	57.31±1.24[b]	38.67±0.70[a]	37.28±2.79[a]
WcSRF	66.97±1.6[a]	63.17±1.11[b]	63.13±1.61[a]	59.33±1.11[b]	40.14±0.20[a]	30.87±0.21[b]
WsCRF	95.25±1.32[a]	87.80±2.92[b]	92.25±1.32[a]	84.80±2.92[b]	40.14±2.72[a]	33.89±3.20[b]
WsGRF	91.37±3.47[a]	79.04±1.12[b]	87.47±3.47[a]	75.14±1.12[b]	33.89±2.41[a]	33.15±1.30[a]
WsSRF	85.56±2.03[a]	79.25±4.45[a]	80.76±2.03[a]	74.45±4.45[a]	44.33±1.16[a]	38.79±0.71[b]

Note: PPCRF, PPGRF and PPSRF are slurries of potato peel and cow, goat and sheep rumen fluid mixture, respectively; SbCRF, SbGRF and SbSRF are slurries of sugarcane bagasse and cow, goat and sheep rumen fluid mixture, respectively; WcCRF, WcGRF and WcSRF are slurries of watercrown and cow, goat and sheep rumen fluid mixture, respectively; WsCRF, WsGRF and WsSRF are slurries of wheat straw and cow, goat and sheep rumen fluid mixture, respectively. Letters compare means across row within characteristics of the slurries. Different letters show significant difference at $p < 0.05$.

3.2. pH of the Slurry

The initial pH was found to range from 6.53 to 8.75. The initial pH for potato peel slurries were 7.24, 6.86 and 6.53 for PPCRF, PPGRF and PPSRF, respectively (Figure 1). The maximum daily biogas yield was achieved in a shorter retention time for potato peel slurries than for the other lignocellulosic slurries (Figure 3, 4 and 5). This might be due to relatively lower pH values of potato peel slurries. The shorter retention time is needed for digestion of lignocellulosic substrate as the pH of the anaerobic digestion medium closer to the optimum pH. Consistent with this suggestion, Lan *et al.* (2013) showed that a pH between 5.2 and 6.2 was needed to obtain maximal hydrolysis of lignocelluloses. Uzodinma and Ofoefule (2009) recorded pH 7 for grass field (*P. maximum*) blended with cow dung at charging. In the present study, pH 8.69 was recorded initially for related slurry (WcCRF).

The final pH ranged from 5.54 to 8.24 (Figure 1). The initial pH values of WsSRF (8.62), WcCRF (8.69) and WsCRF (8.75) were beyond the range 6.5 to 8.5 in which methane fermentation took place in anaerobic digestion systems (Achinas *et al.*, 2019). After the anaerobic digestion, pH of all the slurries was within the range, except the pH of PPSRF. It was reported that the pH less than 6.5 inhibited the growth of methanogenic bacteria and that subsequently the volume of produced biogas was reduced (Food Technology *et al.*, 2016; Achinas *et al.*, 2019). In contrast to this result, the pH of PPSRF fell below the range from 6.53 to 5.54 after anaerobic digestion and it produced a higher cumulative biogas compared to PPGRF which showed higher value of pH. The lower pH of PPSRF might have reduced the toxic level of NH_3. BRC (2014) reported that the amount of NH_3 present in liquid anaerobic digestion medium is dependent on the total $N\text{-}NH_4^+$ levels in combination with pH and temperature, i.e. the higher the pH and/or temperature, the more NH_3.

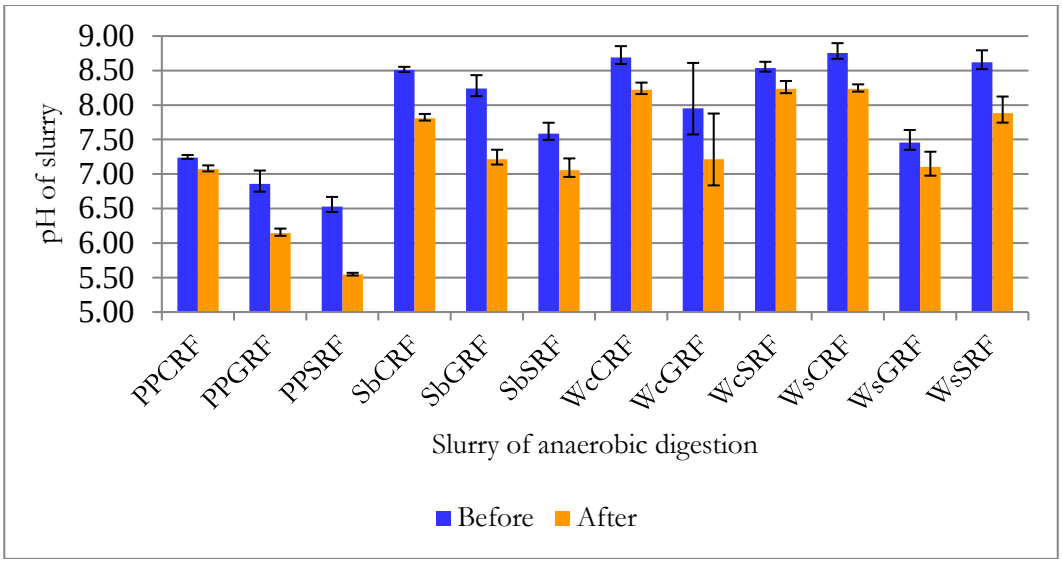

Figure 1. pH of slurry before and after anaerobic digestion.

The effect of change in pH is shown in Figure 2. PPCRF showed the lowest change (0.17) and SbGRF showed the highest change (1.02). From the change in pH, it is expected that SbGRF produced the lowest cumulative biogas yield. But, the lowest cumulative biogas yield was obtained from SbCRF (Table 2) which showed 0.70 value of change in pH. This showed that the decrease final pH of all slurries resulted in a narrow change in which the activities of natural anaerobic microbes in the slurries were maintained. Various research reports have confirmed that the methanogenic microorganisms are highly sensitive to change in pH and survive the pH range of 6.0 to 8.5 i.e. change in pH of 2 (Mösche and Jördening 1999; Wang *et al.* 1999). The narrow change in pH of the slurries shows strong buffer capacity of lignocellulosic substrates blended with rumen fluid. According to Mösche and Jördening (1999) and Wang *et al.* (1999), the pH value decreases by accumulation of VFA in the anaerobic digestion process. But, the accumulation of VFA will often not always result in a pH drop due to the buffer capacity of the substrate. For each lignocellulosic substrate, the lower values of changes in pH, the higher cumulative biogas yield.

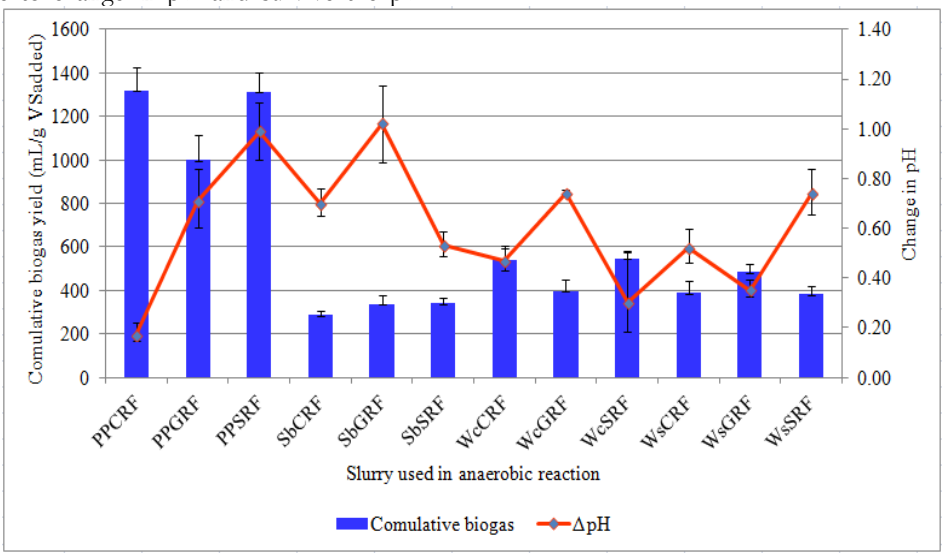

Figure 2. The effect of change in pH on cumulative biogas yield.

3.3. Biogas Production from the Slurries

Experimental data for comparative biogas production of watercrown with other substrates were presented as daily biogas production, daily biogas production rate, cumulative biogas production and energy estimation.

3.3.1. Daily Biogas Production of Watercrown Compared to Potato Peel

The maximum daily biogas yield achieved in shorter retention time for potato peel slurries as compared to other lignocellulosic slurries. This might be due to

relatively lower initial pH values of potato peel slurries (Figure 1). As compared to the slurries of potato peel, all the counterpart slurries of watercrown produced lower maximum daily biogas yield and they took longer period of time to produce the maximum daily biogas yield (Figure 3A).

For the slurries of potato peel, the maximum daily biogas yield reached 64.33 mL g^{-1} VS_{added}^{-1}, 61.95 mL g^{-1} VS_{added}^{-1} and 70.67 mL g^{-1} VS_{added}^{-1} on 12 day for PPCRF, PPGRF and PPSRF, respectively. For the slurries of watercrown, the maximum daily biogas yield reached 28.85 mL g^{-1} VS_{added}^{-1}, 28.36 mL g^{-1} VS_{added}^{-1} and 33.10 mL g^{-1} VS_{added}^{-1} on 17, 11 and 14 days for WcCRF, WcGRF and WcSRF, respectively.

All the slurries of watercrown produced lower daily biogas yield rate during the retention time of anaerobic digestion (Figure 3B). For the slurries of watercrown, the peak yield rate reached 19.42 mL g^{-1} VS_{added}^{-1} day^{-1}, 17.73 mL g^{-1} VS_{added}^{-1} day^{-1} and 21.47 mL g^{-1} VS_{added}^{-1} day^{-1} on 23, 15 and 19 days for WcCRF, WcGRF and WcSRF, respectively. For the slurries of potato peel, the peak yield rate reached 47.86 mL g^{-1} VS_{added}^{-1} day^{-1}, 39.28 mL g^{-1} VS_{added}^{-1} day^{-1} and 49.31 mL g^{-1} VS_{added}^{-1} day^{-1} on 20, 18 and 19 days for PPCRF, PPGRF and PPSRF, respectively.

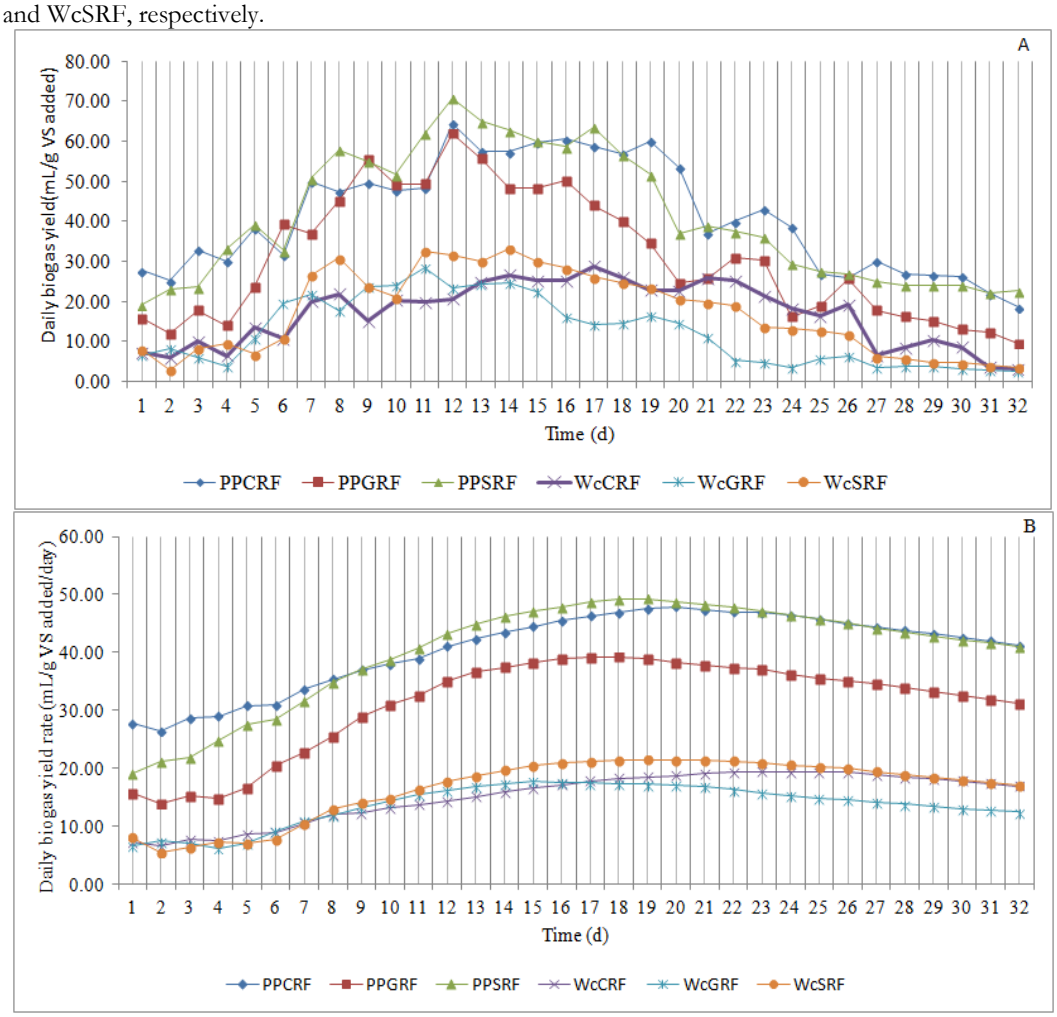

Figure 3. Daily biogas production of watercrown slurry compared to potato peel slurry. Daily biogas yield (A). Daily biogas yield rate (B). PPCRF, PPGRF and PPSRF are slurries of potato peel and cow, goat and sheep rumen fluid mixture, respectively; WcCRF, WcGRF and WcSRF are slurries of watercrown and cow, goat and sheep rumen fluid mixture, respectively.

3.3.2. Daily Biogas Production of Watercrown Compared to Sugarcane Bagasse

Compared to the slurries of sugarcane bagasse, slurries of watercrown maintained producing higher daily biogas yield during 1 to 26, 5 to 19 and 6 to 26 retention times for WcCRF, WcGRF and WcSRF, respectively (Figure 4A). For slurries of sugarcane bagasse, the maximum daily biogas yield reached 12.33 mL g^{-1} VS_{added}^{-1} , 15.82 mL g^{-1} VS_{added}^{-1} and 18.46 mL g^{-1} VS_{added}^{-1} on 25, 20 and 20 days for SbCRF, SbGRF and SbSRF, respectively. The slurries of sugarcane bagasse took longer retention time to reach the highest daily biogas yield and produced lower values of the highest yield.

All the slurries of watercrown produced higher daily biogas yield rate starting from 7-day retention time of anaerobic digestion. WcCRF produced higher daily biogas yield rate than that of SbCRF during entire retention time. WcGRF produced higher daily biogas yield rate than that of SbGRF starting from 6-day retention time. WcSRF produced higher daily biogas yield rate than that of SbSRF starting from 7-day retention time (Figure 4B). For the slurries of sugarcane bagasse, the peak daily biogas yield rate reached 9.50 mL g^{-1} VS_{added}^{-1} day^{-1}, 11.85 mL g^{-1} VS_{added}^{-1} day^{-1} and 12.33 mL g^{-1} VS_{added}^{-1} day^{-1} on 27, 25 and 23 days for SbCRF, SbGRF and SbSRF, respectively. The plot of daily biogas yield rate of the two slurries showed different pattern. The plot of slurries of sugarcane bagasse showed more constant pattern.

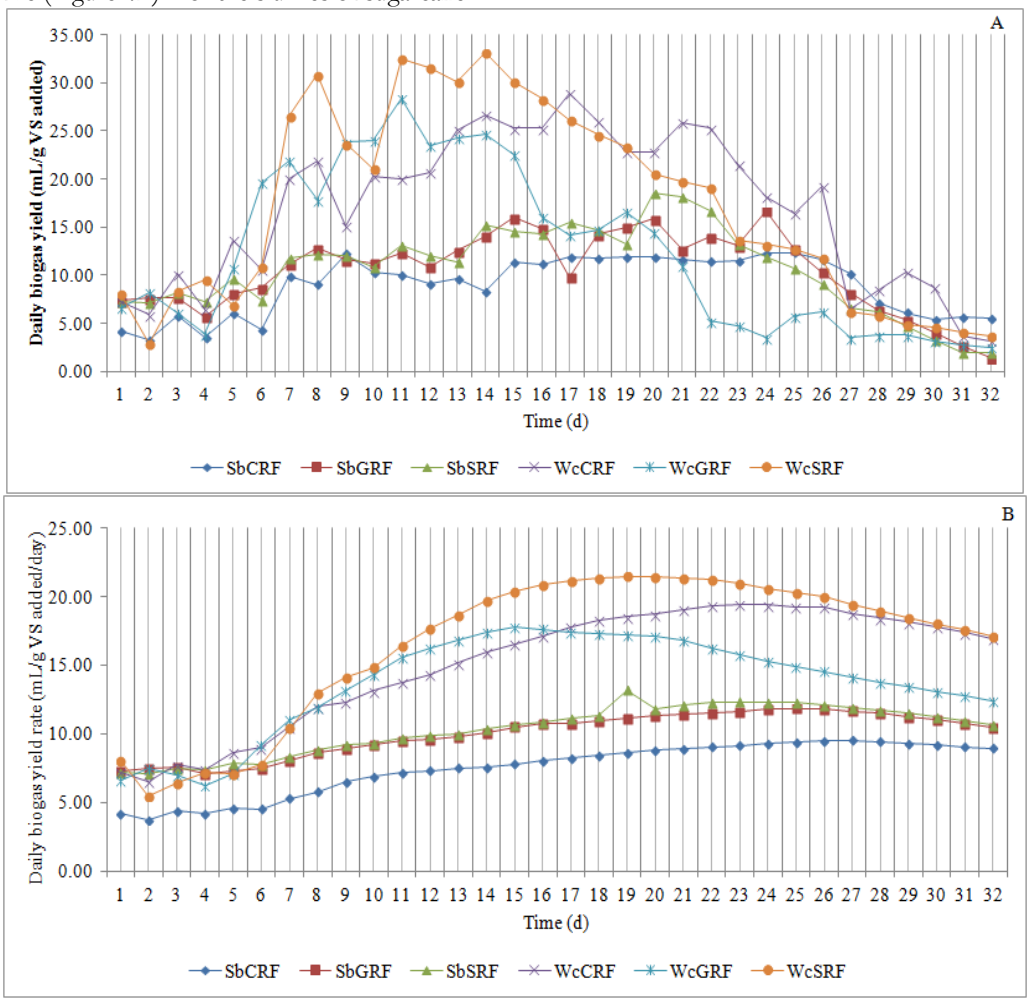

Figure 4. Daily biogas production of watercrown slurry compared to sugarcane bagasse slurry. Daily biogas yield (A). Daily biogas yield rate (B). SbCRF, SbGRF and SbSRF are slurries of sugarcane bagasse and cow, goat and sheep rumen fluid mixture, respectively WcCRF, WcGRF and WcSRF are slurries of watercrown and cow, goat and sheep rumen fluid mixture, respectively.

3.3.3. Daily Biogas Production of Watercrown Compared to Wheat Straw

Compared to the slurries of wheat straw, slurries of watercrown maintained producing higher daily biogas yield in some period of retention times. WcCRF maintained producing higher daily biogas yield than that of WsCRF starting from 5-day of retention time. WcGRF maintained producing higher daily biogas yield than that of WsGRF in the 6 to 15 retention times, then after, it maintained producing lower daily biogas yield. WcSRF maintained producing higher daily biogas yield than that of WsSRF in the 6 to 22 retention times (Figure 5A). For the slurries of wheat straw, the highest daily biogas yield reached 18.44 mL g^{-1} VS_{added}^{-1}, 21.60 mL g^{-1} VS_{added}^{-1} and 21.32 mL g^{-1} VS_{added}^{-1} on 21, 25 and 22 days for WsCRF, WsGRF and WsSRF, respectively. The slurries of wheat straw took longer retention time to reach the highest daily biogas yield and produced lower values of highest daily biogas yield.

All the slurries of watercrown produced higher daily biogas yield rate than that of counterpart slurries of wheat straw starting from 5-day retention time of anaerobic digestion. WcCRF produced higher daily biogas yield rate than that of WsCRF starting from 5-day

retention time. WcGRF produced higher daily biogas yield rate than that of WsGRF and WcSRF produced higher daily biogas yield rate than that of WsSRF during entire retention time (Figure 5B). For the slurries of wheat straw, the peak daily biogas yield rate reached 13.30 mL g^{-1} VS_{added}^{-1} day^{-1}, 15.63 mL g^{-1} VS_{added}^{-1} day^{-1} and 13.73 mL g^{-1} VS_{added}^{-1} day^{-1} on 22, 28 and 22 days for SbCRF, SbGRF and SbSRF, respectively. The plot of daily biogas yield rate of the two slurries showed different pattern.

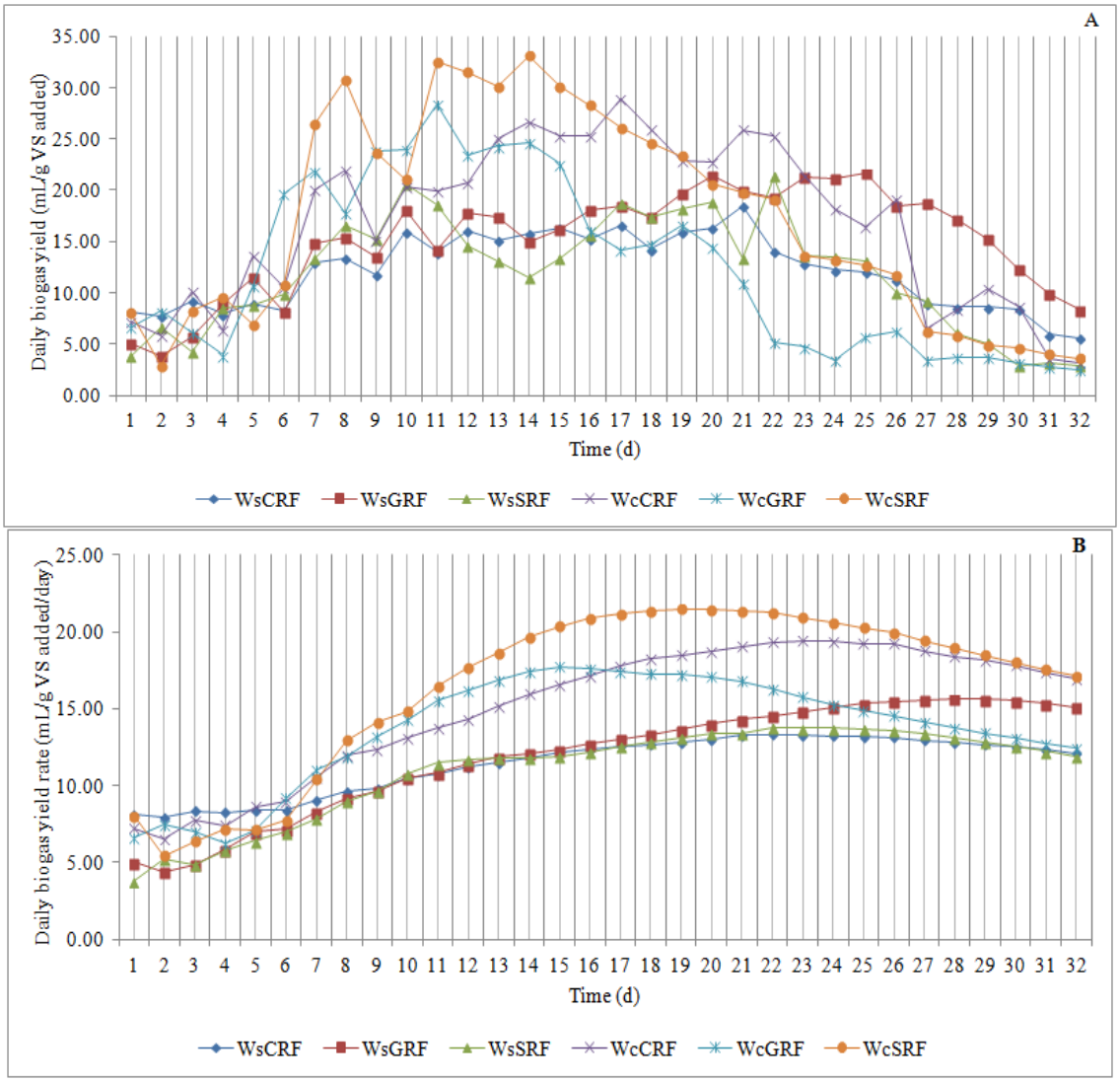

Figure 5. Daily biogas production of watercrown slurry compared to wheat straw slurry. Daily biogas yield (A). Daily biogas yield rate (B). WsCRF, WsGRF and WsSRF are slurries of wheat straw and cow, goat and sheep rumen fluid mixture, respectively; WcCRF, WcGRF and WcSRF are slurries of watercrown and cow, goat and sheep rumen fluid mixture, respectively.

3.3.4. Cumulative Biogas Production and Energy Conversion

The cumulative biogas yield (mL g^{-1} VS_{added}^{-1}) from anaerobic digestion of potato peel, sugarcane bagasse, watercrown and wheat straw inoculated with rumen fluid of cow, goat and sheep were shown in Table 2. After 32 days, the highest cumulative biogas yield (1318.83 mL g^{-1} VS_{added}^{-1}) was obtained from PPCRF and the lowest cumulative biogas yield (286 mL g^{-1} VS_{added}^{-1}) was obtained from SbCRF. The highest cumulative biogas production of slurries of potato peel was might be attributed to C/N ratio close to optimum range and significant removal of percent of volatile solid (Table 1).

The cumulative biogas yield of WcCRF (541.03 mL g^{-1} VS_{added}^{-1}) and WcSRF (547.75 mL g^{-1} VS_{added}^{-1}) were higher than that of all the slurries of sugarcane bagasse and wheat straw. This might be due to a higher lignin percentage in sugarcane bagasse and wheat straw. A review conducted by Cesario and Bolgia (2015) showed 20% lignin content in bagasse and 8.3 to 24% lignin content in wheat straw. Uzodinma and Ofoefule (2009)

reported the cumulative gas yield of 225.80 L per total mass of slurry from anaerobic digestion of *Panicum maximum* blended with cow dung for 30 days.

Inoculums significantly affected cumulative biogas yield of potato peel, sugarcane bagasse, watercrown and wheat straw ($p < 0.05$) (Table 2). PPGRF produced significantly the lowest cumulative biogas yield. The order of cumulative biogas yield was PPCRF > PPSRF > PPGRF. For sugarcane bagasse, SbSRF produced significantly the lowest cumulative biogas yield and the cumulative yield order was SbSRF > SbGRF > SbSRF. For watercrown anaerobic digestion, WcGRF produced significantly the lowest cumulative biogas yield and the order was WcSRF > WcCRF > WcGRF. For wheat straw anaerobic digestion, WsGRF produced significantly the highest cumulative biogas yield and the order was WsGRF > WsCRF > WsSRF. The result showed variation of cumulative biogas production based on inoculums and the type of substrates. These variations were might be due to the difference in feeding habit of cow, goat and sheep. The direct influence of diet on the ruminal microbial community was previously reported indicating that particular groups of microbes are better adapted for fermentation of specific feedstocks (Patra and Saxena, 2009). According to Christopher and Suez (2013), it is practical to feed ruminant animals a preferred substrate and screen a stable microbial consortium that is well adapted to the fermentation of the feed.

Biogas production is usually measured or estimated in cubic meters over a period of time, but it should be converted and reported in energy units (IRENA, 2016). Accordingly, the cumulative biogas produced was converted to energy unit and result was included in Table 2. PPCRF produced the highest cumulative CH_4 (857.35 ± 20.18 mL g^{-1} VS_{added}^{-1}) with total energy estimation of 29.15 ± 0.69 MJ. Whereas, SbCRF produced the lowest cumulative CH_4 (185.90 ± 3.25 mL g^{-1} VS_{added}^{-1}) with total energy estimation of 6.32 ± 0.11 MJ.

Table 2. Cumulative biogas production of the slurries and energy estimation.

Slurry	Cumulative Biogas yield (mL g^{-1} VS_{added}^{-1})	Cumulative CH_4 (mL g^{-1} VS_{added}^{-1})	Total Energy Estimation (MJ)
PPCRF	1318.83 ± 31.31^{g}	857.35 ± 20.18^{g}	29.15 ± 0.69^{g}
PPGRF	997.73 ± 17.84^{f}	648.48 ± 11.41^{f}	22.05 ± 0.39^{f}
PPSRF	1311.94 ± 7.13^{g}	852.80 ± 4.55^{g}	29.00 ± 0.16^{g}
SbCRF	286.00 ± 5.21^{a}	185.90 ± 3.25^{a}	6.32 ± 0.11^{a}
SbGRF	334.33 ± 1.31^{b}	217.32 ± 0.75^{b}	7.39 ± 0.02^{b}
SbSRF	339.70 ± 2.06^{b}	221.00 ± 1.30^{b}	7.51 ± 0.05^{b}
WcCRF	541.03 ± 10.77^{e}	351.65 ± 7.03^{e}	11.96 ± 0.24^{e}
WcGRF	397.43 ± 6.37^{c}	258.48 ± 4.18^{c}	8.79 ± 0.14^{c}
WcSRF	547.75 ± 5.12^{e}	356.20 ± 3.38^{e}	12.11 ± 0.12^{e}
WsCRF	387.15 ± 4.26^{c}	251.77 ± 2.63^{c}	8.56 ± 0.09^{c}
WsGRF	483.33 ± 15.10^{d}	314.17 ± 9.78^{d}	10.68 ± 0.33^{d}
WsSRF	381.45 ± 5.20^{c}	247.87 ± 3.58^{c}	8.43 ± 0.12^{c}

Note: Letters compare means across column. Different letters show significant difference at $p < 0.05$.

4. Conclusions

This study revealed that the use of rumen fluid resulted in fast degradation of lignocellulosic substrate. Potato peel slurries produced the highest cumulative biogas yield in a relatively short period of time. Therefore, it is economical and environmentally friendly to use potato peel waste from domestic and food processing industries for fast and potential biogas production contributing to the solid waste management. The results demonstrate that the second highest biogas yield was obtained from watercrown slurry. Since watercrown is fast-growing, high-yielding, available substrate in plenty, it can be used for sustainable biogas production. Nutrient composition of watercrown slurry should be determined for its application for a large scale biogas production.

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