

Full Length Research Paper

Eggshells – assisted hydrolysis of banana pulp for biogas production

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In this study, pretreatment of banana pulp using eggshells in both calcined and un-calcined forms to examine the extent of hydrolysis was conducted. Reactor C₀ containing banana pulp and inoculum but with no eggshells added was used as the control, while reactors C₁, C₂, C₃, C₄, and C₅ containing banana pulp and inoculum were spiked with 1, 3, 5, 7, and 9 g of un-calcined eggshells and calcined eggshells, for experiment 1 and 2, respectively. Anaerobic digestion was carried out at mesophilic temperature (35°C) for a period of 20 days. Digester C₃ with 5 g of calcined eggshells gave the largest cumulative biogas yield of 2343 mL with 62% CH₄, followed by digester C₅ with 9 g of un-calcined eggshells which gave 2032 mL with 51.9% CH₄. The least biogas yield of 10 mL was obtained in digester C₅ with 9 g of calcined eggshells additive.

Key words: Anaerobic digestion, banana pulp hydrolysis biogas, eggshells.

INTRODUCTION

The energy and global warming crisis, stimulates the need for development of renewable energy worldwide (Buasri et al., 2013). Biogas is one of the desired alternative energy since it is renewable and environmental friendly. Various materials such as agricultural by-product, urban waste and animal by-product can be used as biogas source (Adeyanju, 2008). Although lignocellulosic biomass is the most abundant among these by-products, its huge potential in energy production in form of biogas remains largely unlocked as anaerobic digestion of lignocellulosic materials is too slow in nature for meaningful biogas production (Jaisamut et

al., 2013). This is because lignocellulosic materials are resistant to hydrolysis owing to their polymeric structure and composition (Almoustapha et al., 2009). Poor lignin and cellulose deconstruction and hence poor hydrolysis compounded with the production of toxic substances during digestion limit lignocellulosic materials in biogas production (Čater et al., 2014).

Since lignocellulosic materials are more abundant than non lignocellulosic materials, considerable amount of research efforts have recently been made to make lignocellulosic materials more amenable for biogas production. These efforts entail chemical, physical, and

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biological pre-treatment of the substrate prior to anaerobic digestion (Brodeur et al., 2011; Harmsen et al., 2010). Chemical pre-treatment employs chemical such as acids and alkalis to disrupt the rigid polymeric structure of the lignocellulosic materials (Harmsen et al., 2010; Montgomery and Bochmann, 2014).

Alkaline pre-treatment has been carried out with different alkalis such as sodium hydroxide, potassium hydroxide, calcium hydroxide, and ammonium hydroxide (Park et al., 2010; Taherzadeh and Karimi, 2008). Alkaline disrupts the structure through swelling of lignocelluloses and partial solubilisation of lignin (Cheng et al., 2010; McIntosh and Vancov, 2010).

Physical pre-treatment is achieved by milling and grinding the biomass to reduce its size, degree of polymerization, and crystallinity, so that the accessible surface area and pores size is increased (Brodeur et al., 2011; Harmsen et al., 2010). This technique requires higher energy than the theoretical energy content available in the biomass. In addition, repair or replacement of worn-out mills and cutters is expensive (Brodeur et al., 2011; Montgomery and Bochmann, 2014).

Biological pre-treatment on the other hand employs fungi to degrade lignin and hemicelluloses but not cellulose (Kumar and Wyman, 2009; Shi et al., 2008). Also, compared to other pre-treatment techniques, biological pre-treatment of lignocellulosic biomass is very slow and sensitive to temperature and pH variations (Sun and Cheng, 2002).

Though chemical pre-treatment of substrate is more promising in terms of short resident time and biogas yield, good carbohydrate preservation is still a challenge. Carbohydrate preservation in substrate pre-treatment is imperative for practical biogas yield. Most acids and alkalis such as sodium hydroxide and ammonium hydroxide have been shown to give poor carbohydrate preservation (Liang et al., 2013).

In order to unlock the full potential of lignocellulosic materials in biogas production, there is a need to develop new substrate pre-treatment methods that are cheap and give good carbohydrate preservation. In this study, a cheap and effective banana pulp pre-treatment method premised on the use of eggshells is presented.

The rationale for the use of eggshells is that calcium carbonate (CaCO_3), which constitute 95% of the eggshell total weight, could be transformed into Ca(OH)_2 in-situ to effect cellulose structure disruption. Pre-treatment of lignocellulosic-rich substrates such as switch grass, sugarcane bagasse, and corn stover with commercial Ca(OH)_2 has been reported to improve biogas yield, (Liew et al., 2011; Rabelo et al., 2009). This was attributed to the good carbohydrate preservation afforded by Ca(OH)_2 . The use of eggshells, which is readily available in hotels, baking/confectionary and hatching industries (King'ori, 2011), not only provides a cheap avenue to increased utilization of lignocellulosic materials

in biogas production, but also helps in waste management and hence promote both the public and environmental health. This is because egg waste can cause odours due to microbial action and can also change the quality of soil (Tangboriboon et al., 2012).

MATERIALS AND METHODS

Collection of materials for digestion

The banana pulp was obtained from Banana Investment Limited (BIL) while eggshells were obtained from various cafeterias located in Arusha. The inoculum was obtained from the nearby existing cow dung biogas digester. In order to initiate fermentation, it is important to seed the digesters, with inoculum rich of anaerobic microorganisms within existing biogas digesters. This is because; the populations of anaerobic microorganisms such as hydrolytic bacteria, acidogens, acetogens, and methanogens typically take a significant period of time to establish themselves to be fully effective. The eggshells were washed, sundried and thereafter crushed and ground to obtain fine powder. Some of the eggshells powder was calcined at 700°C for 2 h (Tangboriboon et al., 2012; Buasri et al., 2013). Eggshells calcination was done purposely in order to transform calcium carbonate (CaCO_3) from un-calcined eggshells into lime or calcium oxide (CaO).

Digestion setting up

Two batches of experiments were performed in duplicate, to investigate the effectiveness of eggshells in substrate pretreatment. In batch one, six conical flasks each of 1000 mL capacity were used as reactors herein referred to as C_0 , C_1 , C_2 , C_3 , C_4 , and C_5 . For each batch, 5 kg of BP was mixed with 15 L of distilled water to form banana pulp slurry (BPS).

Reactor C_0 was used as a control and its reactants were the mixtures of 800 mL of BPS and 200 mL of inoculum. The contents for reactors, C_1 , C_2 , C_3 , C_4 , and C_5 , were mixtures of 800 mL of BPS and 200 mL of inoculum followed by the addition of 1, 3, 5, 7, and 9 g of un-calcined eggshells, respectively. 850 mL of the respective mixtures were used for the production of biogas, while the remaining 150 mL were used for pH, volatile solid (VS), Chemical oxygen demand (COD), total solids (TS), and volatile fatty acid (VFA) determination.

All the reactors were fitted with air-tight rubber stoppers and then immersed about one-third in water bath thermo stated at 35°C . Along with the digesters, there were measuring cylinders connected to the digesters by means of rubber tubing. The cylinders were filled with acidified brine solution and were inverted to collect the gas generated by displacement. The acidified brine solution was prepared by dissolving 200 g of NaCl in 1 L of distilled water to form a supersaturated solution. Sulphuric acid was then added to the resultant supersaturated solution until the pH dropped below 2.0. The measuring cylinders were fixed firmly using retort stands. Fig.1 summarizes the digestion setting up.

In batch two, the experimental set up and eggshells concentration, were the same as in batch one, except that calcined eggshells were used. Calcination of the eggshells was conducted at 700°C for 2 h (Tangboriboon et al., 2012; Buasri et al., 2013).

The digestion process was allowed to proceed for 20 days and biogas collected was measured by water displacement method as described by (Iyagba et al., 2009; Adeyanju, 2008). Shaking was done twice a day. In addition to biogas yield the pH, volatile solid (VS), chemical oxygen demand (COD), total solids (TS), and volatile fatty acid (VFA) were determined before and after digestion.

Table 1. pH of raw banana pulp slurry and inoculum (mean \pm SD).

Parameter	Banana pulp slurry (BPS)	Inoculum (I)	BPS + I (substrate)
pH	4.15 \pm 0.03	7.40 \pm 0.06	5.92 \pm 0.04

Table 2. Properties of biodigester content before and after digestion for un-calcined eggshells.

Reactor	C ₀	C ₁	C ₂	C ₃	C ₄	C ₅
Eggshells (g/L)	0	1	3	5	7	9
Initial pH	5.92 \pm 0.04	6.05 \pm 0.05	6.38 \pm 0.06	6.41 \pm 0.07	6.54 \pm 0.05	6.71 \pm 0.07
Final pH	4.46 \pm 0.03	4.80 \pm 0.04	5.31 \pm 0.05	5.68 \pm 0.04	6.01 \pm 0.06	6.52 \pm 0.05
Initial COD (g/L)	13.75 \pm 0.9	17.84 \pm 1	15.62 \pm 0.8	19.21 \pm 0.1	20.43 \pm 0.7	24.11 \pm 1
Final COD (g/L)	6.13 \pm 0.2	7.65 \pm 0.5	6.1 \pm 0.1	7.2 \pm 0.6	6.74 \pm 0.2	7.1 \pm 0.6
% COD removal	55.4	57.1	60.9	62.5	67.0	70.6
Initial TS (g/L)	15.4 \pm 0.2	17.84 \pm 1.2	19.02 \pm 0.6	18.42 \pm 0.8	19.72 \pm 0.16	18.59 \pm 1.7
Final TS (g/L)	5.5 \pm 0.7	6.33 \pm 0.4	5.65 \pm 0.3	1.82 \pm 0.06	1.49 \pm 0.06	1.06 \pm 0.1
% TS removal	64.0	64.5	70.3	90.1	92.4	94.3
Initial VS (g/L)	3.51 \pm 0.7	3.55 \pm 0.52	4.86 \pm 0.4	4.79 \pm 0.7	5.55 \pm 0.1	5.23 \pm 0.9
Final VS (g/L)	2.45 \pm 0.5	2.17 \pm 0.52	2.55 \pm 0.2	2.1 \pm 0.1	2.56 \pm 0.3	1.84 \pm 0.2
% VS removal	30.1	38.8	47.5	56.2	59.3	64.9
Initial VFA (g/L)	0.113 \pm 0.02	0.057 \pm 0.002	0.053 \pm 0.001	0.052 \pm 0.001	0.050 \pm 0.0005	0.032 \pm 0.0001
Final VFA (g/L)	0.169 \pm 0.002	0.086 \pm 0.001	0.08 \pm 0.0004	0.078 \pm 0.0002	0.075 \pm 0.0001	0.048 \pm 0.0002

COD= Chemical oxygen demand, TS=total solids, VS= volatile solids, VFA=volatile fatty acid.

RESULTS AND DISCUSSION

The potential of eggshells in substrate hydrolysis was investigated in the anaerobic fermentation of BPS. Tables 1, 2, and 5 summarize the values obtained for the characteristics of BPS, inoculum, and BPS/Inoculum mixture with and without eggshells. As shown in Tables 2 and 5, banana pulp has a good biogas production potential for its COD ranged from 13.75-24.11 g/L. The volatile solids content of the reactants, which ranged between, 2.97-5.55 g/L, showed that BPS had significant energy content. Before inoculation the mean pH values of BPS was 4.15 \pm 0.03; however after inoculation, the mean pH of the reactants was increased to 5.92 \pm 0.04, Table 1. Further increase in pH was observed after the addition of eggshells into respective reactors, Tables 2 and 5.

Banana pulp hydrolysis using un-calcined eggshells

The effects of un-calcined eggshells to improve hydrolysis, was investigated based on the volume of biogas produced, volatile solid (VS), chemical oxygen demand (COD), and total solids (TS) removal efficiencies. Table 2 shows that TS, VS, and COD removal efficiencies for reactors C₁-C₅, with un-calcined eggshells were higher than for reactor C₀ without un-calcined eggshells, which was used as the control. This indicated that banana pulp has been hydrolyzed by the

eggshells added into respective reactors. The highest TS (94.3%), VS (64.9%), and COD (70.6%) removal rates were observed in reactor C₅, with 9 g of un-calcined eggshells dosage (Figure 1).

Likewise, the biogas yield and methane composition were high in reactors C₁-C₅, spiked with un-calcined eggshells as compared to reactor C₀ as shown in Table 3 and Figure 2. The largest mean cumulative volume of biogas of 2032 mL with 51.9% methane was obtained in reactor C₅. The largest biogas yield obtained in C₅ was probably due to the near neutral final pH (6.52 \pm 0.05) and low VFA in this reactor. Near neutral pH is favorable to methane forming bacteria, (Budiyono et al., 2013; Dioha et al., 2013), while VFA less than 4 g/L indicates that methanogenic bacteria consumed the organoacids produced by acidogenic bacteria (Lee et al., 2015).

Table 4 presents the pH trend in the six digesters: C₀, C₁, C₂, C₃, C₄, and C₅. In digester C₀ the pH decreased slightly from 5.92 \pm 0.04 to 4.46 \pm 0.03 at the end of experimentation. In digester C₅, the pH decreased in the 3rd day and then tends to rise gradually and the final pH value of 6.52 \pm 0.05 was observed. Figure 3 show that the mean cumulative biogas volume might be increasing as un-calcined eggshells dosage is increasing.

Banana pulp hydrolysis using calcined eggshells

The efficiency of calcined eggshells in banana pulp



Figure 1. The digestion set-up.

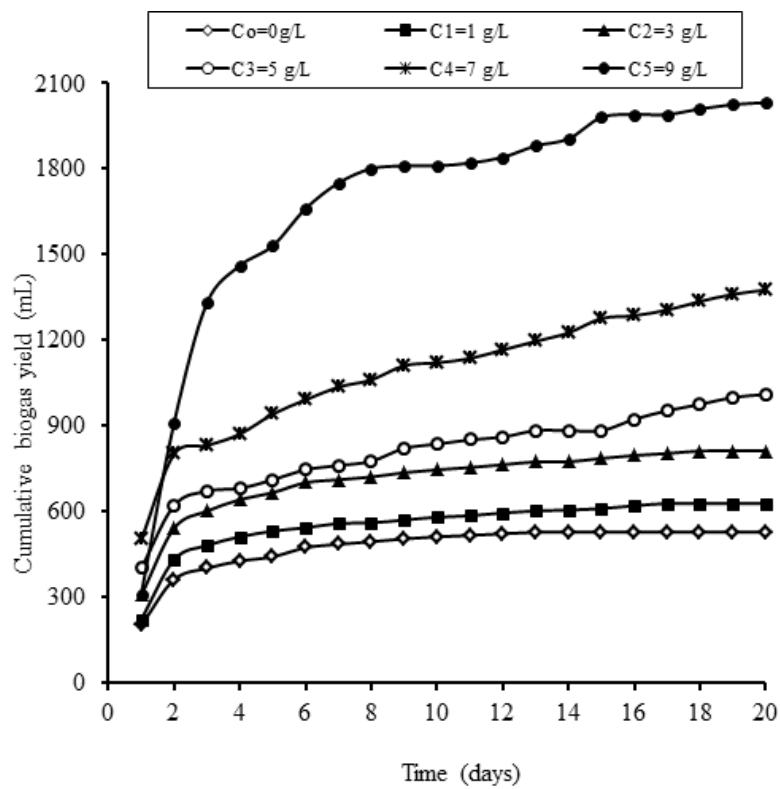


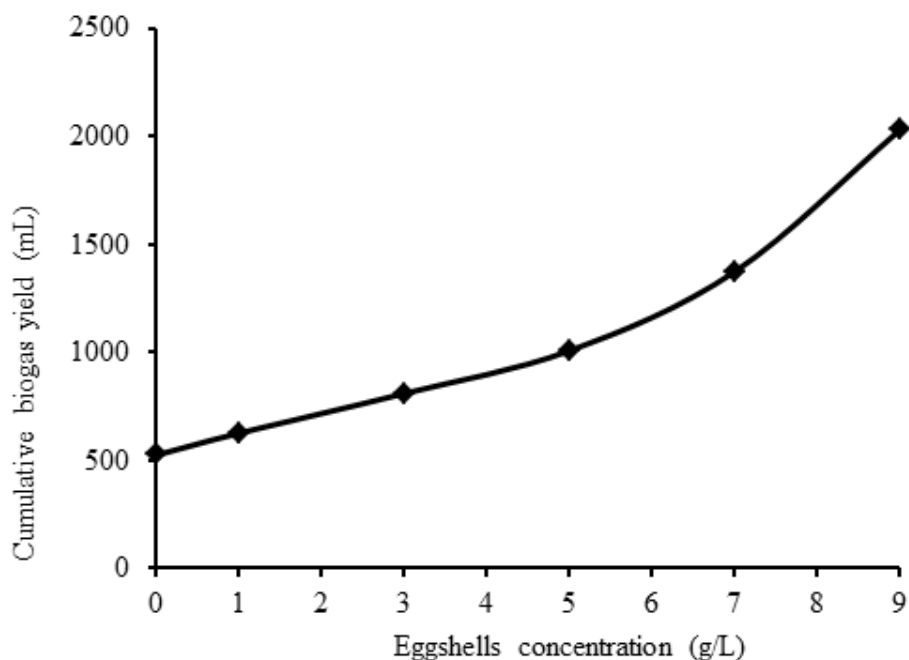
Figure 2. Cumulative biogas yield (mL) versus time (days).

Table 3. Mean cumulative biogas yield and its composition for un-calcined eggshells.

Reactor (g/L)	Biogas yield (mL)	Biogas composition				
		CH ₄ (%)	CO ₂ (%)	O ₂ (%)	NH ₃ (ppm)	H ₂ S (ppm)
C ₀ =0	526	20.9	75.5	2.7	48	73
C ₁ =1	627	24.1	72.4	2.9	13	14
C ₂ =3	811	32.5	63.3	3.1	5	63
C ₃ =5	1009	41.8	53.9	3.4	38	19
C ₄ =7	1375	46.3	51.1	1.6	7	24
C ₅ =9	2032	51.9	45.7	1.2	12	49

Table 4. pH monitoring for banana pulp slurry for selected days for un-calcined eggshells (mean \pm SD).

Reactor (g/L)	pH/Day				
	1 st day	3 rd day	6 th day	9 th day	20 th day
C ₀ =0	5.92 \pm 0.04	5.04 \pm 0.03	4.49 \pm 0.05	4.46 \pm 0.02	4.46 \pm 0.03
C ₁ =1	6.05 \pm 0.05	5.99 \pm 0.04	5.75 \pm 0.07	5.62 \pm 0.01	4.80 \pm 0.04
C ₂ =3	6.38 \pm 0.06	6.26 \pm 0.03	6.04 \pm 0.06	5.95 \pm 0.04	5.31 \pm 0.05
C ₃ =5	6.41 \pm 0.07	6.84 \pm 0.05	5.70 \pm 0.02	5.69 \pm 0.08	5.68 \pm 0.04
C ₄ =7	6.54 \pm 0.05	6.88 \pm 0.02	5.80 \pm 0.04	5.69 \pm 0.06	6.01 \pm 0.06
C ₅ =9	6.71 \pm 0.07	5.91 \pm 0.01	6.07 \pm 0.02	6.2 \pm 0.03	6.52 \pm 0.05

**Figure 3.** Cumulative biogas yield versus un-calcined eggshells concentration.

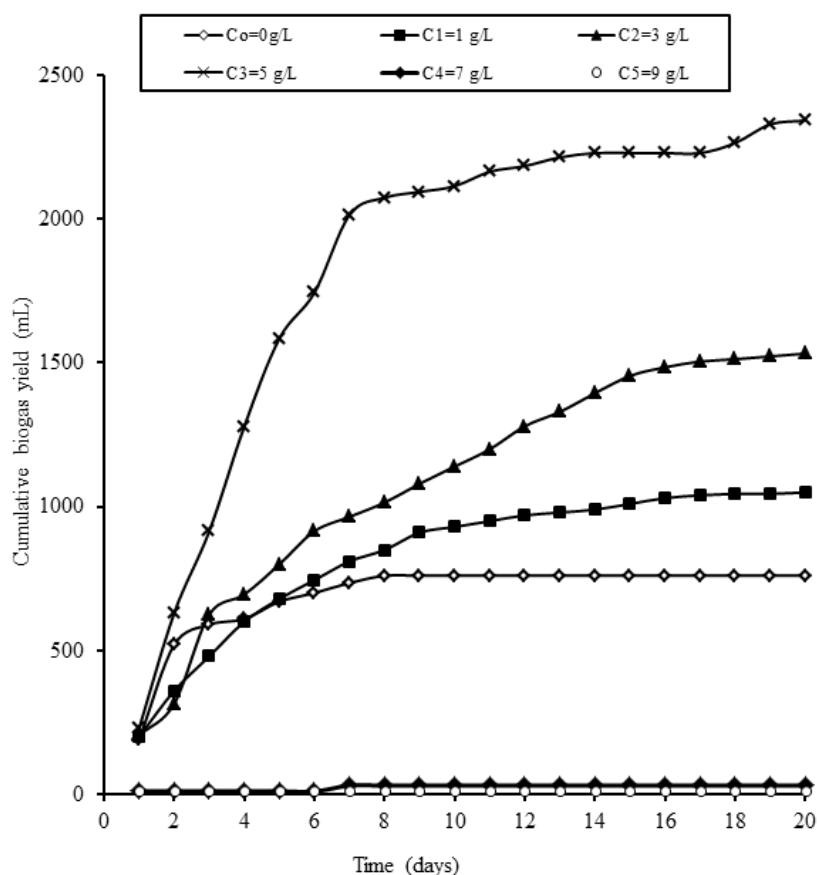
hydrolysis was evaluated in terms of cumulative biogas production, TS, VS, and COD removal as previously discussed.

Table 5 presents the amount of TS and VS biodegradation and COD removal efficiency.

Biodegradation of TS and VS, and COD removal rate was high in digesters C₁-C₃ containing 1, 3, and 5 g of calcined eggshells as compared to digester C₀ with no eggshells additive. The highest TS (89.9%), VS (61.7%) and COD (82.5%) removal rates were observed in

Table 5. Properties of biodigester contents before and after digestion for calcined eggshells.

Reactor	C ₀	C ₁	C ₂	C ₃	C ₄	C ₅
Eggshells (g/L)	0	1	3	5	7	9
Initial pH	5.925±0.04	6.84±0.07	7.19±0.03	7.98±0.09	10.30±0.05	11.95±0.02
Final pH	4.46±0.03	5.55±0.04	5.80±0.05	7.41±0.06	10.17±0.08	11.83±0.04
Initial COD(g/L)	13.75±0.9	8.09±0.2	11.4±0.7	12.31±0.2	16.42±0.7	20.04±0.3
Final COD (g/L)	6.58±0.4	3.04±0.1	3.32±0.3	2.15±0.1	14.75±0.4	18.32±0.5
% COD removal	52.1	62.4	70.9	82.5	10.2	8.6
Initial TS (g/L)	15.4±0.2	17.5±0.7	18.4±1	16.2±0.8	17.9±0.6	16.5±0.6
Final TS (g/L)	8.55±0.1	2.88±0.3	2.28±0.2	1.64±0.1	10.7±0.4	10.77±0.5
% TS removal	44.5	83.5	87.6	89.9	40.2	34.7
Initial VS (g/L)	3.51±0.7	2.97±0.5	3.72±0.7	4.26±0.2	4.29±0.4	5.16±0.2
Final VS (g/L)	2.34±0.01	1.24±0.05	1.54±0.1	1.63±0.1	2.94±0.05	3.72±0.4
% VS removal	33.2	58.3	58.7	61.7	31.4	28
Initial VFA (g/L)	0.113±0.02	0.065±0.002	0.037±0.001	0.028±0.003	0.0156±0.001	0.004±0.0001
Final VFA (g/L)	0.17±0.04	0.056±0.001	0.072±0.002	0.045±0.004	0.024±0.0005	0.006±0.0002

**Figure 4.** Cumulative biogas yield (mL) versus time (days).

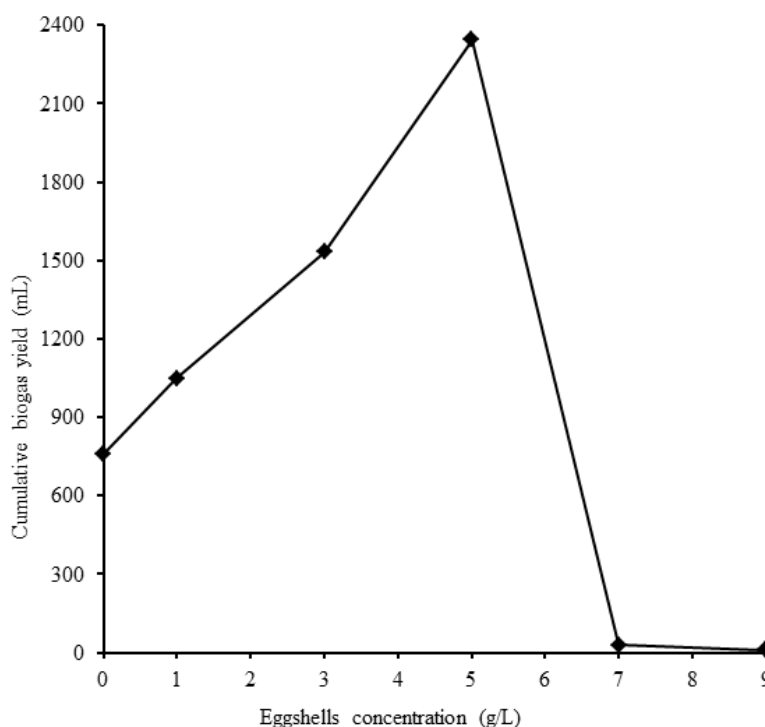
reactor C₃, with 5 g of calcined eggshells as shown in Table 5.

This reactor (C₃) gave the highest mean cumulative biogas yield of 2343 mL, with 60% methane as shown in

Figure 4 and Table 6. The high biogas yield obtained in reactor C₃ was due to the relatively near neutral pH observed over the entire digestion period. The lowest mean cumulative biogas volume was obtained in digester

Table 6. Mean cumulative biogas yield and its composition for calcined eggshells.

Reactor (g/L)	Biogas yield (mL)	Biogas composition				
		CH ₄ (%)	CO ₂ (%)	O ₂ (%)	NH ₃ (ppm)	H ₂ S (ppm)
C ₀ =0	760	26.2	70.6	1.8	8	24
C ₁ =1	1050	37.1	59.5	2.5	24	37
C ₂ =3	1533	40.5	55.9	3.2	33	73
C ₃ =5	2343	62	32.3	3.8	41	43
C ₄ =7	30			Not measured		
C ₅ =9	10			Not measured		

**Figure 5.** Cumulative biogas yield versus calcined eggshells concentration.

C₅, with higher dosage (9 g/L) of calcined eggshells Figures 4 and 5 and Table 6. This was attributed to high pH beyond the optimal value as shown in Tables 5, and 7. The highest CO₂ percentage obtained in reactor C₀ was due to acidic condition of the medium which caused the death of AD bacteria. Figure 5 shows that 5 g/L of calcined eggshells is the maximum dosage that could give the highest volume of biogas.

Generally in Experiment 1 and 2, there was the decrease in TS and VS contents for the pretreated substrate, which contributed to the increase in the amount of water, thus the level of microbial activities was increased. The higher COD removal rate observed, illustrated that the conversion of organic matter into methane was high. Near neutral pH was noticed in reactors C₅ (6.52±0.05) and C₃ (7.41±0.06) for

Experiment 1 and 2, respectively, whereby AD processes in these digesters were improved.

Conclusion

The highest TS and VS removal efficiencies and biogas yield observed from digesters seeded with eggshells indicated that, pre-treating substrates with eggshells improved the degradation of the lignocellulosic substrate. Furthermore, the higher COD removal efficiency, the increase in pH and higher methane content obtained in digesters with eggshells additives showed that eggshells both in calcined and un-calcined form can improve AD processes.

Digester C₃ with 5 g/L of calcined eggshells dose, gave

Table 7. pH monitoring for banana pulp slurry for selected days for calcined eggshells (mean \pm SD).

Reactor (g/L)	pH/Day				
	1 st day	3 rd day	6 th day	9 th day	20 th day
C ₀ =0	5.92 \pm 0.04	5.04	4.49	4.46	4.46 \pm 0.03
C ₁ =1	6.84 \pm 0.07	5.94 \pm 0.05	5.74 \pm 0.03	5.59 \pm 0.05	5.55 \pm 0.04
C ₂ =3	7.19 \pm 0.03	6.41 \pm 0.01	5.92 \pm 0.08	5.92 \pm 0.01	5.80 \pm 0.05
C ₃ =5	7.98 \pm 0.09	5.82 \pm 0.04	6.15 \pm 0.03	6.69 \pm 0.05	7.41 \pm 0.06
C ₄ =7	10.30 \pm 0.05	10.01 \pm 0.02	10.05 \pm 0.04	10.17 \pm 0.08	10.17 \pm 0.08
C ₅ =9	11.95 \pm 0.02	11.53 \pm 0.05	11.74 \pm 0.01	11.80 \pm 0.05	11.83 \pm 0.04

the highest biogas yield (2343 mL) followed by C₅, with 9 g/L of un-calcined eggshells dosage, which produced 2032 mL. The highest TS and VS removal rate was observed in digester C₅, with 9 g/L of un-calcined eggshells, while the highest initial and final pH was observed in digester C₅, with 9 g/L of calcined eggshells. These results indicated that BPS was hydrolyzed significantly into sugars digestible by the hydrolytic, acidogenic and methanogenic bacteria.

Conflict of Interests

The authors have not declared any conflict of interests.

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REFERENCES

- Adeyanju A (2008). Effect of seeding of wood-ash on biogas production using pig waste and cassava peels. *J. Eng. Appl. Sci.* 3:242-245.
- Almoustapha O, Kenfack S, Millogo-Rasolodimby J (2009). Biogas production using water hyacinths to meet collective energy needs in a sahelian country. *Field Actions Sci. Rep.* 2:27-32.
- Brodeur G, Yau E, Badal K, Collier J, Ramachandran K, Ramakrishnan S (2011). Chemical and physicochemical pretreatment of lignocellulosic biomass: a review. *Enzyme Research* 17p.
- Buasri A, Chaiyut N, Loryuenyong V, Wongweang C, Khamsrisuk S (2013). Application of eggshell wastes as a heterogeneous catalyst for biodiesel production. *Sustain. Energy* 1:7-13.
- Budiyo B, Syaichurrozi I, Sumardiono S (2013). Biogas production from bioethanol waste: the effect of pH and urea addition to biogas production rate. *Waste Technol.* 1:1-5.
- Čáter M, Zorec M, Logar RM (2014). Methods for Improving Anaerobic Lignocellulosic Substrates Degradation for Enhanced Biogas Production. *Springer Sci. Rev.* 2:51-61.
- Cheng Y-S, Zheng Y, Yu CW, Dooley TM, Jenkins BM, VanderGheynst JS (2010). Evaluation of high solids alkaline pretreatment of rice straw. *Appl. Biochem. Biotechnol.* 162:1768-1784.
- Dioha I, Ikeme C, Nafi'u T, Soba N, Yusuf M (2013). Effect of carbon to nitrogen ratio on biogas production. *Int. Res. J. Nat. Sci.* 1:1-10.
- Harmsen P, Huijgen W, Bermudez L, Bakker R (2010). Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. pp. 10-13.
- Iyagba ET, Mangibo IA, Mohammad YS (2009). The study of cow dung as co-substrate with rice husk in biogas production. *Sci. Res. Essay.* 4:861-866.
- Jaisamut K, Paulová L, Patáková P, Rychtera M., Melzoch K (2013). Optimization of alkali pretreatment of wheat straw to be used as substrate for biofuels production. *Plant, Soil and Environment (Czech Republic).*
- King'ori A (2011). A review of the uses of poultry eggshells and shell membranes. *Int. J. Poult. Sci.* 10:908-912.
- Kumar R, Wyman CE (2009). Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnol. Prog.* 25:302-314.
- Lee DJ, Lee SY, Bae JS, Kang JG, Kim KH, Rhee SS, Park JH, Cho JS, Chung J, Seo DC (2015). Effect of Volatile Fatty Acid Concentration on Anaerobic Degradation Rate from Field Anaerobic Digestion Facilities Treating Food Waste Leachate in South Korea. *J. Chem.* 501:640717.
- Liew LN, Shi J, Li Y (2011). Enhancing the solid-state anaerobic digestion of fallen leaves through simultaneous alkaline treatment. *Bioresour. Technol.* 102:8828-8834.
- McIntosh S, Vancov T (2010). Enhanced enzyme saccharification of Sorghum bicolor straw using dilute alkali pretreatment. *Bioresour. Technol.* 101:6718-6727.
- Montgomery LF, Bochmann G (2014). Pretreatment of feedstock for enhanced biogas production. *IEA Bioenergy. Ireland.*
- Park J-Y, Shiroma R, Al-Haq MI, Zhang Y, Ike M, Arai-Sanoh Y, Ida A, Kondo M, Tokuyasu K (2010). A novel lime pretreatment for subsequent bioethanol production from rice straw-calcium capturing by carbonation (CaCCO) process. *Bioresour. Technol.* 101:6805-6811.
- Rabelo SC, Maciel Filho R, Costa AC (2009). Lime pretreatment of sugarcane bagasse for bioethanol production. *Appl. Biochem. Biotechnol.* 153:139-150.
- Shi J, Chinn MS, Sharma-Shivappa RR (2008). Microbial pretreatment of cotton stalks by solid state cultivation of *Phanerochaete chrysosporium*. *Bioresour. Technol.* 99:6556-6564.
- Sun Y, Cheng J (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.* 83:1-11.
- Taherzadeh MJ, Karimi K (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review. *Int. J. Mol. Sci.* 9:1621-1651.
- Tangboriboon N, Kunanuraksapong R, Sirivat A (2012). Preparation and properties of calcium oxide from eggshells via calcination. *Mater. Sci. Poland* 30:313-322.