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Substrate-inoculum Ratio Optimization upon the Biogas Production from Peels of Eggplant and Cassava by Biomethanization

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TOM, BMN and SMN performed the experimental work. Authors PVT and PTM wrote the first draft of the manuscript. Authors BMM, PNB and CKM wrote the protocol. Authors DDT and BKM made the literature searches. Author PTM designed the study. Author PVT performed the statistical analysis. Author TOM managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

In this work we have highlighted the importance of substrate-inoculum ratio on the biogas (biomethane) production when using peels of eggplant and that of cassava by biomethanization method. The study has been designed as follow: digestion of pig manure (inoculums) produced under anaerobic conditions; degradation of organic wastes (substrate) and measures of cumulative volumes of biogas produced, respectively. The study was conducted at the Department of Chemistry and Faculty of Oil and Gaz, University of Kinshasa, DR Congo, from February 2013 to May 2016. Standard method of serum bottles liquid displacement systems was used to measure cumulative volumes of biogas produced each day. Digested pig manure was utilized as inoculum and peels of eggplant and that of cassava as substrate sources. Previously, the inoculum was characterized. The inoculum physico-chemical parameters are: (93.27±0.27)% of moisture, (69.46±2.23)% of ash, (30.54±3.27)% of volatil solids and (6.73 ± 0.26)% of total solids. During 14 days of biogas production, the results of cumulative volumes of biogas produced showed that methanogenic bacteria exhibited high biodegradation activity of peels of eggplant than the one of cassava. And for the two cases a substrate-inoculum ratio of 50% produced high amount of biomethane. From the ratio 50% and progressively decreasing the amount of inoculum the yield also decreased proportionately. Weak biodegradation activity of peels of cassava could be explained as an occurrence of cyanide that is toxic to the methanogenic bacteria. Therefore for an optimal production of biomethane from peels of cassava a preliminary deep step of pretreatment is required for the removal of cyanide in order to improve the production yield. The substrate-inoculum ratios as well as pretreatment of substrate are valuable for an optimal yield of biomethanization.

Keywords: Biogas production; biomethanization; substrate-inoculum; peels of eggplant and cassava.

1. INTRODUCTION

Random behavior of oil prices and fossil fuels scarcity, climate change, greenhouse gases emissions, biodiversity carrying and life quality degradation, etc. are all global issues that currently generate large reflections. Furthermore because of many oil crises, the Brundtland report and the Kyoto protocol formalized these issues to environmental challenges in order to ensure a better life quality for future generations [1-3].

In spite of that, demand of energy is keeping on to increase, thus continuously forcing the use of fossil fuels. Among many alternative solutions to these issues biomethanization has shown more effective and has been reported over the past decade to be one of the best ways in terms of security, diversification of energy supply to struggle against environmental issues and especially pollution [4,5].

Biomethanization provides biogas that can be used as fuel and combustible from anaerobic treatment of biodegradable organic solid wastes. It is recognized as an effective technique of organic solid wastes degradation, enabling the reduction of organic solid wastes from households, livestock and food industry and to be among the cheapest of renewable energy processes [2,6-7].

Currently, many countries in the world have been aware of the depletion of fossil resources and because of their harmful effects especially on the climate change, they are gradually implementing new policies to limit the use of fossils energy sources and promote the renewable energy projects which includes biogas production (BP) by biomethanization [6,7].

However, the BP yield is related to the nature of the substrate (biomass) used during anaerobic treatment of organic solid wastes. Recently it has been reported that chemical composition and quantity of the substrate are among the limiting factors of the yield [8,9]. Thus nowadays the choice of substrate and physicochemical parameters characterization that influence BP are attracting researcher's attention in order to get more understanding [5,7,10-15]. Owing to the remarkable structural properties of aromatic molecules [16,17], lately our previous researches have reported the inhibition effects of some aromatic compounds on the BP [6,10], however the ratio of substrate-inoculum has not been reported. Therefore the aim of this work is to report preliminarily quantitative of the optimal ratio of substrate and inoculum on the BP by the use of peels of eggplant and cassava as organic solid wastes and to assess the optimal conditions of these organic solid wastes daily produced in the household and agricultural activities in suburban areas of Kinshasa, in Democratic Republic of the Congo (DR Congo) in biogas production [11,18].

2. MATERIALS AND METHODS

2.1 Biomass

Pig manures from Pigsty of Kinduku (Kinshasa, DR Congo) were used as biomass. As previously reported [6,10,19,20], during 2 months, pig manures were digested in the laboratory scale digester. The digested pig manures (sludge) were used as inoculum.

2.2 Substrates Choice

Choice of organic wastes used for BP is valuable because it represents a food source for the microorganisms (methanogenic bacteria) [21,22]. Therefore, the composition and quantity of substrate has a huge influence of the quality and yield of biogas produced as well as the production stability [22]. The composition also affects the quality of digestate. Indeed it contains either nutrients or potential contamination (metals, organic compounds, organisms responsible of diseases, etc.) [21]. Hence, the choice of a good substrate influences the outcome of the process, maximizes yield and the quality organic fertilizer [18]. Therefore, owing to its composition [23,24], peels of eggplant and cassava collected at 'Somba Zikida' market (Commune de Kinshasa/ DR Congo) were used.

2.3 Reagents

Sodium acetate (CH_3COONa); Chloridric acid (HCl) 3N; Water (H_2O) and Sodium Hydroxide (NaOH) were used to prepare buffer pH=7 and 15% of basic solution that allows to take out CO_2 in the biogas, respectively. All compounds were pure for analysis and supplied by MERCK.

2.4 Inoculum Analysis

Inoculum samples were analyse as reported by earlier [25-29], physico-chemical parameters were evaluated as total solids (TS), rate of moisture (M) or humidity (H), ash contents (A), and rate of organic matter (OM) or volatile solid.

2.5 Total Solids (TS)

Total solids in the inoculum were determined by the loss of weight when heating the sample at 105℃ operated in an oven during 24 hours. Thus, in a previously dried and tared empty pot (crucible) of weight (P0), we weighed a sample of inoculum (P1) and dried it in the oven at 105℃. After 24 hours, the crucible was removed from the oven, cooled in a dessicator and reweighed (P2).TS was evaluated as: TS (%) =[(P2-P0)/P1]x100. P0: weight of dried empty crucible, P1: weight of sample before heating and P2: weight of heated sample and the crucible.

2.6 Moisture (M)

Rates of M were evaluated as: M (%) = 100-TS (%).

2.7 Ash (A)

Ash or mineral matters were obtained by the weight ratio of the obtained powder and represent inorganic substances. Hence, a dried sample was weighed (P2) after being heated at the temperature of 550℃ in the oven during 6 hours. After being cooled in a dessicator, it was reweighed (P3). And Ash were calculated as: A (%) = [(P3-P0)/(P2-P0)]x100. P0 is the weight of dried empty crucible; P2 is the weight of the dried sample and crucible and P3 is the weight of sample heated and that of crucible.

2.8 Volatile Solids (VS)

Once A (%) were evaluated, the following equation allowed to calculate volatile solid (VS) or organic matter rate (MO): VS (%) = 100 – A (%).

2.9 Cumulative Volume of Biogas Pproduction (CVBP)

Standard method of serum bottles was used to determine the biogas amount produced each day during the process. The methane gas volume produced was measured by serum bottles liquid displacement systems (Mariotte flask system). The biogas produced in the biodigester is led by an infusion in a gasometer which contains a 1.5% NaOH solution, the latter is displaced and collected in the beaker and corresponds to the biogas amount produced. Four batch biodigesters were used and were labeled D1, D2, D3 and D4. In each biodigester, we have put 50, 66, 77 and 91 grams of substrate (Solanum melongena peels or that of Manihot esculenta peels) previously soaked in water for two weeks to reduce cyanogenic compounds rate for peels of cassava, against 50, 34, 23 and 9 grams of inoculums, respectively. 5 mL of acetate buffer solution prepared to pH=7 were added, then filled up the biodigester with 800 mL of water and biodigesters were sealed. After the temperature and pressure equilibrium is reached in the biodigester about twenty-four hours, we set the time t = 0 and the volume v = 0, from the t = 0 and at regular intervals of time we started to sampled daily volume of liquid displaced. The volume constitutes that of biogas volume produced. The following data have been sampled: the volume of biogas produced each day, to know the amount of gas produced relative to the displaced volume that was measured and the temperature was measured at the same time than the discharged volume of biogas. For each time after sampling manual agitation of biodigesters were needed.

3. RESULTS AND DISCUSSION

3.1 Inocula Samples Analysis

Table 1 shows the physico-chemical parameters (PPs) analyzed from the pig manure sample used as inoculum. PPs were assessed in triplicate for each trial. So results are expressed as Mean ± Stdv.

As we can notice from Table 1, PPs are ranked as follow: the inoculum contains highest rate of moisture (M), followed by the ash rate (A) in the ratio 1.34:1 with respect to M. Volatile solid (VS) and total solid (TS) showed the lowest values, the ratio with regard to M are 3.06:1 and 13.86:1, respectively. The high amount of M shows the good quality of inoculum. Indeed a high rate of humidity indicates favorable methanogenic bacteria growth in the inoculum medium. The relatively important rate of Ash shows enough inorganic matter as food of microorganisms [30,31]. Despite the small rate of VS, this small amount plays a role of inducing organic matter fermentation process of substrates.

3.2 Cumulative Volume of Biogas Production (CVBP)

Operatory conditions and cumulative volumes of biogas produced during two weeks are summarized in the Table 2. The substrate amount (QS) and that of the inoculum (QI) in the biodigesters (Dx) are given in the second and third columns. In the last but one column and the last column the CVBP and the experimental equilibrium temperature (T) are given, respectively.

The results from Table 2 show that cumulative volumes of biogas produced (CVBP) by different ratio of substrate and inoculum. The biogas production amounts vary with respect to the following sequence: D2a > D2b > D1a > D1b > D4a > D4b > D3a > D3b. From this sequence, we also note that out of the substrate nature used, the proportion of inoculum (or scrotum of methanogenic bacteria) affects the methane gas amount for a given organic substrate [25,32-34]. Indeed biodigesters D2 (D2a and D2b) with 50% of the inoculums and 50% of substrate produced high volumes of methane gas during 14 days of biodigestion; 882.0 mL of CH_4 produced for peels eggplant and 430.2 mL of CH_4 produced for peels of cassava. However, by considering the biodigester D1 (D1a and D1b), D2 (D2a and D2b) and D4 (D4a and D4b) the CVBP decreased and the sequence D1>D2>D4 correspond to the decrease of inoculums rate. This behavior indicates that the biogas production is favorable when using close amount of substrate and inoculums and 50% ratio substrate-inoculum is the optimal ratio. The histogram of Fig. 1 clearly visualizes the exhibited behavior of substrate-inoculum ratios.

The Fig. 1 shows clearly that for all ratios of substrat and inoculum used peels of eggplant have produced a high CVBP than that of peels of cassava. Indeed, this can be explained by the presence of cyanides that are toxic to methanogens bacteria (archaea). Thus acidification of the peels of cassava media did not remove the maximum amount of cyanides reason why in the biodigester of the peels of cassava, the activity of archaea decreased. So for an optimal biogas production from the peels of cassava a deep previous step of pretreatment is required in order to eliminate the maximum amount of cyanide. The biogas production through the organic material decomposition is due to the concerted implication of various floras of microorganisms, where archaea are involved in the final step to produce methane from the substrate acetate. Therefore when we have more archaea widely available, simple fatty acids arising from the degradation of the organic material will be converted to biogas.

With a total cumulative volume of 882.0 mL methane gas produced during 14 days of biodigestion, the digester D2a (50/50: peels of eggplant/inoculum) exhibited the greatest methanogenic activity during our study and therefore we highlighted the influence of the substrate nature on the yield of the biogas, for with the same ratio as substrate/inoculum, the biodigester D2b (50/50: peels of cassava/inoculum) produced less amount of methane gas (439.2 mL) for 14 days. We made the same observation for the biodigesters D1 (D1a and D1b), (D3a and D3b), and (D4a and D4b) that have same proportions substrate/inoculum but the biodigesters of peels of cassava always provided great productivity. The low methane production of biodigesters containing peels of cassava compared to those of peels of eggplant is due to the toxicity of substrate when it is fermented by microorganisms during the biomethanization process. Indeed, with a high concentration of cyanogen compounds, cyanide estimated at 42.269% [32,33,34], peels of cassava biodegradation is inhibited and this inhibition increases with the concentration of the substrate (peels of cassava). This was noticed with the high decrease of methanogenic activity of archaea when the amount of peels of cassava increased (Fig. 1: blue columns). With small amounts of lipids and proteins but high concentrations of antioxidant compounds (anthocyanins, flavonols and the phenolic acid glycoside: quercetin-3-glucoside, quercetin-3-rhamnoside and myricetin-3- galactoside), peels of eggplant substrate that had good anaerobic biodegradability compared to peels of cassava in which out of cyanide compounds, there is huge amounts of cellulose fibers that is unfavorable for a good anaerobic digestion.

Table 1. Physicochemical parameters of inoculum

PPs	TS (%)	M (%)	A (%)*	VS (%)*
1Trial	6.71	93.29	68.42	31.58
2Trial	6.47	93.53	72.73	27.27
3Trial	7.00	93.00	67.23	32.77
Mean ± Stdv	6.73 ± 0.26	93.27 ± 0.27	69.46 ± 2.23	30.54 ± 3.27

**: Based on the dried weight*

Table 2. Maximum cumulative volume of biogas processing

Biodigester		QS (gr)	QI (gr)	CVBP (mL)	T (°C)
D1	D1a	66	34	567.00	28°± 1
	D1b	66	34	400.00	28°± 1
D2	D2a	50	50	882.00	28°± 1
	D2b	50	50	439.20	28°± 1
D3	D3a	91	9	278.00	28°± 1
	D3b	91	9	144.00	28°± 1
D4	D4a	77	23	559.00	28°± 1
	D4b	77	23	233.40	28°± 1

a: Peels of eggplant; b: Peels of cassava

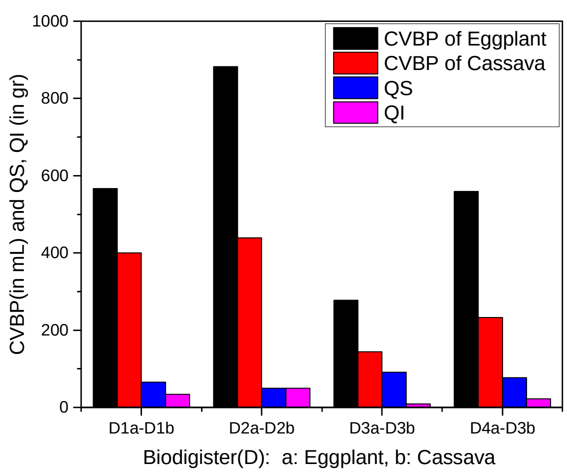

Fig. 1. Variation of the total amounts of biogas production with respect to the substrate-inoculum ratio

4. CONCLUSION

This work aimed to conduct a comparative study of biogas production from peels of eggplant and cassava by biomethanization, in order to highlight the importance of substrate and inoculum ratio upon biomethane process and to consider the conditions to use one of such wastes for biogas production and thereby to open a way that can allow to reduce their amount in the environment of Kinshasa area. The results showed that the biomethanization of peels of eggplant and cassava is possible and has an acceptable yield in the case of the peels of eggplant. However, since this yield also depends on the amount of inoculum used (that is an important source of methanogenic bacteria), thereby for high yield of biomethanization, the 50% substrate: 50% inoculum ratio was required. Therefore, the peels of eggplant biomethanization is possible and profitable in the presence of pig slurry as inoculum, but the ratio of inoculum also plays an important role for an optimal yield. And for the peels of cassava, effective pretreatment in order to eliminate cyanogens compounds is essential in order to increase the yield of biogas.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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