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Potential for methane production from cashew apple residues

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Abstract

Organic matter is a source of bioenergy through anaerobic digestion. The aim of this study is to evaluate the potential energy of cashew apple (CA) abandoned at more than 90% in plantations. Samples of CA residues collected from cashew plantations in Togo were used as substrate. Study of physico-chemical parameters was carried out using standard methods. The potential for methane production was determined by the volume displacement method of a sodium solution of pH 12. Bovine dung was used as an inoculum. The volume of liquid moved, measured daily, was continued until a plateau on the methane production curve was obtained on the 30th day. Despite physical pretreatment, the pH of the substrates remains acidic 6. The high volatile matter content of more than 96 % justifies the choice of anaerobic digestion. The ash content of 3.44 – 3.60 shows mineral salt contents outside the inhibition range. However, the high content of lignin's 23.81 – 28.18% was a constraint to anaerobic digestion and makes theoretical calculations far from being compared with experimental results. The methane potential production from substrates ranges from 67.71 – 74.70 L. CH_4.(kg. MV)$^{-1}$ under conditions where the temperature was 37°C ± 2. Co-digestion, chemical or biological pretreatment in future work could improve the anaerobic digestion process of CA waste.

1. Introduction

The last decades have been marked by a permanent increase in oil consumption in the world, the consequence of which is the depletion of fossil fuel reserves and the increase in the price of petroleum products (Fava and Romanelli, 2023). Similarly, the burning of fossil fuels contributes the most to greenhouse gas (GHG) emissions, which lead to air pollution and global warming (Traoré *et al.*, 2016). In order to ensure the protection of the environment, it is necessary to proceed with the development of bioenergy to create substitutes for fossil fuels. On this, the technique of renewable energy production through biodigesters has found its application through the valorization of animal and plant waste (Okolie *et al.*, 2023; Das *et al.*, 2023)). However, the huge amount of some agricultural waste produced each year, which could usefully be exploited in this sense, is often abandoned and rotten at the beginning of plantations and agro-food industries. CP is the most common and most encountered example in Togo.

Indeed, the cashew tree being cultivated for its nut, its false fruit PC representing 9 to 10 times the weight of the nut (Soro *et al.*, 2017), is often neglected. This allows (Antony *et al.*, 2020) to estimate

the loss of cashew apples at 90% worldwide. These losses are linked to several factors such as inadequate road infrastructure, inadequate processing infrastructure and the astringency of cashew apples, which hinders their food value. However, the organic matter contained in these residues is a source of biogas through anaerobic digestion (Djaâfri *et al.*, 2009). Natural and spontaneous, anaerobic digestion is a biological process of degradation of organic matter by microbial flora that is triggered under anaerobic conditions (Moletta, 2005); it produces not only biomethane but also carbon dioxide, hydrogen sulfide and other trace elements. Especially since large cashew plantations are found in rural areas, the production of biomethane from this energy resource would reduce the loss of raw material but constituted a renewable energy source (Thaiki *et al.*, 2023; Aouled Ali et al. 2017). In addition to the availability of sufficient quantities of the substrate which is an essential condition, cashew apple is an agricultural residue rich in reducing sugars (fructose and glucose), vitamins, minerals, and certain amino acids (Silveira *et al.*, 2012) and can be a suitable low-cost substrate for anaerobic digestion studies. The characterization and control of physico-chemical parameters were key factors in this study. The implementation technique for a better determination of the biomethanogenic potential of the substrate is simple and topical.

The objective of the present work is then to evaluate the methane potential production by anaerobic digestion of CA waste at the laboratory scale and this by monitoring the evolution of the volume of methane produced through the volume of sodium solution moved.

2. Methodology

2.1 Sampling

The plant biomass used as substrate in this study consisted mainly of CA residues, including cashew bagasse and raw cashew apples, all of which were unfit for human consumption. These residues (raw cashew apples after harvesting nuts) were collected from February to March 2021 in the cashew plantations of Koudjoudjou, Sarakawa, Banjeli and Bouladè located respectively in the prefectures of Dankpen, Kozah, Bassar and Assoli which represent the very favorable areas of cashew in the Kara region as shown in Figure 1.

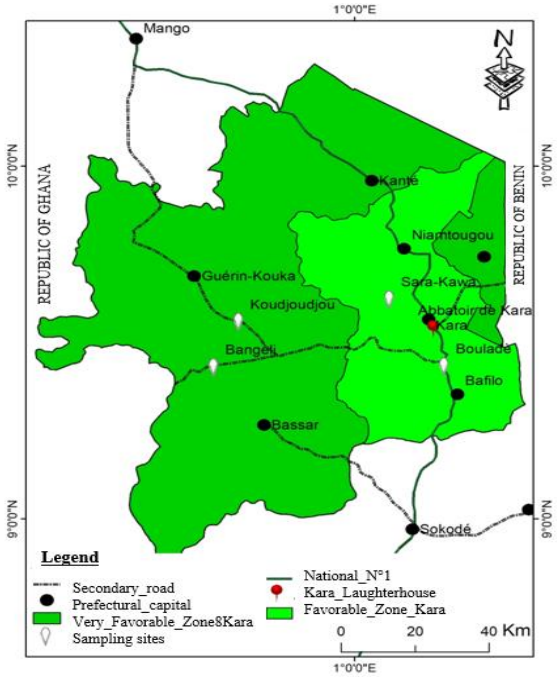

Figure 1: Study area with sampling points from the Kara region (Togo)

2.2 Sample processing

These substrates of acidic characteristics with a pH between 4 and 5, very fibrous, after collection, were pretreated to reduce the effects of these two characteristics which are inhibitory factors during their anaerobic digestion (Kata *et al.*, 2020). They were stored at laboratory temperature on a bench for 10 days. After drying, each type of waste was ground in a mortar and then passed through a sieve with a diameter of 1 mm mesh and the powders obtained were stored in sterile bags in the laboratory at a temperature of 32 ± 2 °C. Three types of cashew apple samples were made, namely: raw cashew apple dried RCAD; DCBA dried cashew bagasse after pressing the juice and a mixture of 50 % RCAD and 50 % DCBA. This mixture was carried out in order to value the apples that are discarded for lack of deterioration. Greenish in color, residues from the bovine dung freshly collected from cattle at the Kara slaughterhouse were used as an inoculum in our study. Once in the laboratory, these residues were diluted (m/v, 4/5) with a buffer solution consisting of 2 g of K_2HPO_4 + 2 g of NH_4Cl/1000 mL of distilled water to prevent a drop in pH (Traoré *et al.*, 2016). The inoculum thus treated was distributed in 500 mL vials with manual stirring for one hour. After this homogenization phase, the vials containing the inoculum were separately incubated for 10 days respectively at laboratory temperature 32-35 °C before the start of the experiments, in order to exhaust the starting organic matter. As a result, the gas produced was quantified daily. The contribution of inoculum activity was subtracted when drawing the curves.

2.3 Physico-chemical analyses of the substrate

2.3.1 pH measurement

5g of samples were dissolved in 10 mL of distilled water. The mixture was stirred for 30 minutes. The suspension is left to stand for 10 minutes. The PT-10 probe of the Sartorius pH meter was immersed in the beaker containing the mixture and the pH reading was made after stabilization.

2.3.2 Determination of dry matter

The dry matter was determined from 5 g of samples, by differential weighing, after passing through the oven made in Germany type Narbertherm at 105 ± 0.5 °C for 24 hours.

2.3.3 Determination of organic matter and ash content

After drying in an oven at 105 ± 0,5 °C, the sample consists only of organic and mineral dry matter which does not burn at 500 °C. A sample previously steamed at 105 ° C is put in the oven at 550 ° C for 4 H (Koledzi *et al.*, 2011). The content of organic matter OM or volatile solid is obtained by the difference in weighing between the mass of dry samples (105 °C) and the mass of calcined samples. It shall be expressed as % in relation to the dry mass of samples. The ash content was obtained by weighing, after evaporation of water and removal of organic constituents by calcination of the sample using equation **Eqn.1**.

$$\%\text{Ash} = 100 - \%\text{OM} \qquad \textbf{Eqn.1}$$

2.3.4 Total organic carbon

The organic carbon content was derived from the volatile dry matter content after calcination of the sample in a muffle furnace at 550°C for two (2) hours using the equation Eqn.2 (Koledzi *et al.*, 2011) :

$$\%C = \frac{\%MO}{1{,}724} \qquad \textbf{Eqn.2}$$

2.3.5 Protein determination

The Kjeldahl method was used for the determination of proteins. A test portion of 0.2 g of samples with 10 mL of concentrated sulfuric acid and catalysts (3.5 g of K_2SO_4 and 0.4 g of $CuSO_4$) was mineralized at 400 °C for$_2$hours. The mineralization diluted with 40 mL of distilled water was neutralized with 10 N soda in the presence of phenophthalein. The solution obtained was distilled and collected in 20 mL of boric acid containing 5 to 6 drops of helianthin and bromocresol green. 0,1 N sulfuric acid was used to titrate the distillate. A blank was made by performing the same procedure without the test taking. The protein content (P) was determined by equation Eqn.3.

$$P = \frac{(V_1 - V_0) \times N \times 14 \times 0{,}001 \times 6{,}25 \times 100}{m} \qquad \textbf{Eqn.3}$$

With $\mathbf{V_0}$ = Volume of H_2SO_4, having been used to titrate the blank; V_1 = Volume of H_2SO_4 used for the titration of the sample; **N** = Normality of sulfuric acid; m = mass of the sample; 6.25 = Nitrogen-to-protein conversion coefficient; 14 = molar mass of nitrogen; 0.001= Volume conversion factor (mL) to L.

2.3.6 Lipid determination

The lipid content was determined according to the method described by (Afilal *et al.*, 2014). This method is based on differential solubility of lipids in organic solvents. It uses the SOXLET method to extract lipids from 10 g of samples using hexane as an organic solvent.

2.3.7 Lignin content

The lignin content was determined according to the method of (Sluiter *et al.*, 2008) used by (Amenan *et al.*, 2022). This method uses dried biomass extracted after lipid analysis. It hydrolyzes and solubilizes carbohydrates in a 72 % sulfuric acid solution. Dilution followed by filtration makes it possible to determine the insoluble lignin content after calcination at 575 °C taking into account the ash content. The acid-soluble lignin fraction was determined by measuring the absorbance of acid-hydrolyzed samples at 320 nm. Lignin content was calculated as the sum of acid-insoluble lignin and acid-soluble lignin.

2.3.8 Determination of carbohydrates associated with hemicellulose and cellulose

It was determined theoretically by referring to (Melzer, 2013) using equation **Eqn.4**.

$$\mathbf{H\ \&\ C} = 100 - (\%\text{protéine} + \%\text{lipide} + \%\text{lignine} + \%\text{cendre}) \qquad \textbf{Eqn.4}$$

With H & C: hemicellulose and cellulose content.

2.4 Mineral Analysis

Mineral salts such as Na^+, Mg^{2+}, K^+ and Ca^{2+}, were determined with the SAA atomic absorption spectrophotometer (brand 3000 thermo fiscer series). The solubilization method that is used is nitric acid attack mineralization. It is carried out in a closed environment and hot (150 °C). In 1g of sample of dried and finely ground PC waste was added 10 mL of hydrogen peroxide (H_2O_2) to 9 % and left to act for 24 hours before the attack with nitric acid 16 N (4 mL).

Total phosphorus was determined directly with the Skallar auto-analyser after mineralization of the sample using sulfuric acid (H_2SO_4) and $C_7H_6O_3$ in the presence of hydrogen peroxide (H_2O_2).

2.5 Theoretical biomethanogenic potential and biodegradability

2.5.1 Estimation of Theoretical Biomethanogenic Potential (TBMP)

Referring to the work of (Triolo *et al.*, 2011), the theoretical biomethanogenic potential (TBMP**)** in L. CH_4.(kg. MV)[1] was evaluated using equation **Eqn.5**.

$$PBMT = (\%Lipid \times 1014 + \%Protein \times 496 + \%Carbohydrates \times 415 + \%Lignin \times 727) \times 0{,}001. \quad \textbf{Eqn.5}$$

2.5.2 Experimental device of Biomethanogenic Potential (EBMP)

The experimental device Figure 2 consists of digesters (0.5 L vials), 0.5 cm diameter rubber line pipes, which collect gas from the digester to tanks containing the acidic pH 2 and sodium solutions pH 12 of 5 L.

The tests were carried out in batch reactors. They were based on the measurement of methane and biogas production, in hermetically sealed 500 mL reactors in which a known quantity of test sample (1.5 g MV/reactor, MV: volatile matter, corresponds to organic matter) and anaerobic microorganisms from the treated inoculum were brought into contact. (0.5 g MV/reactor). The ratio S/X, (quantity of substrate to quantity of microbial biomass) was therefore 3. In order to ensure good microbial activity, the reactors are placed in optimal conditions, by adding a bicarbonate buffer solution to adjust the pH to 7.2 after 5 days of pre-fermentation. The volume of each reactor is then completed to 400 mL with distilled water during pH adjustment. Each vial has been hermetically sealed with septum caps to ensure perfect gas tightness. The incubation of the vials was done at a temperature of 37 ° C ± 2 in order to be in anaerobic mesophilic condition. Each bioreactor underwent manual stirring daily. The study was carried out in the period from March to April which corresponds to the heat period in the Kara region (Northern Togo). Fifteen (15) reactors at a rate of five (5) per substrate placed under the same conditions were used as a test for monitoring the pH variation. The duration of the experiment was 30 days. To do this, the liquid displacement method (Figure 2) was used. An acidic solution of pH 2 contained in a 5 L can will prevent the dissolution of the gases so that the volume of the liquid displaced can correspond to the volume of biogas that will be stored at the top of the canister. This aqueous solution allows by the displacement of the liquid, to quantify the production of biogas (Akpaki *et al.*, 2016). The sodium solution of pH 12 contained in a 5 L tank, will allow the dissolution of carbon dioxide and some gases except methane so that the volume of liquid displaced can correspond approximately to the volume of methane stored at the upper part of the tank. For each sample, the test was performed in triplicate; the volume of liquid moved was measured daily and continued until a plateau was obtained on the methane and biogas (mL) production curve. The values obtained are the average of the triplets on each digester.

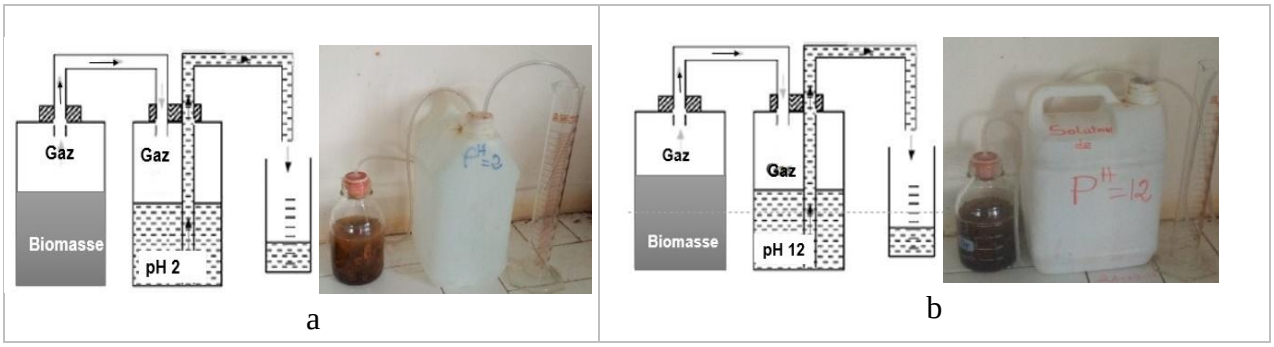

Figure 2: experimental device

2.5.3 Anaerobic biodegradability

It was determined by the ratio between the experimental methanogenic potential and the theoretical methanogenic potential using equation **Eqn.6.**

$$BA = BMEP/TBMP \times 100 \qquad \textbf{Eqn.6}$$

With BA: Biodegradability in Anaerobic; EBMP: Experimental Biomethanogenic Potential and TBMP: Theoretical Biomethanogenic Potential

3. Results and Discussion

3.1 Physico-chemical characteristics of study substrates

Table 1 indicates that the substrates used are at acidic pH despite pre-treatment. This justifies the use of a bicarbonate buffer solution to adjust the pH to 7.2 after 5 days of pre-fermentation in order to be in the optimal range of pH values (6.8;7.8) (Esposito *et al.*, 2011) favorable to methanization. The pre-treatment phase resulted in self-regulation of the pH of substrates that were more acidic. The cashew apple in its initial state has a pH between 4.2 and 5.

The substrates analyzed have almost little variable levels of dry matter (%DM) and organic matter (%OM). There is an abundance of OM in substrates. The latter is considered to be the part of the substrate that is likely to be transformed into biogas (Darwin *et al.*, 2014). This indicates that our substrates are suitable for use as raw material in the anaerobic digestion process (Nurika *et al.*, 2023). This organic matter content is quite important considering the protein composition between (9.31 and 10.75) and carbohydrates (59.28 and 63.54). Fruit and vegetable waste usually contains a high percentage of DM due to its high organic matter content; fraction favorable to the process of anaerobic digestion (Calabrò *et al.*, 2016). The lipid fraction is particularly less important in all three substrates and is a peculiarity of fruit waste (Prabhudessai *et al.*, 2013). The carbon content of our three substrates is high and practically identical due to the fact that they are of the same nature about 56%. This carbon content is in perfect agreement with the work of (Kata *et al.*, 2020) whose carbon content was 56.65%. This carbohydrate-rich biomass guides the choice for biogas production by anaerobic digestion. However, variability in the nitrogen content of substrates influences the C/N ratio. The DCBA have a nitrogen content 1.98 ± 0.21% higher than RCAD 1.49 ± 0.35%. The mixture of the two substrates in the same proportion gave a nitrogen content of 1.78 ± 0.47%.

The C/N ratio of the three substrates DCBA, RCAD and the mixture are 28.27 ± 0.42; 37 ± 0.38 and 31.46 ± 0.56, respectively. The optimal C/N ratio for anaerobic digestion is in the range 20 to 30 according to the literature. Only the DCBA substrate has its C/N ratio in this range. A high C/N ratio is an indication of rapid nitrogen consumption by methanogen and leads to a reduction in nitrogen consumption with low methane production (Ojikutu and Osokoya, 2014). Although we know that the further a substrate moves away from this range, the more its methane production decreases, the use of an inoculum could improve methane production. The ash content of high RCAD compared to that of DCBA are perfectly correlated with the mineral salt contents of the substrates. Similarly, the amount of different mineral elements present in these organic substrates is sufficient and non-toxic, in comparison with the reference inhibitory values according to the work of (Darwin *et al.*, 2014). However, the high lignin content of 23.81 - 28.18% could be a constraint. Indeed, according to (Hammado *et al.*, 2020), lignin can inhibit the hydrolysis process in anaerobic digestion and affect the volume of biogas production.

Table 1: Physicochemical and macromolecular characteristics of study substrates

Parameters	Types of substrates		
	RCAD	DCBA	Mixture
pH	6.28 ± 0.10	6.23 ± 0.13	6.42 ± 0.12
%Dry matter (DM)	21.81 ± 2.40	31.41 ± 3.21	30.20 ± 1.81
%Organic matter (OM)	96.40 ± 1.8	96.52 ± 2.00	96.56 ± 1.00
%Ash	3.6 ± 0.16	3.48 ± 0.14	3.44 ± 0.20
%N	1.49 ± 0.35	1.98 ± 0.21	1.78 ± 0.47
%C	55.91 ± 0.9	55.98 ± 1.00	56.00 ± 0.77
C/N	37 ± 0.38	28.27 ± 0.42	31.46 ± 0.56
Phosphorus (mg/g DM)	0.42 ± 0.02	0.38 ± 0.01	0.40 ± 0.00
Na^+ (mg/g DM)	12.42 ± 0.03	11.12 ± 0.08	11.23 ± 0.01
K^+ (mg/g DM)	21.13 ± 0.08	19.42 ± 0.05	20.18 ± 0.10
Ca^{2+} (mg/g DM)	12.36 ± 0.22	13.11 ± 0.17	13.21 ± 0.13
Mg^{2+} (mg/g DM)	8.17 ± 0.12	9.28 ± 0.26	8.52 ± 0.24
% Lipid	3.24 ± 0.30	4.17 ± 0.50	3.39 ± 0.42
%Protein	9.31 ± 1.50	10.75 ± 1.21	10.21 ± 1.11
%Lignin	23.81 ± 0.35	28.18 ± 0.48	26.41 ± 0.50
%H & C	63.54 ± 1.20	56.90 ± 1.00	59.28 ± 1.13

3.2 pH variation during anaerobic digestion of the three substrates

The pattern of the curves in Figure 3 shows a decrease in the initial pH values of the three samples from 6 to 5 during the 5 days of anaerobic pre-fermentation. Indeed, in the presence of soluble or rapidly hydrolyzable substrate, fast-growing acidogenic bacteria tolerant to acidic pH generated significant amounts of volatile fatty acids lowering pH (Traoré *et al.*, 2016). This period therefore corresponds to the phase of hydrolysis and acidogenesis. This is the stage where we witness the decomposition of polymers into monomers and the fermentation of monomers into amino acids; the sugars and fatty acids obtained during hydrolysis are converted into volatile fatty acids using acid-forming microorganisms (Amani *et al.*, 2010). In the interval of 5 to 10 days, after pH adjustment, there was self-regulation of pH. The work of (Kata *et al.*, 2020) showed this self-regulation beyond the third day without the addition of a buffer solution, but with rector thermostats. The temperature variations during our work may explain this difference because the temperature of the experiment room is not stable. This pH self-adjustment step towards neutrality and more is characterized by acetogenesis. Beyond 10 days, the observed pH range shows that it is the methanogenesis itself. It is characterized by a small variation in pH due to the addition of the buffer.

3.3 Experimental measurements

3.3.1 Methane and biogas production

The average kinetics of methane production **Figure 4** makes it possible to appreciate the time after which the maximum quantity of methane is produced. In this study, the kinetics of daily methane

production lasted 30 days until methane production was no longer observed. Methane production started immediately on day one and peaks in daily methane production were observed after the first day on all three substrates. The largest peak was achieved with RCAD with a maximum daily methane production rate of 128 mL on 6th day. An analysis of the graph shows that between the 6th and 11th day, all three substrates showed their peak maximum. This is the period of transformation of volatile fatty acids into methane and carbon dioxide. This period corresponds to the day after the pH adjustment and the period of pH self-regulation.

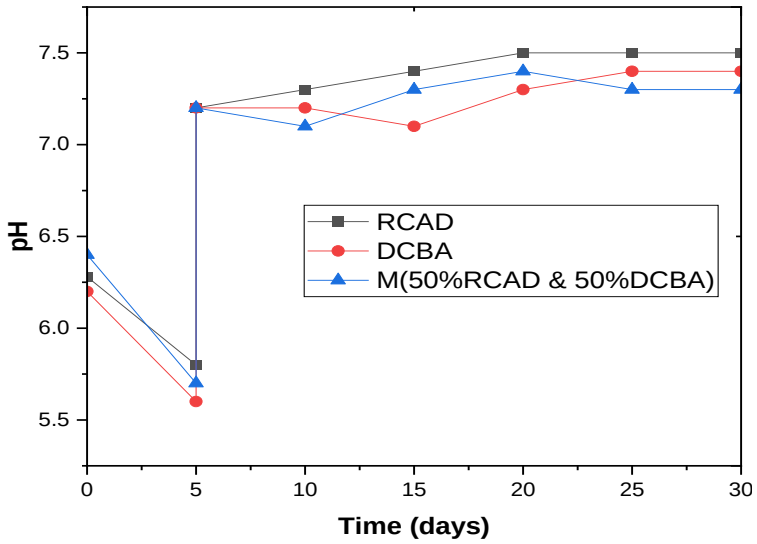

Figure 3: pH variation during digestion

The work of (Ojikutu et Osokoya, 2014) showed that the variation in biogas production reached its peak during the first five days in the methanization of food waste. Beyond the11th day, there is a drop in all methane production. It corresponds to the acetogenic phase whose reaction rates are generally slow and subject to inhibition problems related to the presence of hydrogen which modifies the thermodynamic equilibrium of the reactions (Kata *et al.*, 2020). Beyond the 15th day, the decrease in methane production shows that we are entering the phase of methanogenesis which is considered the limiting step in the degradation process of dissolved compounds (Nikiema *et al.*, 2020). This phenomenon can be explained by a depletion of the substrate in general. From the 28th to the 30th day marking the end of the anaerobic digestion process, the volumes of methane recorded are almost zero on all three substrates. **Figure 5** shows the cumulative methane production in ascending order: The mixture of 50% RCAD and 50% DCBA; DCBA and RCAD with volumes respectively 938; 918 and 817 mL. The option of extracting the juice before anaerobic digestion would seem to be the most cost-effective in terms of methane volume.

The biogas accumulations from the process of displacement of the acidic solution of pH 2 are recorded in Figure 6. The mixture of 50% RCAD and 50% DCBA produced the largest amount of biogas 1767.42 mL, comes in second place with RCAD with a production of 1702.54 mL and finally RCAD 1436.33 mL of biogas. The methane content in the RCAD substrate 56.88 % is higher than those of the DCBA and the mixture achieved 53.96 and 53.07 % respectively **Table 2**. This could be explained by the high sugar content of RCAD compared to DCBA which lost sugar during juice pressing. A methane content of 62.95 % was found by (Kata *et al.*, 2020) with substrates of the same nature. Although the diagram in **Figure 7** shows that RCAD produced less biogas and methane compared to other substrates, its methane content is higher.

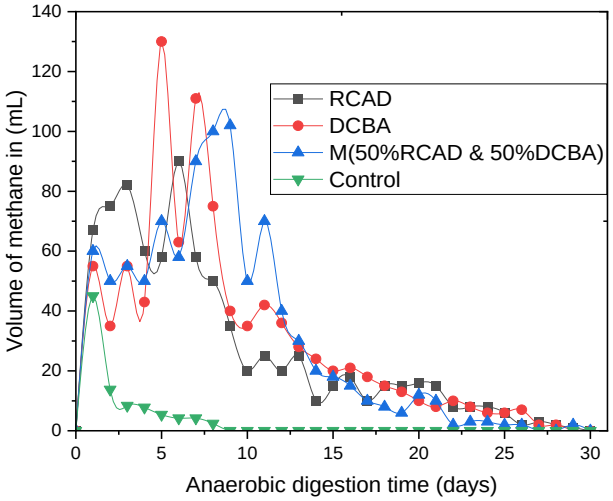

Figure 4: Daily methane production

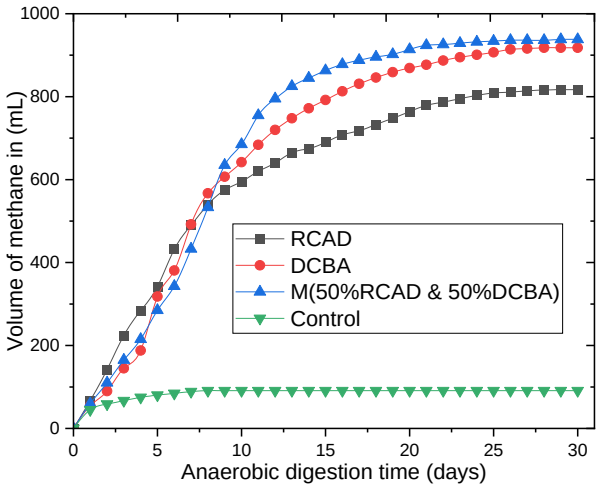

Figure 5: Cumulative methane production

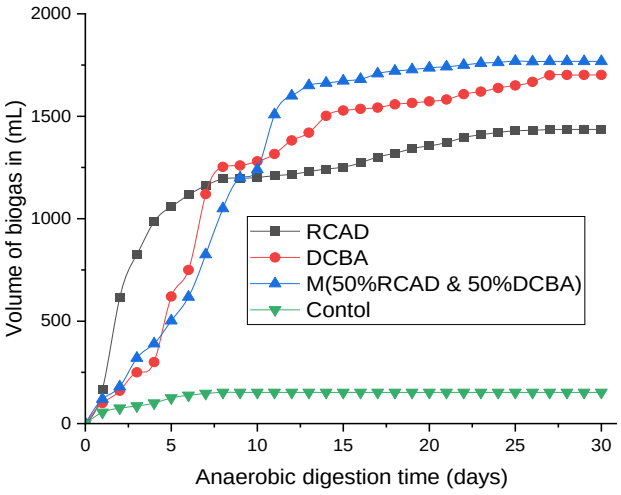

Figure 6: Cumulative biogas production

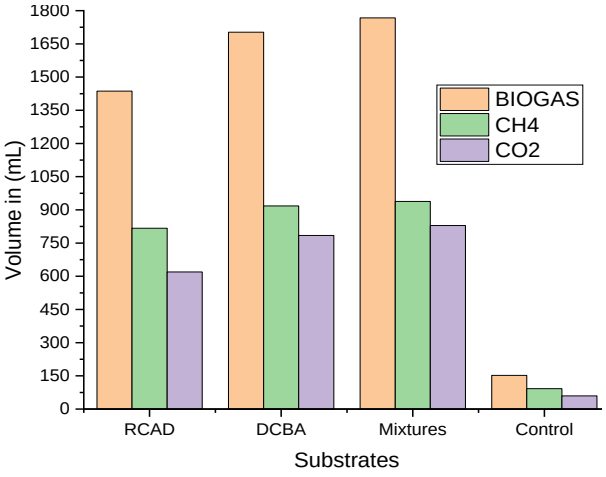

Figure 7: Cumulative diagram of Biogas, Methane and Carbon Dioxide

Table 2: CH_4 percentage of substrates

Substrates	RCAD	DCBA	Mixture	Control
% Methane	56,88	53,96	53,07	60,65

3.3.2 Specific methane potential

The assessment of the biomethanogenic potential of substrates is contained in **Table 3**. The results obtained by theoretical calculations are far from being compared with experimental results. The differences between theoretical and experimental values could be explained by constraints during anaerobic digestion (Akpaki *et al.*, 2016). This influenced the rate of biodegradability. This low biodegradability of about 22.16% can be explained by either an inhibition or a strong difficulty of degradation of organic matter given the non-stability of temperature during the anaerobic digestion process. Lignin is a recalcitrant compound that is associated with hemicellulose forming covalent and non-covalent bonds in the cell wall which makes it difficult to separate from polysaccharides (Semhaoui *et al.*, 2017; Nishimura *et al.*, 2018; Terret *et al.*, 2019; ; Zoghlami & Paës, 2019).

Table 3: BMP of substrates

	Average production of CH_4 L. (kg. MV)$^{-1}$		
Samples	RCAD	DCBA	Mixture
EBMP	67,71	71,95	74,70
TBMP	352,39	340,86	362,55
Biodegradability	20,32 %	22,16 %	21,61 %

Conclusion

The potential of PC waste as a substrate for anaerobic digestion was investigated. The physico-chemical parameters of different CP samples have shown that these substrates can be used in anaerobic digestion. Anaerobic digestion of 1.5 g MV of each of the DCBA, RCAD substrates and the mixture produced in 30 days' maximums of 817; 918 and 938 mL of methane respectively. These results show that the recovery of PC waste into methane is of economic interest. It has been proven that the EBMP of the substrates in our study is between 67.71 and 74.70 L CH_4. (kg. MV)$^{-1}$ with a biodegradability that varies between 20.32 and 22.16%. EBMP is far from being compared to TBMP (352.39 and 362.55 L.CH_4. (kg. MV)$^{-1}$ shows inhibition that could be related to lignin content or excessive acidity of substrates. A judicious choice to carry out chemical and biological pre-treatments in future work could limit this constraint. The optimization of the chemical composition of PC waste with a system of pre-treatment, co-digestion and continuous reactor use could be interesting and expected for a better anaerobic digestion performance. However, energy recovery from cashew apple biomass in Togo would reduce its post-harvest loss in plantations and could bring a gain to producers.

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