



Influence of Inoculums Source and Pretreatment on Biogas Production from Cashew Nut Shells (*Anacardium occidentale*)

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Received: 29 Sep 2021; Received in revised form: 09 Nov 2021; Accepted: 16 Nov 2021; Available online: 22 Nov 2021

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Abstract— Bioenergy recovery from cashew nut shells was investigated throughout using efficient inoculums source and appropriate pretreatment. Physicochemical characteristics of shells and inoculums including pH, volatile fatty acid (VFA, total solid, volatile solid, ash were determined using standard methods. Total anaerobes and methanogenic archaea from inocula were determined by MPP method. Wastewater (WW), sludge from bioreactor (SBR), bovine dung (BD) and mixed inoculums (MIX) were used to evaluate inoculums source in batch system. Biochemical methane potential of pretreated shells was evaluated. Biogas was measurement by liquid displacement. CH₄ and CO₂ were performed by GC. MIX and WW showed high concentration of methanogenic bacteria (2.3 10² CFU/mL). Best biomethane levels 70.38% with yields of 55.52 L biogas. (Kg VS)⁻¹ was observed with old shells inoculated by MIX. Low productivity was noted with thermal and biological treatment of old and fresh shells, 11.20 and 0.02 L CH₄. (Kg VS)⁻¹, respectively. Inoculums source has significant effect on biogas production. Mixed inoculums exhibited significantly high yields of biomethane. Thermo-biological pretreatment seems to be not appropriate for a better biomethane production. Combination of thermochemical and biological pretreatment could be necessary for best biomethane production yield.

Keywords— Cashew nut shells, methanogenic bacteria, thermo-biological pretreatment, biomethane.

I. INTRODUCTION

Worsening international food problems have created the need to develop agri-food sector in developing countries. Development of agro-food industries releases an important amount of organic waste or biomass which can be used as a feedstock source for bioenergy and or bioproduction. However, this technological way for biomass valorization remains still underused in large-scale condition in these countries. And their outdoor accumulation leads to environment and population health

problems as highlighted by several studies (WHO, 2015, Franchitti *et al.*, 2020). Nevertheless, some lab- and pilot-scale studies have been carried out for converting into bioenergy, biofuels and bio-based products (Mahdy *et al.*, 2015, Na *et al.*, 2021). Agricultural residues depend on locality. The third largest agricultural product for export, after cotton and sesame, is cashew nut which is still a poorly performing sector marked by low yields, processing and marketing. However, cashew production increased by 13% in 2018 (85,000 tons) and the

processing rate from 40% (7,000 tons) (COMMODAFRICA, 2019). In Burkina Faso, cashew nut sector is experiencing increasingly significant development with production estimated at 81,000 tons in 2017 and expected to reach 200,000 tons in 2030 (Somé, 2014). Processing units generate a large amount of waste consisting of approximately 73% hulls and 6% skins (Tagutchou and Naquin, 2012). According to Lacroix (Lacroix, 2003), cashew shell contains a toxic acid, cashew nut shell liquid (CNSL) or balm, which makes production of almonds painful (during shelling). Improper drying procedures can create potentially hazardous situations for environment following release and infiltration of CNSL into soil. This would cause the death of trees in the area and infertility of the soil for several years. Cashew processing units are confronted with recurring energy problems. They use unsustainable energy sources such as wood and butane gas at excessive costs, leading them to resort to cashew shells as fuel in the processing chain, in particular for the embrittlement of nuts, cooking with steaming and drying almonds (Thiombiano *et al.*, 2011). Godjo *et al.* (2015) showed cashew nut shells burning causes significant damage to environment and human health. Cashew nut consists of hard woody shell containing liquid (Cashew Nut Shell Liquid CNSL). CNSL is composed of 70 to 90% anacardic acid, 10 to 18% cardol and about 5% cardanol (Das, Sreelatha, & Ganesh, 2004; Patel, Bandyopadhyay, & Ganesh, 2006). Most cashew nut upgrading work is oriented towards CNSL extraction processes, some only talking about thermochemical treatment, in particular pyrolysis and gasification (Das *et al.*, 2004, Singh *et al.* 2006, Tsamba *et al.* 2006). Faced to increasingly growing energy demand in processing units, and environmental problems linked to burning and release of shells into environment, a more ecological recovery of hulls is required. Several authors such as Saka *et al.* (2009) and Chandel and Singh (2011) showed difficulties during the bioconversion of plant species due to the structure and components of cell walls, which certainly influences digestibility. Indeed, Mahato *et al.* (2021) was reported possibility of citrus waste biotransformation, bio-waste which is antimicrobial in nature and inhibits fermentation process. The systematic process of bioproducts production from citrus biomass requires pretreatment steps including physical, chemical, physicochemical and biological pretreatment. Leitão *et al.* (2011) studied

anaerobic digestion of cashew bagasse, but found no conclusive results given the complexity of this substrate. Nikiema *et al.* (2020) indicated the feasibility of biogas production from cashew nut shells and found experimental biochemical methane potential (BMP) was $46.84 \text{ CH}_4 \text{ L. (Kg VS)}^{-1}$ and $1.98 \text{ CH}_4 \text{ L. (Kg VS)}^{-1}$ for old and fresh shells, respectively. However, theoretical values could reach up to $526.206 \text{ CH}_4 \text{ L. (Kg VS)}^{-1}$ and $666.937 \text{ CH}_4 \text{ L. (Kg VS)}^{-1}$ for old and fresh shells, respectively. The presence of certain substances including anacardic acids, cardol and cardanol could explain lower yields observed in experimental study, since these substances constitute a limit to the bioconversion of cashew shells into biogas. Our study aims to contribute to ecological elimination of cashew nut for environment protection and at the same time to agro-resources valorization by bioconversion into bioenergy. Specifically, it aimed at to find out suitable source of inoculum and appropriate way of pretreatment of cashew nut shells for a better biogas production.

II. MATERIAL AND METHODS

2.1. Sampling of cashew nut shells and microbial inoculums

The samples required for biogas production have been collected at ANATRANS Company, a cashew scale transformation units, located in Bobo-Dioulasso, Burkina Faso, West Africa. Two types of waste samples were used: eight-year-old shells (OS) and fresh shells (FS) freshly produced. Four (04) types of inoculums were used: wastewater (WW), sludge from bioreactor (SBR), bovine dung (BD), mixed (MIX) consisting of three (03) inoculums combination. Slaughterhouse effluents (wastewater and bovine dung) were sampled in anaerobic basin of Ouagadougou refrigerated slaughterhouse ($12^\circ 24'59'' \text{N}$; $1^\circ 28'29'' \text{W}$). Sludge from biodigester was sampled from biogas production unit of fecal sludge treatment center (CTBV) of Zagtouli in Ouagadougou. Sludges were sampled in 20 L flasks containing nitrogen gas (N_2) to maintain anaerobic conditions.

2.2. Mechanical pretreatment of cashew nut shells

Old hulls samples were crushed to obtain particles size $\leq 1 \text{ mm}$ in diameter while new hulls were grounded to obtain cake (Figure 1).



Fig. 1- Physical aspect of samples after mechanical treatment of cashew nut shells: (a) Grounded fresh shells; (b) old shells particles

2.3. Physicochemical characteristics of cashew nut shells

pH was determined using pH meter (WTW pH340) previously calibrated with buffer solutions at 25 °C. Five (5) gram of old shells particles and grounded fresh shells were homogenized in 45 mL of distilled water after measurement (Noutb *et al.*, 1989). Total solids content (TS) was determined by drying 5 g sample in an oven at 105 °C until constant weight. Volatile solids (VS) and ash content were obtained by weight difference between dried waste and burnt waste at 550 °C for 4 hours (AFNOR, 1985).

2.4. Physicochemical characteristics of inoculums

pH was determined as described above. Total solid (TS), ash (As), volatile solid (VS) in sample were determined according to Sakaki (2014) methods. Titration assay method was used to determine volatile fatty acid (VFA) content. A volume of 25 mL of reactor supernatant was collected in a beaker and stirred. Initial pH was read. Using a 1/10 mL burette, a volume of sulfuric acid (0.1 mol. L⁻¹ H₂SO₄) was poured until pH = 4. The volume poured was noted (V1) then the liquid was boiled 3 min. After cooling, Na₂CO₃ (0.05 mol. L⁻¹) was poured to pH = 7, and volume V2 poured in noted. Equation 1 shows formula using for determine VFA value.

$$\text{VFA} = 6.10^4 \times [(\text{C2V2} / \text{Ts})] \text{ (mg (acetic acid). L}^{-1}\text{)} \quad (1)$$

V1: volume of sulfuric acid

V2: volume of sodium bicarbonate

Ts: Test sample (25 mL)

Viable total anaerobic and methanogenic bacteria were enumerated by the three-tube most probable number (MPN) technique during anaerobic digestion using modified medium of by Angelidaki *et al.* (2009). Cellulose 1g/L, glucose 1g/L, casein 1g/L, propionic acid 0.5g/L, n-butyric 0.5g/L, methanol 10 mM, sodium acetate 20 mM and sodium formate 20 mM were added as

substrate. Tubes showing methane production at the GC were considered positive for methanogenic archaea. Tubes showing turbidity are considered positive for total anaerobes.

2.5. Thermal and biological pre-treatment of cashew nut shells

Thermal and thermobiological pretreatment was realized using method described by Fadil *et al.* (2003) and Aissam (2003) for biodegradation of effluents from olive oil production was adapted for the pretreatment of cashew shells. The fungus strains *Aspergillus niger* isolated from old shells matrices on sabouraud medium with chloramphenicol was used as inoculums for biological pre-treatment. Liquid culture medium (100 mL) with 2% (v/w) of shells placed in 250 mL Erlenmeyer flasks was used for improving aerobic fermentation. Medium was composed of yeast extract (0.1% w/v), (NH₄)₂SO₄ (0.5% w/v), KH₂PO₄ (0.4% w/v), MgSO₄ (0.05% w/v), NaCl (0.05% w/v). 25%, and pH was adjusted to 5. Erlenmeyer flasks were sterilized by autoclaving at 121 °C for 15 minutes. After fungi inoculation, cultures were incubated during 14 days at 30 °C with shaking at 150 rpm. Controls were uninoculated shells. The experiments were carried out in triplicate.

2.6. Assessment of biogas and gaseous metabolites production from cashew nut shells anaerobic digestion

Anaerobic digestion was realized on pretreated shells according to batch method described by Angelidaki *et al.* (2009). Estimation of biogas production was carried out using liquid displacement method. CH₄ and CO₂ were determined using a gas chromatograph (Girdel series 30 with catharometer equipped with thermal conductivity detector [TCD] and linked to the potentiometric recorder SERVOTRACE of type Sefram Paris 1mV). The chromatographical conditions for CH₄ and CO₂ measurement were as follows: injector temperature 90 °C, column temperature 60 °C, detector temperature 100 °C,

filament current 150 mA, N₂ carrier gas pressure 1 bar, attenuation 32, paper speed 10 mm/min. Volume of gas phase 1 mL was injected into chromatograph using a sealed syringe graduated. CH₄ and CO₂ contents were determined using a standard curve established from CH₄ and CO₂ standards (Sawadogo et al., 2012). The yield of substrate-specific methane (YSM) was calculated from the Equation 2.

$$\text{YSM} = \frac{\text{Quantity of biomethane product (L)}}{\text{Quantity of volatile solide (Kg)}} \quad (2)$$

2.7. Data processing

Statistical analysis of data was realized using XLSAT 7.5.2 software. Analysis of variance (ANOVA) was performed to compare mean values of the different variables using Fisher's tests at probability $p = 5\%$. Principal Component Analysis was carried out for distribution of biogas production variables from new and old hulls, CO₂ and CH₄ proportions depending on source of inoculums and pretreatment.

III. RESULTS AND DISCUSSION

3.1. Characteristics of feedstock and inoculums

Physicochemical of cashew nut shells feedstock and inoculums characteristics are pictured in Table 1. pH of fresh and old shells samples was respectively around 4.20 and 6.41. The acidic pH of dry hulls could be explained by high levels of acidic compounds such as anacardic acid (70 to 90%) and other phenolic compounds including 10 to 18% cardol and 5% cardanol (Das et al., 2004; Patel et al., 2006). Over time these compounds are degraded under the action of abiotic and biotic factors. Abiotic factors such as temperature, wind, rains as well as biotic factors (insects, microorganisms) can have an impact on

the integrity of the shells. This explains the degraded condition of the old shells. Joutey *et al.* (2013) reported efficiency of microbial degradation depends on many factors, including chemical nature and the concentration of pollutants, their availability to microorganisms, and the physicochemical characteristics of the environment. High ash content of old shells could be explained by dilapidated condition of old hulls associated with presence of dust and dead insects. Volatile Solids content in fresh shells (88.74% VS) was significantly higher ($P = 0.0001$) than old shells (84.61% VS). High volatile solid indicated a preferred substrate for anaerobic digestion microorganisms according to Milaiti *et al.* (2003) and Nikiema *et al.* (2015). Significant difference as to parameters pH, VFA and methanogenic archaea concentration ($p < 0.0001$) was observed. The SBR, MIX, WW inoculums exhibited pH close to neutral (pH 7) with VFAs values of 1592, 1484 and 1756 mg (acetic acid). L⁻¹, respectively. Bovine dung (BD) inoculum had a slightly acidic pH (6.31) with high VFA concentrations of 2840 mg (acetic acid). L⁻¹, respectively. According to Vedrenne (2007) concentrations from 2000 to 3000 mg (acetic acid). L⁻¹ of total VFA could inhibit anaerobic digestion process. Total anaerobic bacteria were higher than 10⁷ CFU/mL for all inoculum. According to Wang *et al.* (2017) microbial community plays a role in process performance and stability. Mixed inoculums and wastewater exhibited high concentration of methanogenic archaea (2.310² CFU/mL) compared to SBR and BD inoculums. Low concentration of methanogenic archaea in bovine dung (2.3 10¹ CFU/mL) could be explained by unfavorable physicochemical conditions, namely low pH (6.3) and high VFA (2840 mg/L). It should be noted that number of bacteria still does not confirm effectiveness of inoculums.

Table 1. Characteristics of cashew nut shells feedstock and inoculums

Parameters	Feedstock			Inoculums				
	Old Shells	Fresh Shells	<i>p</i> value	SBR	MIX	WW	BD	<i>p</i> value
pH	6.41 ^a	4.29 ^b	0.0001	7.67 ^a	7.63 ^a	7.11 ^b	6.31 ^c	< 0.0001
TS g/L	90.66 ^a	90.916 ^a	0.067	13.44 ^b	13.90 ^{ab}	20.49 ^a	12.37 ^b	0.027
VS g/L	84.61 ^b	88.74 ^a	0.0001	90.76 ^b	90.60 ^b	92.90 ^{ab}	94.83 ^a	0.027
Ash g/L	6.07 ^a	2.18 ^b	> 0.0001	9.24 ^a	9.40 ^a	7.09 ^a	5.17 ^b	0.027
VFA (mg (acetic acid)/L)	--	--	--	1592 ^b	1484 ^b	1756 ^b	2840 ^a	< 0.0001
MA (CFU/mL)	--	--	--	2.30 10 ^{2b}	4.30 10 ^{2a}	4.30 10 ^{2a}	2.30 10 ^{1c}	< 0.0001
TA	--	--	--	> 10 ⁷	> 10 ⁷	> 10 ⁷	> 10 ⁷	---

(CFU/mL)

SBR: Sludge from Bioreactor; WW: Wastewater; BD: Bovine dung; MIX: Mixed of the three inoculums; TS: Total solid; VS: Volatile solid; VFA: Volatile Fatty Acid; MA: Methanogenic archaea; TA: Total Anaerobes

3.2. pH variation during the anaerobic digestion of cashew nut shells

Figure 2 shows pH variation during the anaerobic digestion process of shells with regard to inoculums source (SBR, WW, BD and MIX). From first days of anaerobic digestion, pH variation trended towards acidity

(pH 6.5). Stability was obtained after 10th day for all inoculums. This stability depends on inoculums source, because with wastewater inoculum cultures continued to acidify after 20 days. This shows a strong activity of acidogenic bacteria of wastewater on old shells.

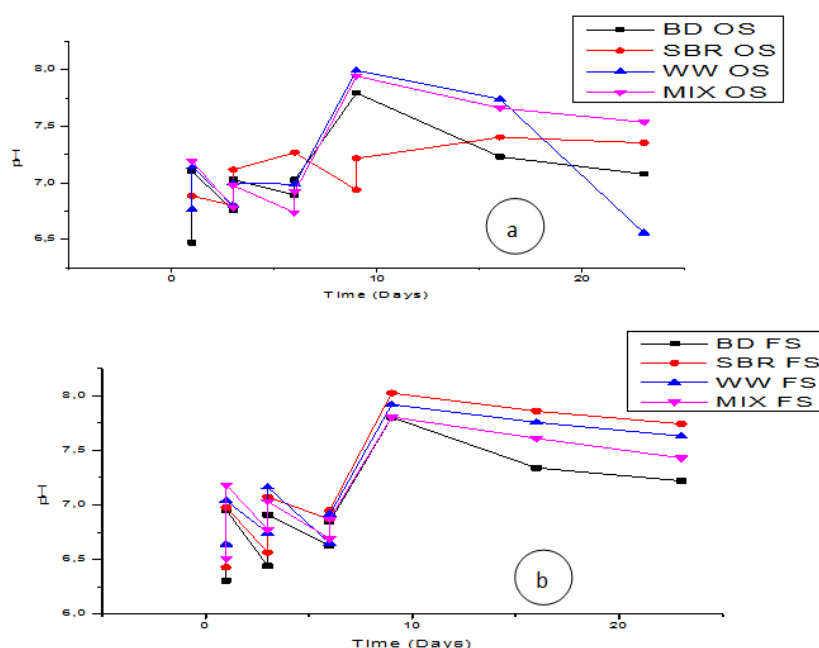


Fig. 2- pH evolution over the anaerobic digestion of cashew nut shells: (a) old shells, (b) fresh shells

3.3. Biogas production with regard to inoculums source

Biogas and biomethane production ranged according to inoculums source and type of shells as presented in Table 2. A significant difference of biogas and biomethane values was denoted between different inoculums ($p < 0.05$). Depending on type of shells, mixed inoculums and wastewater have best yields. SBR, BD and MIX inoculums gave best biogas yields for the old shells 64.18, 59.12 and 55.52 biogas L. (Kg VS)⁻¹, respectively, comparatively to values on fresh shells. The mixed inoculums showed a significantly high biomethane proportions with a value of 70.38%. There was no significant difference between biogas production with wastewater and mixed from fresh shells with 43.87 L biogas. (Kg VS)⁻¹ and 27.78 L biogas. (Kg VS)⁻¹, respectively. The high proportions of biomethane on fresh shells digestion were at the order of 10.30% and 9.81%

CH₄ with wastewater and mixed inoculums, respectively. Similar results was obtained by our previous study (Nikiema *et al.*, 2020) with yields of 46.840 CH₄ L. (Kg VS)⁻¹ and 1.982 CH₄ L. (Kg VS)⁻¹ upon old and fresh shells, respectively. Indeed, wastewater contains a microbial consortium very active in the degradation of complex substrates. The prediction in figure 3 shows that mixed inoculums is suitable in the production of biomethane from cashew nut shells compared to other types of inoculums. Nikiema *et al.* (2017) was showed possibility to realize a activated sludge for optimization of biomethane production by mixing several types of inoculums like wastewater and bovine dung. The low yields with old and fresh shells could be explained by low proportion of inoculum used which was 10%. Codigestion systems could increase biomethane production yields. Pouan (2011) found a production of 50 L biogas. (Kg ST)⁻¹ for *Jatropha curcus* cake, and showed that this

production could reach 206 L biogas. (Kg ST)⁻¹ in codigestion with cattle manure. Singh et al. (2008) obtained a biomethane yield of 333 L. (Kg TS)⁻¹ from seed hulls of *Jatropha curcus* in co-digestion with bovine

excreta. In addition to co-digestion, an appropriate pre-treatment could boost the anaerobic digestion process thus the production of biomethane (Forgacs *et al.*, 2012, Wikandari *et al.*, 2014, Mahato *et al.*, 2021).

Table 2. Biomethane production from old and new shells according to the type of inoculums

Cultures	Mean values	
	Biogas L CH ₄ . (Kg SV) ⁻¹	CH ₄ (%)
SBR OS	64.18 ^a	20.37 ^b
BD OS	59.12 ^{ab}	17.23 ^b
MIX OS	55.52 ^{ab}	70.38 ^a
WWFS	43.87 ^{abc}	10.30 ^b
WW OS	32.42 ^{abc}	29.19 ^b
MIXFS	27.78 ^{abc}	9.81 ^b
BDFS	18.67 ^{bc}	2.94 ^b
SBRFS	4.86 ^c	1.03 ^b

In a column the values which have a letter in common do not present a significant difference according to the Fisher LSD test at the probability threshold $p = 0.05$. OS: old shells; FS: fresh shells; WW: Wastewater; SBR: Sludge from Bioreactor; BD: Bovine dung; MIX: Mixed of inoculums

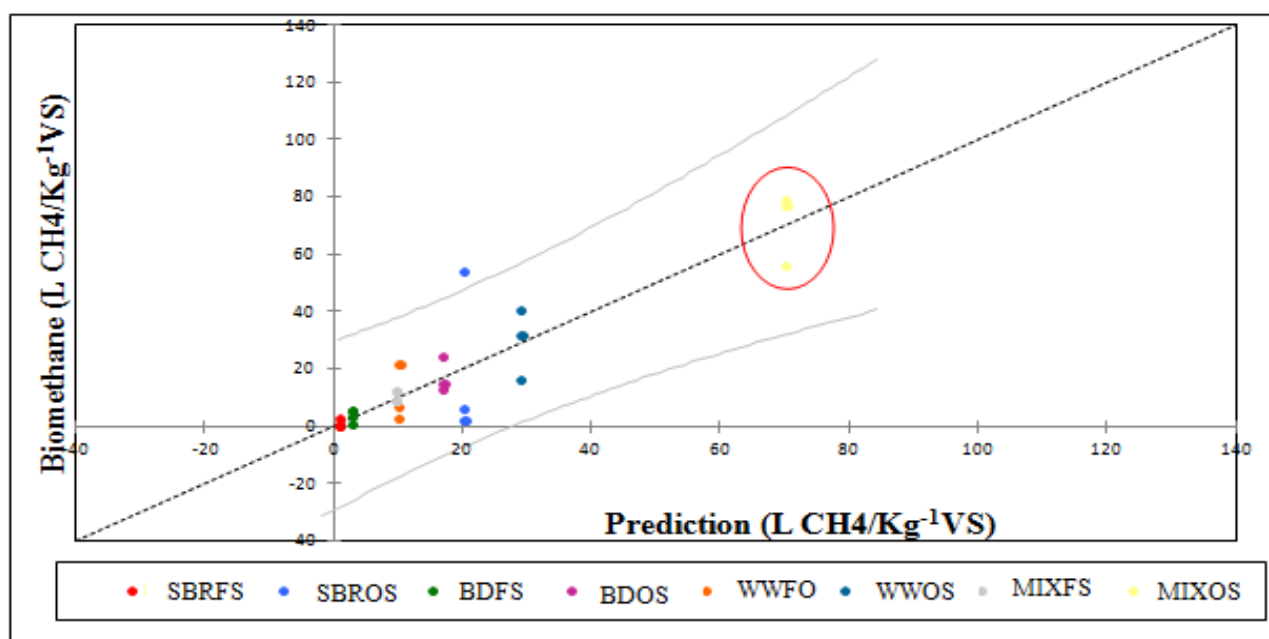


Fig. 3-Prediction of the biochemical methane potential of inoculums according to shell types

The typologies of the variables (Biogas, CH₄ and CO₂) and cultures according to the inoculums and the type of cashew shells on the factorial plans constituted by axes 1 and 2 are presented in Figure 4a and 4b. In this figure, only the variables close to the correlation circle need to be taken into account. In Figure 4a, there are clearly three groups of variables close to the circle (Biogas, CH₄ and CO₂), so that the projections on the axes F1 and F2 are

93.47%). Indeed, biogas is made up of CH₄ and CO₂. The representation of cultures as a function of inoculum and type of shell on the two factorial planes described by the axes F1x F2 (Figure 10a) allows them to be compared according to the production of biogas, CH₄ and CO₂. BD_{VC} crops have a significant production of biogas. MIXOS and WWOS cultures have high yields of CH₄. SBR OS crops produce a high CO₂ production. The last

batch formed by the cultures from the new hulls shows

low production of biogas.

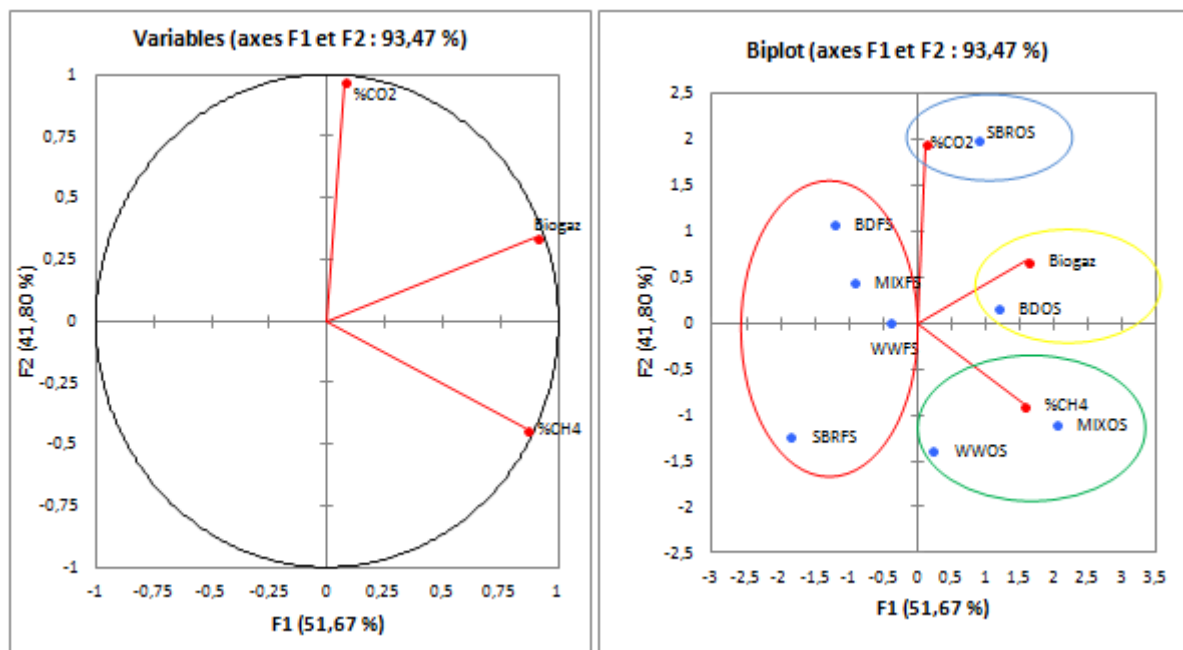


Fig. 4-Principal component analysis (a) plot of biogas production variables from old and fresh shells (OS and FS), CO₂ and CH₄ proportions (b) distribution of treatment types

3.4. Influence of pretreatment on anaerobic digestion of cashew nut shells

The results of pretreatment influence on shells anaerobic digestion are presented in Table 3. Thermo-biological treatment of cultures of old shells shows a significantly high production ($p < 0.0001$) of biogas (40.04 L. biogas (Kg VS)⁻¹) compared to control T-TTBOS (24.82 L. biogas (Kg VS)⁻¹) having only undergone heat treatment. This indicates that biological treatment improves yield after old hulls heat treatment. Same observation is made with new hulls. Yield was 16.55 L. biogas (Kg VS)⁻¹, a significant improvement ($p < 0.0001$) is remarkable at the end of biological treatment (27.63 16.55 L. biogas (Kg VS)⁻¹). The biomethane yield also underwent a significant improvement ($p = 0.001$) from 6.30 to 11.20 L. CH₄ (Kg VS)⁻¹. The opposite is remarkable for the new hulls with a significant decrease in biomethane productivity from 4.05 to 0.02 L. CH₄ (Kg VS)⁻¹. The biomethane yield values compared to work without thermobiological pre-treatment showed a decrease. Anaerobic digestion activities depending on inoculums source yielded 55.52 L. biogas (Kg VS)⁻¹ with 70.38% CH₄. Previous work had made it possible to find higher biogas yields than 70.38 L biogas. (Kg VS)⁻¹ with an also higher biomethane yield of 46.840 L CH₄. (Kg VS)⁻¹ with conditions similar to old untreated (Nikiema *et al.*, 2020). Nikiema *et al.* (2020) had found yields of 1.982 L CH₄. (Kg VS)⁻¹ with the new untreated hulls. That indicates an improvement biomethane yield

with thermal pretreatment only (4.05 L CH₄. (Kg VS)⁻¹), the biological treatment decreases value to 0.02 L CH₄. (Kg VS)⁻¹. Radziejewska-Kubzdela *et al.* (2020), enzymatic and thermal treatment of must made it possible to obtain highest content of phenolic compounds in *Berberis amurensis* Rupr juice, a plant whose total content of phenolic compounds is much higher high ranging from 261 to 1074 mg / 100 g (Hassanpour and Alizadeh, 2016). Thermal treatment weakens cell structuring and facilitates enzymatic activity through biological treatment. The differences in biogas production with untreated shells could be explained by the fact that treatment forms improve productivity of CO₂ contained in biogas. The low of biomethane yields obtained with old and new hulls as substrate could be explained by their physicochemical composition. In fact, cashew nut shells contain cashew nut shell liquid (CNSL) which is a liquid composed of 70% - 90% anacardic acid, 10% - 18% cardol and about 5% cardanol including new hulls contain abundantly. According to authors such as Liu *et al.* (2018), Zhang *et al.*, (2019) and Liu *et al.* (2020), heat treatment degrades the cell structure and facilitates the extraction of soluble solids, polysaccharides, phenolic compounds from fruit and vegetables. These soluble sugars and other bioaccessible compounds will likely be used for microorganisms during the associated biological treatment. Heat treatment also results in the release of substances toxic to the microbial groups in anaerobic digestion. Bisaria and Ghose (1978) and Boulanger

(2011) showed thermo-chemical and physico-chemical treatments of organic matter could produce compounds such as hydroxyfulfurals and fulfural which can have a toxic effect for microorganisms. Liu *et al.* (2020) reported increase total phenols in finished product (wine) to 2.97–105.41% following heat treatment of *Crataegus* spp. fruits (Atanackovic *et al.*, 2012). Lodish *et al.* (2000) showed with increasing temperature, thermal energy increases, thus facilitating cleavage of covalent bonds, resulting in the release of bound polyphenols. During the heat treatment, the ester and glucoside bonds can be broken, resulting in the release of the bound polyphenols. During the heat treatment, the ester and glucoside bonds can be broken, resulting in the release of the bound polyphenols. High temperatures can also lead to the formation of Maillard reaction products, altering the color of the material and contributing to the production of compounds such as hydroxyfulfurals and fulfural which can have a toxic effect for microorganisms (Bisaria and Ghose, 1978; Lavelli *et al.*, 2009). Papoutsis *et al.* (2018) showed higher increase in total polyphenol contents (171.0 μM) compared to unheated control (71.8 μM) with treatment at 150 °C for 40 minutes of citrus peels extracts. At the same time, heat can lead to the transformation of polyphenols explaining why the content of some phenolic compounds increases while the content of others decreases (Buchner *et al.*, 2006). Radziejewska-Kubzdela *et al.* (2020) showed with enzymatic and thermal treatment it possible to obtain highest content of phenolic compounds in *Berberis amurensis* Rupr juice, a plant with a much higher total content of phenolic compounds ranging from 261 to 1074 mg / 100 g (Hassanpour and Alizadeh, 2016). Decrease in our yields compared to previous work could be explained by inappropriate

pretreatment which would contribute to production substances toxic to anaerobic bacteria. These pretreatment conditions cause an increase in the content of phenolic compounds and probable release of hydroxyfulfural and fulfural compounds. Saenab *et al.* (2017) worked on effects of anacardic acid isolated from cashew nut shells on production of methane and other products in rumen fermentation, found that the inhibition of methane production by 1 Anacardic acid was lower than that of biogas (crude extract of shells). However, as part of the bioactive compounds present inside the biogas, the contribution of anacardic acid to the total reduction of methane amounted to 77.36% while the contribution of other compounds amounted to 22, 64%. The other compounds besides anacardic acid are cardol, cardanol, dimethyl cardol according to Gandhi *et al.* (2013) and Njuku *et al.* (2014). Other forms of pretreatment could reduce the levels of these toxic compounds and thus promote the production of biomethane. Whether old or fresh shells, pretreatment is an essential step in increasing accessibility and biodegradation of macromolecules by anaerobic microorganisms. Indeed, Ghaderi-Ghahfarokhi *et al.* (2017) investigated the effects of different treatment processes like boiling, autoclaving, roasting and soaking in solutions (water, acetic acid, NaOH and NaCl) on the removal of polyphenolic compounds from varieties of acorns, namely *Quercus brantii* var. persica and *Quercus castaneifolia* var. castaneifolia. According to these authors all the processes applied, with the exception of roasting, resulted in a significant decrease ($p < 0.05$) in the polyphenol content. Boiling reduced the polyphenol concentrations by approximately 52%. A considerable reduction in anti-microbial substances could lead to a significant improvement in biomethane production.

Table 3. Biogas, CH₄ and CO₂ production from pretreated shells

Culture	Mean values (L. Kg VS ⁻¹)		
	Biogas	CH ₄	CO ₂
TTBOS	40.04 ^a	11.20 ^a	7.15 ^a
TTBFS	27.63 ^b	0.02 ^c	2.07 ^b
T-TTBFS	16.55 ^c	4.05 ^b	5.75 ^a
T-TTBOS	24.82 ^b	6.30 ^b	1.85 ^b
<i>p</i> value	< 0.0001	0.001	0.002

In a column, the values which have a letter in common do not present a significant difference according to the Fischer test (LSD) at the 5% threshold, TTBOS: Thermal and biological treatment of old shells, TBOS: Biological treatment of old hulls, T-TTBOS: Control-TTBOS, T-TTBFS: Control-TTBFS.

IV. CONCLUSION

The source of inoculums has a significant effect on biogas production from cashew shells. Mixed inoculums

exhibited significantly high biomethane productions with old and new cashew shells. Thermobiological pretreatment of old and new shells performed was not

effective in biogas production. It would be advisable either to experiment other forms of pretreatment and to find optimal time of biological pretreatment in order to increase yields or to examine agronomic possibility.

ACKNOWLEDGMENTS

All authors are grateful to the International Foundation for Science (IFS_ I-3-E-6204-1) through individual research grant to support this research.

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