ANAEROBIC DIGESTION OF LIGNOCELLULOSIC BIOMASS PRE-TREATED BY SUB AND SUPERCRITICAL CONDITIONS

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Abstract

Large-scale anaerobic digestion applications are limited in the food industry due to difficulties related to operational instability, generally produced by different types of macro-pollutants. In this scenario, the use of co-substrates or additives have been introduced as cost effective methods that allow the effective stabilization of organic matter, with a high methane yield gain. Vegetable crop residues, despite their limited biodegradability, are potential materials to integrate anaerobic processes due to their low cost, high availability, sugar content, porosity and adsorption capacity. Thus, regarding the physical, chemical and biodegradability properties of sugarcane bagasse, different supercritical and near critical CO_2 pre-treatments were evaluated with and without the addition of NaOH: (i) 40˚C/70 $Kgf.cm^{-2}$ (ii) 60˚C/200 $Kgf.cm^{-2}$ and (iii) 80˚C/200 $Kgf.cm^{-2}$. The methanogenic production by anaerobic digestion of sugarcane bagasse increased in all cases in which the material was pre-treated, with the exception of the case in which NaOH was used together with a high temperature. The second condition of CO_2 at 60˚C/200 $Kgf.cm^{-2}$ stood out with a lignin removal of 8.07% and an accumulated methane production of $0.6498 \pm 0.014\ {}_{N}L$, 23.4% higher than the obtained with the untreated material.

Keywords: Sugarcane bagasse, renewable energy, methane production, supercritical and near critical CO_2, vegetable crop residues

1. Introduction

Global energy demand is increasing each year, and therefore, the need to develop alternative energy production systems is critical. Residues and effluents from the food industry can be the key to a sustainable energy system based on renewable sources and decentralization. By means of anaerobic digestion (AD), residues can be stabilized with the production of biogas, a mixture of carbon dioxide, methane, water vapour, traces of sulphuric gas and ammonia, in proportions of 50-75%, 25-50%, 1-5%, 0-5.000 ppm and 0-500 ppm, respectively [1].

Nevertheless, anaerobic digestion applications in the food industry are complicated due to difficulties related to operational instability produced by different types of inhibitors such as long chain fatty acids (LCFA), volatile fatty acids (VFA), ammonia, and metal elements [2, 3]. To improve these applications, the use of co-substrates and additives, has gain interest. Co-substrates can promote nutritional balance, help achieve a desired moisture content and improve methane yield by increasing the organic load [3–6]. While additives, stimulate microbial growth and reduce the concentration of inhibitory agents [7].

Vegetable crop residues, such as sugar cane bagasse, are by-products with great potential for the production of biogas because of their low cost, high availability and high sugar content. However, their physicochemical characteristics brings many limitations to microbial hydrolysis [8]. This residues consist mainly of cellulose, hemicellulose and lignin, as well as starch, ashes, proteins, oils, and other minor compounds, all bounded together by covalent bonds, intermolecular bridges, and Van-der-Waals forces [9]. In this matrix, lignin makes up as the most recalcitrant component of the plant cell wall, providing stability and making it resistant against water, microbial attack and oxidative stress [10]. Yet, different researchers have reported positive impacts of lignocellulosic materials in AD processes. Angelidaki and

Ahring [4] used wastes with high content of lignocellulosic fibers in co-digestion with an effluent from an oil industry, which helped control the pH of the system. Lehtomäki et al. [11] observed a positive effect of grass silage, sugar beet tops and oat straw, in the nutrient balance and C/N ratio of the anaerobic digestion processes of bovine manure. Nielsen and Ahring [12], demonstrated that digested straw addition can minimize the adsorption of LCFA into the microorganisms of the anaerobic consortium, mitigating its inhibitory effect. Palatsi et al. [13], was able to recover a manure thermophilic digestion process inhibited by LCFA employing digested fibres as adsorbents, this, in a shorter time than with other methods such as dilution of the inhibitor load.

Different pre-treatment methods have been developed in the aim to reduce the recalcitrance of lignocellulosic materials, with main focus on biofuel production, still lacking evaluation in AD processes, although this process has being recognized as a cost-effective bioconversion technology [14]. For the AD of residues, an ideal pre-treatment method would not only increase the biodegradability of the material, it will also improve its adsorptive capacity and expose cellulose to favour microbial growth. Supercritical fluids offer a mass transfer higher than conventional organic solvents, as they present the density of liquids and the diffusivity and viscosity of gases [15]. Therefore, they have the capacity to penetrate different matrices more easily, disrupt cellular structures and extract different compounds more efficiently [16].

Sugarcane bagasse (SCB) is an especially attractive biomass due to its worldwide availability. According to the Food and Agriculture Organization of the United Nations (FAO), in 2014 the production of sugarcane crop reached 1.884 billion tons [17], that could generate around 607,9 billion tons of bagasse [18]. This work evaluates the effect of sub and supercritical CO_2 pre-treatment in SCB biodegradability and physicochemical characteristics, visualizing future uses of this material as an additive on the AD of effluents with elevated concentrations of complex substrates.

2. Materials and Methods

2.1. Raw material

Sugarcane bagasse was obtained from a sugar and alcohol processing company located in the state of São Paulo (Brazil) and characterized as shown in Table 2.

2.2. Sub/supercritical pre-treatment of sugarcane bagasse

Sugarcane bagasse was pre-treated with CO_2 following the conditions established in Table 1. This parameters were selected based on results obtained by Souza Melo et al. [19]. Thus, one near critical and two supercritical conditions were evaluated, both with pure CO_2 (T2, T3, T4) and with CO_2 enriched with NaOH as a polarity modifier (T5, T6 and T7). Pre-treatments were executed using a supercritical extractor Thar-SFC (Pittsburgh, USA) programed for 5 hours of static flow and 1 hour in dynamic flow ($5 g.min^{-1}$). In the cases including the addition of the polarity modifier, the SCB was submerged in a NaOH solution of 2 g/L, before its introduction to the equipment's reaction chamber.

After treatment, the material was cooled and then stored in plastic bags at room temperature. The SCB derived from the treatments performed with NaOH addition, was washed to lower its pH and dried at room temperature.

2.3. Analytical Methods

Chemical composition of the pre-treated and raw materials were characterized by the Van Soest method [20], while solids and organic matter (as chemical oxygen demand - COD) were determined in accordance with the Standard Methods [21], the latter with modifications according to Yadvvika et al. [22]. Materials' morphology was analysed in a scanning electron microscopy (SEM) with a 15kV beam (Hitachi-TM 3000, Tokyo, Japan), the contact angle (ϴ) was determined a tensiometer DCAT11 (Dataphysics, Filderstadt, Germany) which uses the Wilhelmy board technique. Real density (ρ_r) was measured in a pycnometer equipped with helium gas (Quantachrome-UltraPycnometer 1000, Florida, USA). Apparent density (ρ_{ap}) was determined by gravimetric method and the porosity (ε) was calculated using the real and apparent density values according to equation 1.

$$\varepsilon = \frac{\rho_r - \rho_{ap}}{\rho_r} \qquad (1)$$

Table 1. Sugarcane bagasse pre-treatment conditions*

	Pre-treatment	T (˚C)	Pressure ($Kgf.cm^{-2}$)	Polarity Modifier
T1	SCB raw	-	-	-
T2	Sub-CO_2	40	70	-
T3	Sup-CO_2-I	60	200	-
T4	Sup-CO_2-II	80	200	-
T5	Sub-CO_2+NaOH	40	70	NaOH
T6	Sup-CO_2-I+NaOH	60	200	NaOH
T7	Sup-CO_2-II+NaOH	80	200	NaOH

*All pre-treatments were executed during 5h + 1h of dynamic flux.

2.4. Biomethane potential (BMP) tests

Methane production was assessed by triplicate batches following the recommendations of Angelidaki et al. [23]. Thus, 120 mL reactor vials were fed with 1.0 g of SCB, 25 mL of inoculum, 20 mL of buffer and 0.2mL of macroelement solution and 0.1 mL of oligoelement solution, both prepared according to Zehnder et al.[24]. Vials with no bagasse were employed as control.

Nitrogen gas was employed to guarantee the anaerobiosis and vials were incubated at 37°C and 100 rpm in an incubator table. The inoculum was degassed until negligible production of biogas. The BMP trials were ended when the biogas production rate became similar to that in the control vials. At that moment, the remaining mixture in each bottle was characterized by COD and Suspended Solids (SS) analysis.

The inoculum source was from a UASB reactor treating effluents from a poultry slaughterhouse, later adapted in an anaerobic reactor fed with a milk and cellulose based substrate with 3g/L of total COD. This reactor was operated in sequential batches of 48-hour cycle times, during a period of 60 days. After the adaptation, the inoculum presented a semi-granular appearance with 70.23± 4.6g/L of total solids (TS), 13.01± 0.30 g/L of fixed solids (TFS) and 57.22 ± 4.76g/L of volatile solids (STV) and a specific methanogenic activity (SMA) of 1.129 g .DQO / g.SSV.d. Microscopic analysis of the inoculum allowed to infer, based on the morphology of the microorganisms observed, the presence of *Methanosarcinas* sp, *Methanosaetas* sp. and sulphur reducing bacteria.

2.5. Volume and composition of the produced biogas

During the BMP tests, biogas production was measured by pressure transduction in a Datalogger GN200. The obtained pressures (y) were converted into volume of biogas (V_B) considering the headspace (HS) of the vials using Equation 2, obtained from the calibration curve of the equipment.

$$y = 13{,}066\frac{V_B}{HS} - 0{,}2549 \qquad (2)$$

The biogas composition was analysed by a gas chromatograph GC-2014 (Shimadzu, Kyoto, Japan), equipped with a micro-packed column with a length of 1,0 m and an internal diameter of 1 mm, using helium as carrier gas at 10 $mL.min^{-1}$. The temperature at the injector port and the detector were set at 100 and 120°C, respectively. While the column temperature was programed at 40°C (3 min hold) and a heating ramp of 60°C.min^{-1} until 150°C (1 min hold). The area obtained by the injection of a standard containing CH_4 and CO_2 (50.032:49.968 cmol:cmol) was used as conversion factor to calculate the mass of methane present in the samples. The injections were made under environmental pressure and temperature (24°C and 1,002 atm).

2.6. Estimation of kinetic parameters

Methane production kinetics were obtained from the BMP assays and adjusted by a modified Gompertz equation (Equation 3), following the recommendations of Chen et al. [25]. Where $P_{CH4}\ (t)$ is the accumulative methane production (mmol/STV) in an expecific time (t), P_{CH4} the specific methane production potential, λ the duration of the lag phase, and e is 2.71828. This way, the methane production potential, the methane production rate and the duration of the latent phase, were estimated using Origin 9.0^{TM}.

$$P_{CH_4}(t) = P_{CH_4} exp\left\{-exp\left[\frac{k.e}{P_{CH_4}}(\lambda - t) + 1\right]\right\} \quad (3)$$

3. Results and discussion

3.1. Physicochemical effects of sub and supercritical CO2 pre-treatments on SCB.

Table 2 presents the physicochemical characteristics of the SCB obtained after each pre-treatment. No bigger difference between the values obtained for the treated samples and the raw material was observed. It was expected that the pre-treatments evaluated would increase accessible surface area as an effect of the pressure employed and the consequent disruption of the hemicellulose structure [26]. Although this was not manifested in the porosity of the material, the compositional analysis of the material, presented in Table 2, shows structural modifications.

Considering that the pre-treatments sought, among other things, to reduce the lignin content of the material; the addition of NaOH as polarity modifier conducted in the cases denominated as T5, T6 and T7, generated a negative effect. These treatments showed an increase in the lignin content possibly due to an exposure facilitated by the polarity of NaOH, as the component was dissolved but was not dragged or eliminated. In addition, there was also a slight decrease in the percentage of cellulose. Still, it was noted that NaOH can significantly expose hemicellulose, this effect seems to be assisted by high temperatures, since, as stated by Agbor et al. [10] it is a highly thermo-sensitive compound. These results contrast with publications that report lignin removal rates from 80.2% [27] up to 89.9% [28] in sugarcane bagasse, through alkaline pre-treatments with NaOH, however this publications employed temperatures higher than those evaluated in this work, i.e. 184ºC and 121ºC, respectively. Thus, it is detected that the effectiveness of NaOH in the removal of lignin depends on the temperature. Still, the temperature alone does not seem to have sufficient effect for the total removal of this compound, in T4 (pure CO_2 at 80ºC and 200 Kgf.cm^{-2}) the percentage of lignin also increased.

In addition to temperature, treatment time also seems to be a factor that influences lignin removal, even more than pressure, as it was observed a greater lignin removal in the conditions T2 and T3, compared to the supercritical pre-treatment evaluated by Gao et al. [29]. This author pre-treated rice straw with CO_2 at 110ºC and 300 Kgf.cm^{-2} for 30 min detecting changes in the morphology, yet no lignin removal. Thus, larger studies are required to determine whether this variable generates a significant effect, or if the difference is due to the particularities of each material.

Structural changes can also be observed in the micrographs of pre-treated SCB, presented in Figures 1 and 2. While in the micrographs obtained from the raw material, a compact and rigid structure was observed, in the pre-treated samples distorted structures are present. Similar results were reported after SCB pre-treatment with formic acid [30], diluted sulfuric acid [31], and an alkaline solution [28].

Pre-treatment with pure CO_2 at 60ºC and 200 Kgf.cm^{-2} (T3) was, in general, the most effective in reference to morphology alteration and lignin removal (8.07%.). By the results obtained, it is possible to confirm the existence of a relation between lignin and cellulose content, were the exposure of later is favoured by the elimination of the former According to Sindhu et al. [28], the rupture of the lignin structure weakens its carbohydrate bonds, increasing the accessible surface area and increasing cellulose exposure. In the obtained images, cellulose, hemicellulose and lignin exposure can be observed in the form of spiral, grids and thin wires or films. According to Yu et al. [32], cellulose acts as a structure of vascular bundles with highly oriented chains.

Table 2. Physicochemical characteristics of raw and pre-treated SCB

	T1	T2	T3	T4	T5	T6	T7
Humidity(%)	4.8	4.3	4.6	3.6	3.9	4.7	5.5
Real density (g/cm3)	1.480 ±0.033	1.146 ±0.022	1.206 ±0.065	1.378 ±0.085	1.063 ±0.066	1.391 ±0.037	1.456 ±0.048
Apparent density (g/cm3)	0.056 ±0.001	0.068 ±0.003	0.061 ±0.003	0.063 ±0.003	0.049 ±0.002	0.061 ±0.001	0.060 ±0.002
Porosity (%)	96.219	94.042	94.962	95.422	95.423	95.647	95.891
Contact angle	114.747 ±15.51	110.823 ±12.89	89.755 ±8.13	92.795 ±7.81	87.219 ±10.40	101.009 ±13.39	88.988 ±7.45
COD (g.DQO/g)	0.857 ±0.834	0.948 ±0.200	0.903 ±0.025	1.025 ±0.004	0.833 ±0.052	0.966 ±0.260	0.935 ±0.051
TS (mg/g)	941.457 ±3.097	938.161 ±6.940	935.311 ±4.652	944.757 ±0.154	866.602 ±95.201	874.586 ±85.640	929.339 ±12.033
TFS (mg/g)	8.402 ±2.198	6.574 ±2.003	6.283 ±1.296	6.844 ±1.339	6.331 ±3.470	6.307 ±1.522	6.787 ±2.798
TVS (mg/g)	933.056 ±0.899	931.587 ±4.936	929.027 ±3.355	937.913 ±1.185	860.270 ±91.731	868.278 ±84.118	922.553 ±9.236

Mean values ± standard deviation

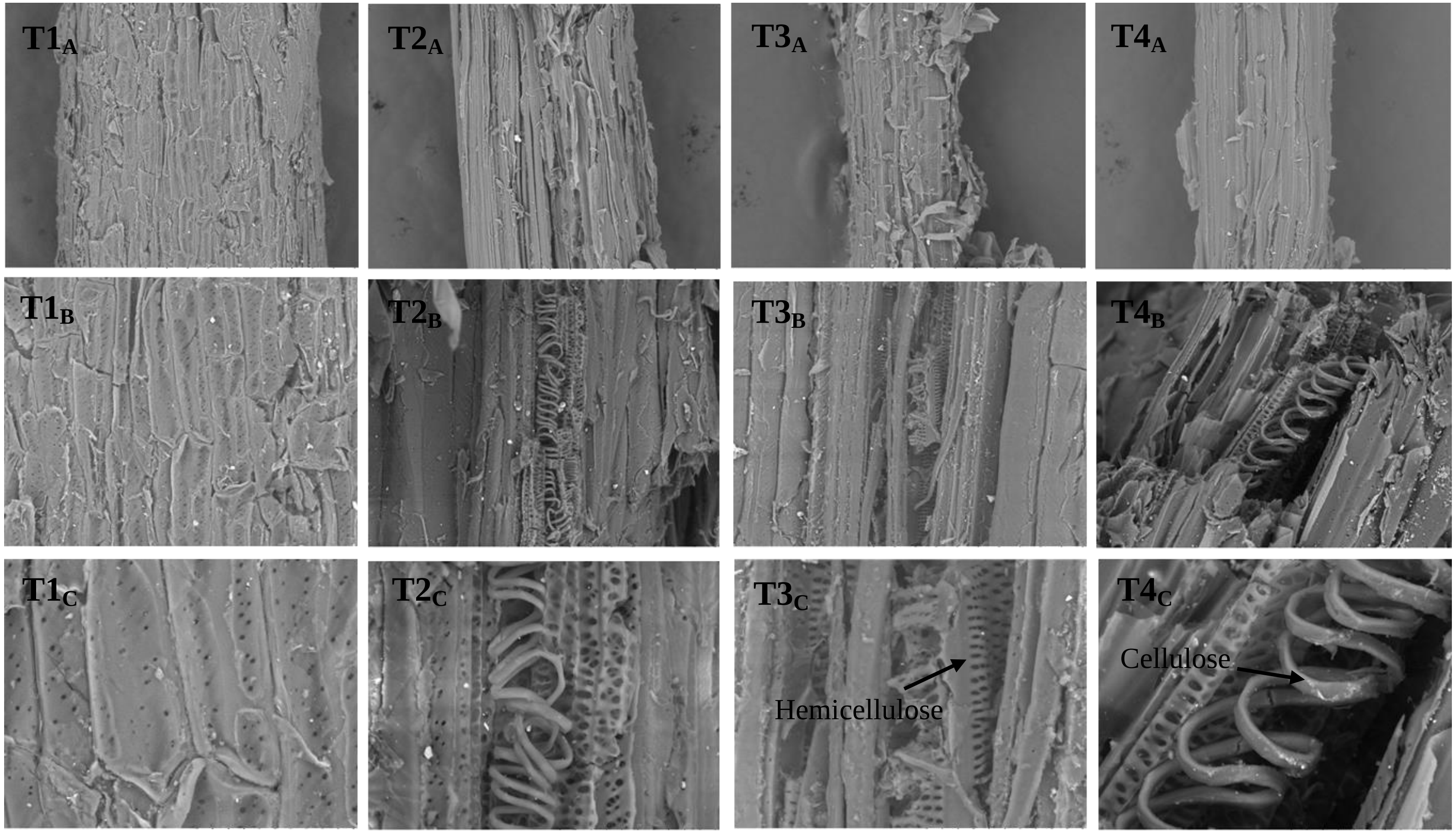
T1: raw SCB; T2: SCB pre-treated with CO_2 at 40°C & 70 Kgf.cm^{-2}; T3: SCB pre-treated with CO_2 at 60°C & 200 Kgf.cm^{-2}; T4: SCB pre-treated with CO_2 at 80°C & 200 Kgf.cm^{-2}; T5: SCB pre-treated with CO_2 + NaOH at 40°C & 70 Kgf.cm^{-2}; T6: SCB pre-treated with CO_2 + NaOH at 60°C & 200 Kgf.cm^{-2}; T7: SCB pre-treated with CO_2 + NaOH at 80°C & 200Kgf.cm^{-2}.

Table 4. Lignocellulosic composition of raw and pre-treated SCB

	T1	T2	T3	T4	T5	T6	T7
Acid Lignin (%)	10.53	10.11	9.68	12.14	15.73	12.54	13.80
Cellulose (%)	49.81	50.26	50.42	49.52	49.14	50.23	49.37
Hemicellulose (%)	25.71	25.62	27.22	29.44	28.09	28.68	29.63

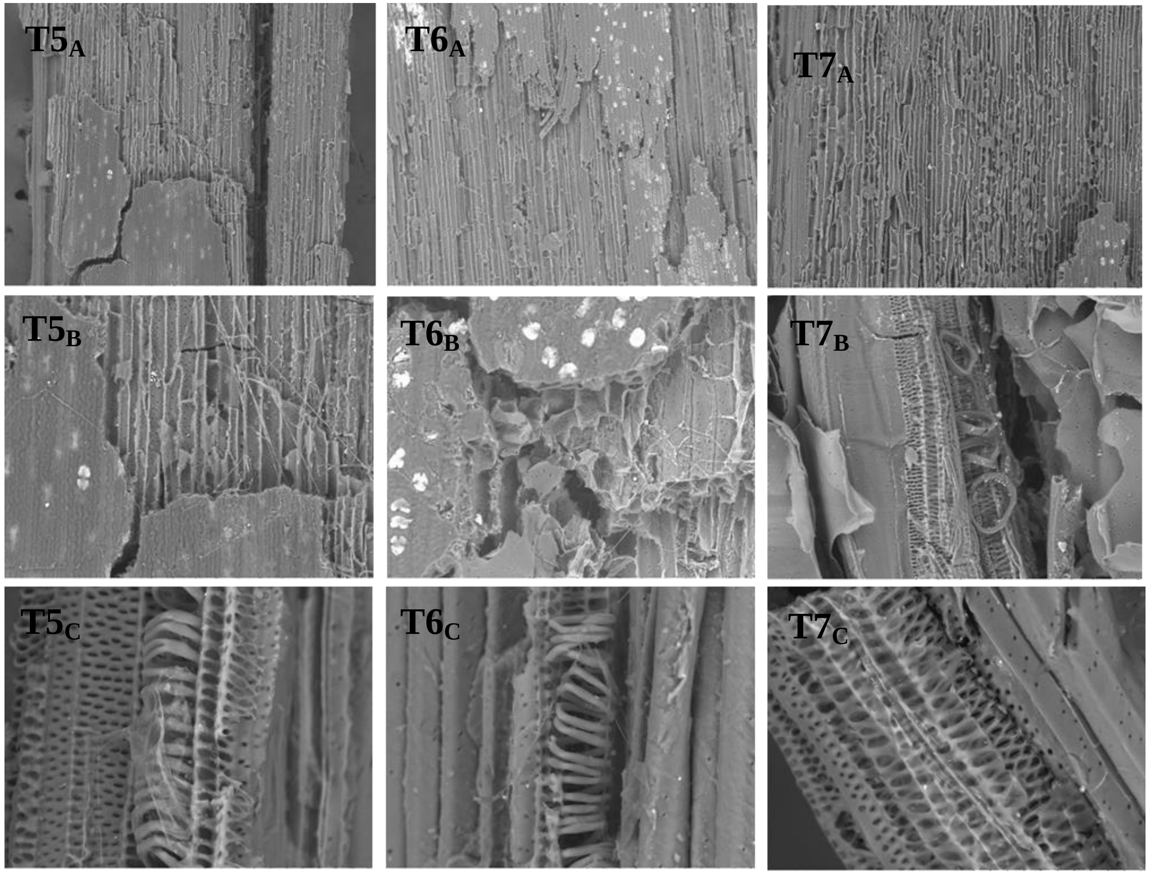
T1: raw SCB; T2: SCB pre-treated with CO_2 at 40°C & 70 Kgf.cm^{-2} ; T3: SCB pre-treated with CO_2 at 60°C & 200 Kgf.cm^{-2}; T4: SCB pre-treated with CO_2 at 80°C & 200 Kgf.cm^{-2}; T5: SCB pre-treated with CO_2 + NaOH at 40°C & 70 Kgf.cm^{-2}; T6: SCB pre-treated with CO_2 + NaOH at 60°C & 200 Kgf.cm^{-2}; T7: SCB pre-treated with CO_2 + NaOH at 80°C & 200 Kgf.cm^{-2}

Figure 1. Scan electron microscopy (SEM) of raw and pre-treated SCB



T1: raw SCB; T2: SCB pre-treated with CO_2 at 40°C & 70 Kgf.cm^{-2}; T3: SCB pre-treated with CO_2 at 60°C & 200 Kgf.cm^{-2}; T4: SCB pre-treated with CO_2 at 80°C & 200 Kgf.cm^{-2}; amplified by x200 (A). x500 (B) e x1.5k (C)

Figure 2. Scan electron microscopy (SEM) of pre-treated SCB



T5: SCB pre-treated with CO_2 + NaOH at 40°C & 70 Kgf.cm^{-2}; T6: SCB pre-treated with CO_2 + NaOH at 60°C & 200 Kgf.cm^{-2}; T7: SCB pre-treated with CO_2 + NaOH at 80°C & 200 Kgf.cm^{-2}; amplified by x200 (A). x500 (B) e x1.5k (C)

3.2. BMP of sugarcane bagasse.

Accumulative methane production obtained from BMP assays are presented in Figure 4, together with the kinetic adjustment. All pre-treatments, except T7, exceeded the methane production of the raw SCB of 0.5267±0.003 $_{N}L.CH_4$, confirming that changes in the morphology and the composition of these materials and the consequent increase in their surface area, does improve the hydrolysis efficiency and favours the methane production. During the pre-treatments, the SCB was heated in contact with CO_2 at high pressure, and then decompressed due to pressure release, producing organic acids. Such acids, together with the carbonic acid, formed from CO_2 dissolution, catalyse the hydrolysis of hemicellulose into mono-sugars [26, 33].

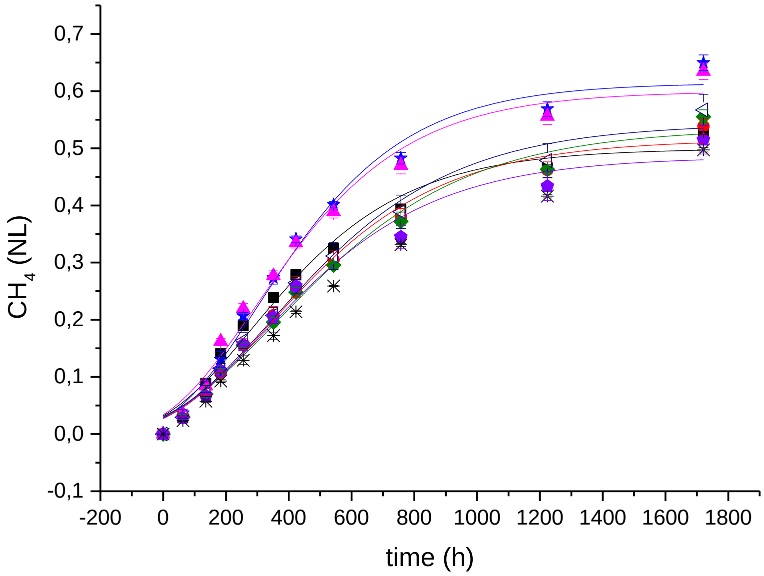
The highest methane production, of 0.6498±0.014 $_{N}L.CH_4$, corresponds to the bagasse pre-treated with pure CO_2 at 60ºC and 200 $Kgf.cm^{-2}$ (T3), surpassing the production of the untreated material by 23.4%. As stated before, this treatment also achieved the higher lignin removal and cellulose exposition. It's been indicated that lignin removal may leave the material vulnerable to microbial attacks [14].

When contrasting the accumulated methane production obtained from each material against its hemicellulosic composition, it was not possible to establish a clear relation. Although, according to Yue et al. [34], in AD processes of lignocellulosic biomass, the hemicellulose is digested before the cellulose and lignin. In the tests with pure CO_2, it was observed that the methane production was actually higher when the hemicellulose content was higher; this can be motivated by the consumption of the sugars constituent of this compound. However, the methane production obtained from the materials pre-treated with addition of NaOH did not follow the same relation; it is worth recalling that these materials presented higher hemicellulose content. This raises the suspicion of the generation of an inhibitory effect that apparently, could have been potentiated by the increase in temperature. In fact, different researches have reported the formation of inhibitory compounds, such as furfural, hydroxymethylfurfural (HMF), and phenolic compounds, from the excessive degradation of hemicellulose and/or cellulose [33]. Specifically, alkaline conditions can lead the formation of many compounds like carboxylic acids from carbohydrate degradation, acetic acid formed by saponification of acetyl groups, as well as saccharinic and formic acid from polysaccharides deterioration [35], which can be inhibitory in high concentrations or can present low degradation rates.

The specific methane production achieved with pre-treatment T3 exceeded the values reached by Rabelo et al. [36] who evaluated lime and alkaline hydrogen peroxide pre-treatments, both combined with an enzymatic hydrolysis step, obtaining yields of 58.7 and 72.1 $L.CH_4$/kg bagasse at STP conditions, respectively. The yield in T3 when expressed in the same units and conditions was 168.99 $L.CH_4$/kg bagasse. Moreover, Badshah et al. [37] studied the methane potential of SCB after a combined pre-treatment of sulphuric acid and enzymatic hydrolysis, managing to increase the methane yield by 16% for a total of 200 mL/g.VS, in front of the 173 mL/g.VS achived with the acid pre-treatment alone (181.9 $mL.CH_4$/g.VS for T3). It is important to highlight that the composition of SCB can vary due to many factors such as harvest time, type of soil and genetic variety, between others; thus, affecting its methane potential.

An analysis of the kinetic parameters obtained (Table 5), allowed to observe higher methane rates (*k*) in conditions T3 and T4 of $8.43x10^{-4}$ and $7.99x10^{-4}$ $_{N}L.h^{-1}$, respectively. Even though the lag (λ) phase in these pre-treatments was slightly higher than that presented in the raw SCB digestion (281.5±20.7 for raw, 303.78±16.9 for T3, and 291.30±20.63 for T4), once considering the standard deviation, the differences are not significant. In the other pre-treatments the lag phase increased, being an indication of difficulties of the inoculum to adapt and to degrade the substrate. This difficulty was overcome in most pre-treatments and high methane yields were reached. The rate of methane production (*k*) was lower in the pre-treatments with addition of NaOH. By calculating the specific methanogenic production relative to the substrate (Yp/S), a higher efficiency in T3 and T4 pre-treatments is confirmed, as well as the existence of an inhibitory effect on T7.

Figure 4. Methane production obtained from raw and pre-treated SCB



Mean values ± standard deviation (—),

Control (*), T1 (■), T2 g (●), T3 (★), T4 (▲), T5 (♦), T6 (◁), T7 (⬟)

Table 5. Kinetic parameters obtained by BMP assays of raw and pre-treated SCB

	Pre-treatment	Pmáx (${}_{N}$L)	λ (h)	*k* (${}_{N}$L/h)	R^2	Yp/S*
T1	SCB raw	0.499±0.020	281.51±20.68	$6.59 x 10^{-4}$	0.980	0.615
T2	CO_2 40ºC & 70 Kgf.cm^{-2}	0.515±0.018	335.02±20.18	$6,11 x 10^{-4}$	0.986	0.568
T3	CO_2 60ºC & 200 Kgf.cm^{-2}	0.615±0.020	303.78±16.88	$8.43 x 10^{-4}$	0.987	0.720
T4	CO_2 80ºC & 200 Kgf.cm^{-2}	0.600±0.023	291.30±20.63	$7.99 x 10^{-4}$	0.980	0.620
T5	CO_2 +NaOH 40ºC & 70 Kgf.cm^{-2}	0.536±0.023	361.93±25.50	$5.69 x 10^{-4}$	0.983	0.666
T6	CO_2 +NaOH 60ºC & 200 Kgf.cm^{-2}	0.543±0.022	346.87±23.62	$6.13 x 10^{-4}$	0.983	0.587
T7	CO_2 +NaOH 80ºC & 200 Kgf.cm^{-2}	0.485±0.025	323.57±31.06	$5.75 x 10^{-4}$	0.974	0.551

Mean values ± Standard deviation * Expressed in ${}_{N}$L.CH_4/g.STV$_{substrate}$

4. Conclusions

The results showed that pre-treatments with sub and supercritical fluids have the potential to reduce structural obstacles of composition and increase the biodegradability of lignocellulosic materials. The methanogenic production of the anaerobic digestion of sugarcane bagasse was increased in all cases in which the material was pretreated with sub and supercritical CO_2, with exception of the cases in which NaOH was used as polarity modifier in combination with high temperatures. It is recommended more research with the aim of increasing the effectiveness of this pre-treatment by evaluating with greater amplitude variables of temperature, pressure, time and addition of co-solvents, as well as an economic analysis to determine the cost/benefit balance.

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