

SUSTAINABLE RESEARCH

METHODOLOGY ON THE EFFECT OF THE REUSE OF BLACK LIQUOR IN THE ALKALINE PRE-TREATMENT OF GARDEN RESIDUES FOR THE PRODUCTION OF BIOGAS

DOI: 10.5585/GEAS.V8I3.15780

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ABSTRACT: Biogas is an important renewable source of energy, which converts organic material into energy. Lignocellulosic biomass emerges as a strategy to increase biogas production through pre-treatments that provide organic matter to the anaerobic environment. Among the chemical pre-treatments available, the alkaline is the most used one because it presents the highest yield in biogas production. However, the effluent (black liquor) it generates is an alkaline byproduct that, if disposed irregularly, can cause environmental problems. In this context, the objective of the present study is to present the methodology employed to evaluate the effect of alkaline pre-treatment with potassium hydroxide solution (KOH) applied to garden waste in the production of biogas and the reuse of black liquor as a new alkaline medium. The pre-treatment was divided into seven subsequent batches, with the first batch consisting of a 5% KOH solution and dry and crushed substrate (garden pruning waste). For the other batch the black liquor from the liquid fraction of the separation process was reused. The Biochemical Methane Potential (BMP) tests were carried out with the fraction sifted in mesophilic conditions (35° C) for 25 days, following the German standard VDI 4630. The results showed that the second batch had the highest biogas production (620 LN biogas/SV-1 kg) and an efficiency of 30% when compared to the non-pretreated substrate. A positive effect was also observed in the biogas yield after reusing the black liquor twice, presenting an average efficiency of 20%. In this sense, this study demonstrates that the reuse of the remaining black liquor from pre-treatment with KOH is a viable and sustainable technique for pre-treatment of garden waste and contributes to the reduction of costs in real scale.

KEYWORDS: methodology; sustainability; biogas; methane; lignin; lignocellulose.

RESUMO: O biogás é uma importante fonte renovável de energia, que converte material orgânico em energia. A biomassa lignocelulósica surge como uma estratégia para aumentar a produção de biogás através de pré-tratamentos que fornecem matéria orgânica ao ambiente anaeróbio. Entre os pré-tratamentos químicos disponíveis, o alcalino é o mais utilizado, pois apresenta o maior rendimento na produção de biogás. No entanto, o efluente gerado (licor negro) é um subproduto alcalino que, se descartado irregularmente, pode causar problemas ambientais. Nesse contexto, o objetivo do presente estudo é apresentar a metodologia empregada para avaliar o efeito do pré-tratamento alcalino com solução de hidróxido de potássio (KOH) aplicado a resíduos de jardim na produção de biogás e a reutilização de licor negro como novo meio alcalino. O pré-tratamento foi dividido em sete bateladas subsequentes, com a primeira batelada composta por uma solução de KOH a 5% e substrato seco e triturado (resíduo de poda de jardim). Para as demais bateladas, o licor negro da fração líquida do processo de separação foi reutilizado. Os testes de Potencial Bioquímico de Metano (PBM) foram realizados com a fração peneirada

em condições mesofílicas (35°C) por 25 dias, seguindo a norma alemã VDI 4630. Os resultados mostraram que a segunda batelada teve a maior produção de biogás (620 LN biogas/SV-1 kg) e uma eficiência de 30% quando comparado com o substrato não pré-tratado. Efeito positivo também foi observado no rendimento de biogás após a reutilização do licor negro duas vezes, apresentando uma eficiência média de 20%. Nesse sentido, o estudo demonstra que o reuso do licor negro remanescente do pré-tratamento com KOH é uma técnica viável e sustentável para o pré-tratamento de resíduos de jardim e contribui para a redução de custos em escala real.

PALAVRAS-CHAVE: metodologia; sustentabilidade; biogás; metano; lignina; lignocelulose.

RESUMEN: El biogás es una importante fuente renovable de energía, que convierte el material orgánico en energía. La biomasa lignocelulósica surge como una estrategia para aumentar la producción de biogás a través de pretratamientos que proporcionan materia orgánica al ambiente anaeróbico. Entre los pretratamientos químicos disponibles, el alcalino es el más utilizado porque presenta el mayor rendimiento en la producción de biogás. Sin embargo, el efluente (licor negro) que genera es un subproducto alcalino que, si se elimina de manera irregular, puede causar problemas ambientales. En este contexto, el objetivo del presente estudio es presentar la metodología empleada para evaluar el efecto del pretratamiento alcalino con solución de hidróxido de potasio (KOH) aplicado a los desechos del jardín en la producción de biogás y la reutilización del licor negro como un nuevo medio alcalino. El pretratamiento se dividió en siete lotes subsiguientes, y el primer lote consistió en una solución de KOH al 5% y sustrato seco y triturado (residuos de poda de jardín). Para los demás lotes, el licor negro de la fracción líquida del proceso de separación se reutilizó. Las pruebas de Potencial Bioquímico de Metano (PBM) se llevaron a cabo con la fracción tamizada en condiciones mesofílicas (35°C) durante 25 días, siguiendo la norma alemana VDI 4630. Los resultados mostraron que el segundo lote tenía la mayor producción de biogás (620 LN biogas/SV-1 kg) y una eficiencia del 30% en comparación con el sustrato no pretratado. También se observó un efecto positivo en el rendimiento de biogás después de reutilizar el licor negro dos veces, presentando una eficiencia promedio del 20%. En este sentido, este estudio demuestra que la reutilización del licor negro restante del pretratamiento con KOH es una técnica viable y sostenible para el pretratamiento de los desechos del jardín y contribuye a la reducción de costos a escala real.

PALABRAS-CLAVE: metodología; sostenibilidad; biogás; matano; lignina; lignocelulosa.

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I. INTRODUCTION

Biogas is an important renewable and sustainable source of energy, which has been increasingly used in several countries. This study will work with the so-called “third generation biomass”, because it is an organic matter of difficult degradation, that is lignocellulose, derived from green grasses. Brazil has enormous potential for this type of biomass, but there are not many methodologies applied to green grasses that make this material accessible and with adequate degradability to be treated in the anaerobic reactors.

The objective of the implemented methodology in this study considered three aspects related to sustainability: 1) the pre-treatment of the grass, turning this organic matter more bioavailable to be used in the reactor, 2) reuse of the effluent, allowing economy in the pretreatment process 3) application of a novel chemical compound by replacing a sodium hydroxide solution (NaOH) – which is traditionally used but which can inhibit biological activity in reactors – by a potassium hydroxide (KOH) solution, which, in addition to being less inhibitory, can be reused as a biofertilizer in agriculture.

The organic fraction of solid residues presents potential for conversion into biogas by means of microbial transformations, such as anaerobic digestion (A.D). According to Raposo, De La Rubia, Fernández-Cegrí and Borja (2012), anaerobic digestion is a technological and biochemical process for the treatment of organic substrates, which involves the degradation and stabilization of organic matter by means of microorganisms that leads to the generation of biogas. Biogas is an energy-rich gas that can be used to replace traditionally used fossil fuels and thus reduce the emission of greenhouse gases (GHG) and their negative environmental impacts. Biogas can be used in several ways: directly as thermal energy for boilers, heaters and dryers; in engines for the generation of electrical and mechanical energy, and as the biomethane used in vehicles in substitution of natural gas. One of the main advantages of using biogas is the minimization of environmental impacts caused by municipal solid waste and animal waste when irregularly arranged.

With the technological advance, it was possible to incorporate into the A.D. process more complex

residues in terms of macromolecular composition (carbohydrates, proteins and lipids) and ones that have important potential to increase energy efficiency in terms of yield, for example, lignocellulosic residues such as pruning of urban vegetables, agricultural straw (rice and wheat) and silage. This source of waste is thought to be the third generation of biomass, mainly composed of cellulose, hemicellulose and lignin, the latter being considered difficult in terms of anaerobic degradation due to the rigid and complex structure. Due to the limited biodegradability, these substrates need treatment prior to the conventional anaerobic process, aiming to increase the bioconversion efficiency by means of lignin breakage and rupture of the crystalline structure of cellulose, improving the accessibility of the components contained in the biomass and consequently increasing the production of biogas.

Among the currently used methods, alkaline pre-treatment has shown to be effective in several types of biomass. This method uses a basic solution—such as NaOH, Ca(OH)₂, KOH and NH₃·H₂O—to improve the digestibility of lignocellulosic biomass, in addition to helping avoid the pH drop during the acidogenesis process, to increase the efficiency of methanogenesis, and to provide lower production costs when compared to other methods of chemical pre-treatment (Liew; Shi; Li, 2011; Liu, X.; Zicari; Liu, G.; Li; Zhang, 2015; Zheng; Zhao; Xu; Li, 2014).

Although it seems efficient, the demand for chemical compounds and water during the pre-treatment process of this type of biomass raises the operational costs and limits the application in real scale. For this reason, methodological and technological improvements are necessary, such as the use of alternative chemical compounds or the improvement of efficiency and yield of traditional compounds (Muryanto; Triwahyuni; Hendarsyah; Abimanyu, 2015). Thus, the reuse of the effluent generated by the chemical pre-treatment (black liquor) becomes attractive and can be reused and applied to subsequent pre-treatment, until the moment the action of the chemical compound mentioned loses efficiency due to dilution.

The objective of this work was to determine the effect of alkaline pretreatment with the use of potassium hydroxide (KOH) applied to garden waste and the efficiency of black liquor reuse

in biogas production through the Biochemical Methane Potential (BMP). In this sense, the study's premise was to identify sustainable techniques for converting waste into value-added byproducts.

2. THEORETICAL REFERENCE

2.1. ANAEROBIC DIGESTION

Anaerobic digestion (A.D.) is a biological treatment process of degradation of organic matter that occurs in the absence of oxygen. During the process, most of the organic load in the biomass (substrate) is converted into biogas by means of biochemical reactions that integrate in several steps until methane (CH₄) is obtained. In order for the AD process to occur completely and efficiently, a wide variety of microorganisms are required, which perform complex biochemical reactions, generating biogas as a byproduct (Montgomery; Bochmann, 2014). These actions are divided into four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1).

Obtained from a process of degradation of organic matter and whose advantage is the production of thermal and electrical energy, biogas is inexorably available to provide new applications for the residues of agricultural exploration, industrial activity and sewage. The composition of biogas can vary according to the type and amount of biomass employed, climatic factors, dimensions of the biodigester, among others. When the environmental conditions for the processing of waste by the microorganisms are met, the biogas obtained must be composed of a mixture of gases, with about 50 or 75% of the total volume consisting of methane, while the remainder consists mainly of carbon dioxide and smaller amounts of other gases (Surendra; Takara; Hashimoto; Khanal,

2014). To Galbiatti et al. (2010), it is not enough to have data only with reference to the amount of biogas produced: it is also essential to know the quality of the biogas produced.

2.2 SUBSTRATE

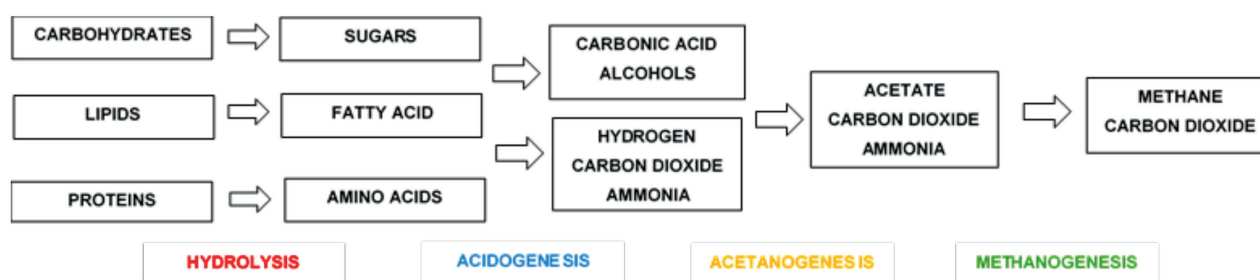
There are several types of biomass that can be used as substrate (raw material) in the anaerobic digestion process for the production of biogas (Adekunle; Okolie, 2015). However, the substrate must meet the nutritional requirements of microorganisms taking into account the energy sources and vital components for the formation of new cells (Aslanzadeh; Berg; Taherzadeh; Sárvári Horváth, 2014).

Lignocellulosic materials are known as fibrous materials and form complex matrices of cellulose, glucose polymers, hemicellulose, pectins and other elements. In addition to this matrix, there is lignin, which can be considered as a plastic resin cover covering the entire complex matrix of these materials (Brodeur et al., 2011). According to Sawatdeenarunat, Surendra, Takara, Oechsner and Khanal (2015), the lignocellulosic biomass consists of 35 to 50% cellulose, about 20 to 35% hemicellulose, and 10 to 25% lignin. A small amount of ash and extractive are also part of this biomass.

- Cellulose

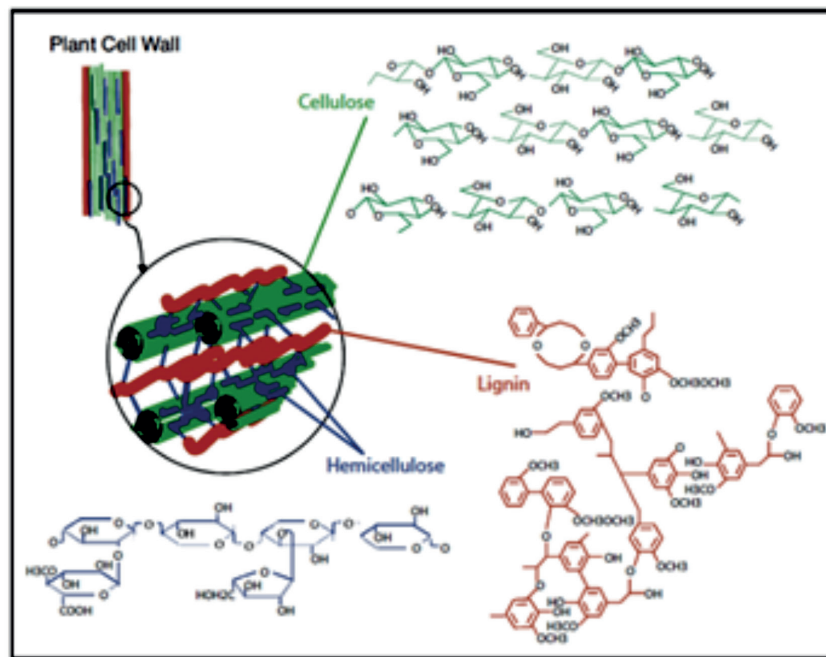
Cellulose plays an important role in vegetal organisms, accounting for about 40% of all the carbon stock that is available for nutrient synthesis. According to Mood et al. (2013), the cellulose chain is formed by β-glucose units, which guarantees a polymer of high molecular weight, with a linear structure and constituted by a single type of sugar. Such chains in the cell wall of plants are compactly

Figure 1 – Sequence of anaerobic digestion



Source: Adapted from Seadi et al. (2008)

Figure 2 – Internal structure of lignocellulosic biomass



Source: Bioful webgarden (2019)

arranged through hydrogen bonded bonds, which results in a strong interaction between their molecules, presenting fibers with distinctly crystalline regions and some amorphous regions.

- Hemicellulose

The hemicellulose or polyose is also formed by polysaccharides, but the difference between it and cellulose lies in the variability of types of sugars, since the cellulose presents only one type. Another important factor is the branching in the hemicellulose chain and the lower degree of polymerization due to the low molecular weight when compared to cellulose (Hendriks; Zeeman, 2009). Based on its structure, hemicellulose is more similar to cellulose than to lignin, in addition to being deposited in the cell wall before the lignification step (Mood et al., 2013; Zheng et al., 2014).

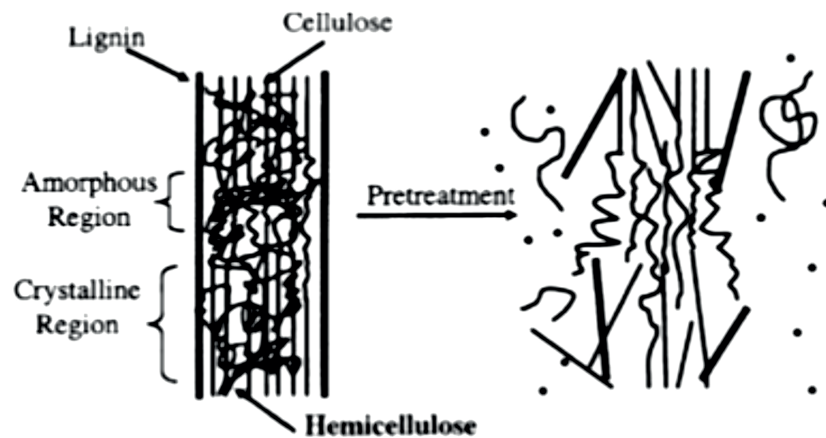
- Lignin

The lignin structure, on its turn, is composed of amorphous molecules, extremely complex and formed by aromatic units of phenylpropan, and these are characterized by fouling (Rowell; Petterson; Han; Rowell; Tshabalala, 2005). The amount of lignin molecules and their structure can vary depending on the plant species. They can be obtained in physical-chemical processes that allow their use as an energy source. Due to

its three-dimensional structure, lignin is a rigid component resistant to compression forces, which allows for resisting impacts, compression and breakage of the cell wall, as well as being responsible for the connection between one cell and another. Therefore, lignin has an important role in plants, providing resistance to attacks by microorganisms and acting in the transportation of water and nutrients (Figure 2), which is the major barrier in the use of lignocellulosic biomasses in anaerobic digestion (Hosseini Koupaie; Dahadha; Bazyar Lakeh; Azizi; Elbeshbishy, 2018; Sawatdeenarunat et al., 2015; Zheng et al., 2014).

2.3 PRE-TREATMENTS

The application of pre-treatment aims to improve the use of lignocellulosic biomass by separating its main components: cellulose, hemicellulose, and lignin. The exposure of cellulose allows for the access of hydrolytic agents, whether enzymatic or acidic, so that the process of transforming cellulose into glucose can occur easily (Manochio; Andrade; Rodriguez; Moraes, 2017). The pre-treatments can use acids, bases, steam, pressure or even the combination between them, which allows for the breakage or separation of lignin from the other components, facilitating A.D. (Sun; Ye; Cheng; Jay J., 2005) (Figure 3).

Figure 3 – Structural alteration of the molecule due to the use of pre-treatments

Source: Mosier et al. (2005)

- Physical pre-treatment

The physical pre-treatment aims to modify the structure of the biomass to increase the accessibility of the enzymes to the cellulose through the surface area, defibrillation and sometimes the reduction of the degree of polymerization and crystallization, without interfering in the chemical composition of the biomass (Alvira; Tomás-Pejó; Ballesteros; Negro, 2010). The types of physical pre-treatments include extrusion, disc milling, ball milling, microwaves, and freezing (Mood et al., 2013).

- Chemical pre-treatment

The chemical pre-treatments differ by the organic or inorganic compounds used, as well as in the mechanisms responsible for the structural and chemical modifications of the cell wall. They may use organic acids, bases or solvents (Brodeur et al., 2011). According to Chen, Pen, Yu and Hwang (2011), pre-treatment promotes the hydrolysis of hemicellulose and other polysaccharides into monosaccharides, such as xylose, mannose, galactose, glucose, among others. As hemicellulose consists primarily of xylan, the main product of acid treatment is xylose.

The acid pre-treatment can be conducted under two different conditions: concentrated acid or dilute acid. The first one is not as attractive as

the second, because it presents several problems such as formation of inhibitors, toxicity, corrosion and the need for resistant equipment (Singh; Suhag; Dhaka, 2015). The second one employs acid with a concentration between 0.1 and 2% (m.v-1). It is usually more effective, since it presents a lower sugar degradation and consequently lower formation of inhibitors (Behera; Arora; Nandhagopal; Kumar, 2014).

The alkaline pre-treatment, on its turn, aims to increase cellulose porosity and reduce its degree of polymerization and crystallinity (Zhang; Keshwani; Xu; Hanna, 2012), providing cellulose accessibility through the removal of lignin (Balat; Balat; Öz, 2008). The most used bases for this type of pre-treatment are: sodium hydroxide, calcium hydroxide, ammonia and urea, with diluted calcium hydroxide being the most used and advantageous because of its easy handling and low cost (Montgomery et al., 2014). In addition, sodium hydroxide presents a more efficient result in biomass with a low lignin content (10 to 18%) (Zhang et al., 2012).

- Biological pre-treatment

Biological pre-treatments usually use fungi and some species of bacteria. During the process, these microorganisms secrete extracellular enzymes—such as lignin peroxidases and laccases—that help remove a considerable amount

Table 1 – Substrates used to adapt the inoculum

COMPONENTS	%
Powdered milk	25
Soy protein	10
Corn flour	20
Dry grass	25
Vegetable oil	20

of lignin from biomass (Ogeda; Petri, 2010). Removal of the lignin makes the cellulose more accessible, favoring the hydrolysis step. Despite its low energy consumption, the biological pre-treatment requires a longer period to deconstruct the biomass and also presents a high cost (Balat et al., 2008; Mood et al., 2013).

2.4 REUSE OF BLACK LIQUOR

Although the chemical pre-treatment is the most used in research, the high production of polluting effluents mainly containing alkali-soluble lignin (whith high COD content), along with the excessive demand for water durint the pretreatment, has limited its application in commercial scale. For this reason, alternatives such as the reuse of the remaining black liquor from pre-treatment need to be considered (Wang et al., 2017).

In addition to the environmental issue, the alkaline pre-treatment uses a large amount of base, therefore, the reuse of the black liquor increases the economic efficiency by reducing the cost associated with using the base (Muryanto et al., 2015). In this sense, the remaining alkaline liquid from the pre-treatment with potassium hydroxide (KOH) can be used for pre-treatment in place of a new KOH solution (Liu et al., 2015).

3. METHODOLOGICAL PROCEDURES

3.1 CHARACTERIZATION OF THE INOCULUM

The inoculum used for the BMP tests was provided by the International Center for Renewable Energies – Biogas (CIBiogás). It is composed of two different types of digestates: swine biodigester sludge, bovine biodigester sludge and bovine waste on a wet basis in a 1:0,5:0,5 proportion, respectively. The purpose of the mixture was to

initiate the process of acclimatization of the inoculum, grouping several species of bacteria used to degrade different types of residues, since the sludge presents different physicochemical compositions. After mixing the residues, the inoculum was acclimated with substrates rich in proteins, carbohydrates, lipids and lignocellulosic residues, to adapt the inoculum for it to degrade components present in the samples (Table 1).

3.2 CHARACTERIZATION OF SUBSTRATE

The substrate came from the pruning of grass and gardens of the Itaipu Binacional Hydroelectric Power Plant (Foz do Iguaçu/Brazil). The green area has 411 hectares of *Panicum maximum* and three species of grasses, consisting of 18% of *Zoysia japonica*, 80% of *Stenotaphrum secundatum*, and 2% of *Axonopus compressus* (Figure 4).

The drying of the substrate was carried out in a greenhouse with forced air circulation at 50° C for a period of 24 hours, in order to guarantee the homogeneity of the sample to be incubated, and thus facilitating the grinding process to reduce the particle diameter (< 3 mm), since the degradation is directly linked to the size of the substrate to be treated. After drying, the samples were stored at 4° C to prevent the proliferation of microorganisms and the degradation of the sample. The characterization was determined according to physical parameters such as: total solids content (TS) and volatile solids (SV) (APHA, 2005).

3.3 ALKALINE PRE-TREATMENT AND REUSE OF BLACK LIQUOR

The alkaline pre-treatment of the dried and crushed substrate was carried out in seven subsequent batches. In the first batch, the substrate was pre-treated with a 5% potassium hydroxide solution (KOH) and a mixture containing 10% ST (Equation 1) was established. After a 6-hour contact period at

Figure 4 – *Zoysia japonica* (I), *Stenotaphrum secundatum* (II), *Axonopus compressus* (III) and *in natura* substrate (IV)



Source: Mosier et al. (2005)

room temperature (25° C), the liquid fraction (black liquor) was separated using a 2 mm granulometry sieve, and then the black liquor was set aside as the alkaline medium for a new pre-treatment (using a new sample of grass pruning).

The sieved fraction (pre-treated substrate) was washed with about 100 mL of distilled water, and the recovered liquid was combined with the black liquor to total a 200 mL volume, maintaining the same ratio as the initial batch. The sifted fraction was washed again with running water until neutral pH was obtained, and then dried at 50° C for 24 hours to be submitted to the BMP test (Figure 5). Drying the solid fraction after pre-treatment and prior to the anaerobic digestion process carried out in batches is important to reduce the moisture content of the sample and ensure its heterogeneity, resulting in triplicates with statistically acceptable values of variation.

Where:

$$TS_{mix} = \frac{M_{sub} \cdot TS_{sub}}{M_{sub} + M_{sol}} \quad (\text{Equation 1})$$

TS_{MIX}: total solids content of the mixture (adopted as 10% or 0.1)

TS_{SUB}: total solids content of the substrate, %

M_{SUB}: substrate mass, g

M_{SOL}: alkaline solution mass, g (adopted density = 1:1)

The identification of BMP of the substrates and inoculum was carried out with the anaerobic digestion in batch, according to the German standard VDI 4630 (2006) (Figure 6).

The system consists of a 300 mL glass reactor (A), 500 mL eudiometer tubes (B) and leveled flasks (G). A connecting pipe (C) inside the eudiometer connects the reactor (A) so that the volume of gas produced can be measured. A barrier solution was used to avoid the dissolution of biogas components, such as CO₂, allowing for the determination of the actual concentration of these components afterwards. The volume of liquid displaced from the pressure generated by the biogas is transferred to a glass vessel (G) connected to the eudiometer tube. The eudiometer and the glass vessel can be connected with silicone hoses (F). Once the gas is produced, it can be collected for analysis and discarded by the valve (H).

The barrier solution was prepared according to DIN 38414 (1985), in which for each 1 L of distilled water it was added 30 mL of H₂SO₄, 88.21 g of Na₂SO₄ and 3 drops of methyl orange. According to the standard, this solution should be stored at room temperature due to sodium crystallization and, if necessary, should be heated for solubilization of the crystals.

For each sample unit, 200 mL of acclimated inoculum was added together with the substrate mass, calculated based on the amount of VS, and 95 mL of headspace, as established. For the assembly and incubation of the samples it was necessary to meet parameters established by the VDI standard

Figure 5 – Illustrated flowchart of the methodological procedure for the reuse of black liquor.

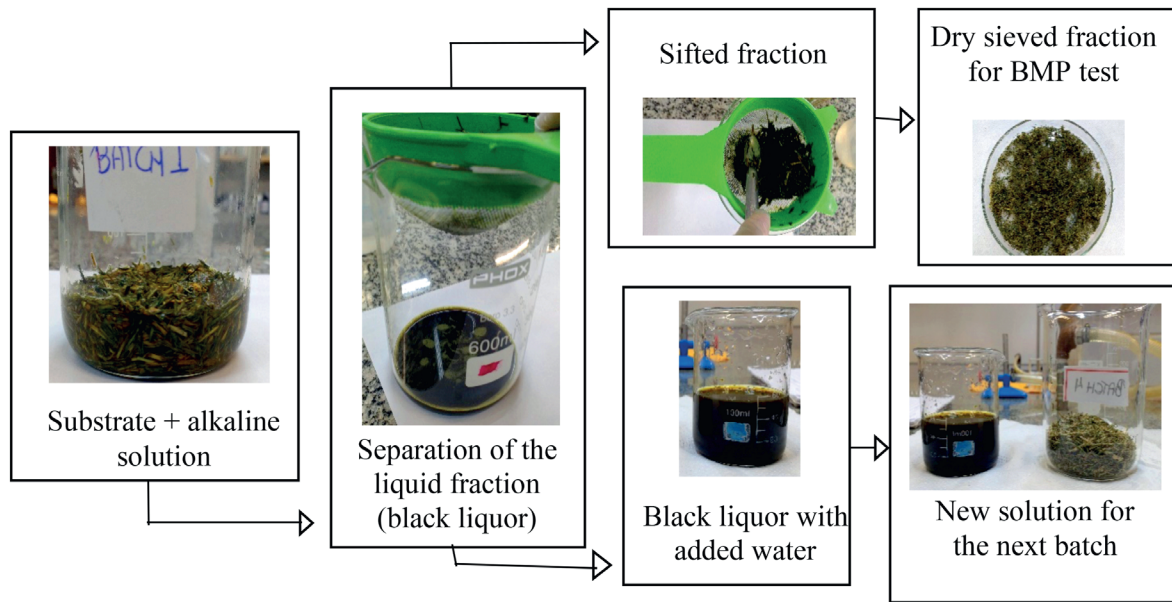
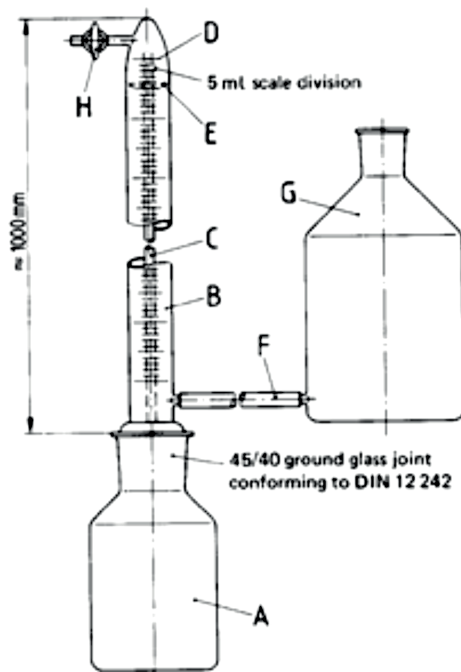


Figure 6 – Equipment for BPM tests.



Source: DIN 38414 (1985)

4630 (2006) (Table 2). The tests were carried out for a period of 25 days at mesophilic temperature (37° C). The test period was defined as the period in which the ratio of accumulated production to daily biogas production was below 1%.

A triplicate containing only inoculum was incubated to identify the endogenous production of biogas, and the volume generated specifically by the inoculum was subtracted from the production containing inoculum and substrate. A second triplicate isolated from reactors and containing inoculum was incubated, and a sample of known composition (microcrystalline cellulose) was used

as a positive control. This procedure is important to evaluate the biological activity of the inoculum and to ensure that the accumulated biogas production of the tested substrates is not inhibited by micro-biological limitations. Considering the tests with inoculum, microcrystalline cellulose, *in natura* grass and the 7 pre-treatment batches, the experiment was assembled with a total of 30 reactors.

The volume of biogas generated was recorded daily at the same times, as well as the ambient temperature and atmospheric pressure for the standardization of the biogas volume (Equation 2). The ambient temperature data was measured in degrees Celsius (° C) and then converted into Kelvin (K = ° C + 273.15).

$$V_0 = V \cdot \frac{(P_L - P_W) \cdot T_0}{P_0 \cdot T} \quad \text{Equation (2)}$$

Where:

- Vo = volume of standardized biogas, mL;
- V = volume of biogas registered in the eudiometer, mL;
- PL = atmospheric pressure at time of recording, mbar;
- PW = water vapor pressure, mbar;
- T0 = standardized temperature, T0 = 273 K;
- P0 = standardized pressure, P0 = 1013 mbar;
- T = room temperature, K.

According to Strömberg, Nistor and Liu, (2014), water vapor generates overestimated values between 2-8% of the biogas volume under normal conditions of temperature and pressure.

Table 2 – Specifications according to VDI 4630 (2006).

PARAMETERS	VDI 4630	UNIT
TS in reactor	≤ 10,0	%
VS in reactor	1,5 a 2,0	%
$VS_{\text{sample}}/VS_{\text{inoculum}}$	≤ 0,5	%

Thus, the vapor pressure (P_w) was considered to obtain accurate measurements of biogas production, since the ambient temperature (T) was considered as the temperature of the gas and not of the inside of the reactor. The calculation of the water vapor pressure can be done using the Antoine Equation (Equation 3).

$$P_w = 10^{8,1962 - \frac{1730,63}{T - 39,724}} \quad \text{Equation (3)}$$

3.4 ANALYSIS AND COMPOSITION OF BIOGAS

The biogas composition analysis was carried out using gas chromatography (PerkinElmer Chromatograph - Clarus 680) (Figure 7). The levels of methane (CH_4), carbon dioxide (CO_2) and hydrogen sulfide (H_2S) were obtained from the conversion of the areas established in the chromatograms in comparison with the biogas areas of known concentration (standard biogas).

To that end, the standard biogas present in the gas cylinder (Mark White Martins) containing the known concentration of methane (CH_4) and carbon dioxide (CO_2) was transferred to an air balloon in order to equalize the gas pressure with the atmospheric pressure, thus minimizing

possible errors. Subsequently, 0.5 mL of standard biogas was transferred to a glass syringe, always being careful that there is no air entering the syringe (Figure 8).

The glass syringe was then positioned in the chromatograph reader, and all the volume present therein was entirely injected during the 5-second countdown on the equipment. The same procedure was done for reading the samples, and the final value was correlated with the values of areas of the chromatogram of the standard biogas.

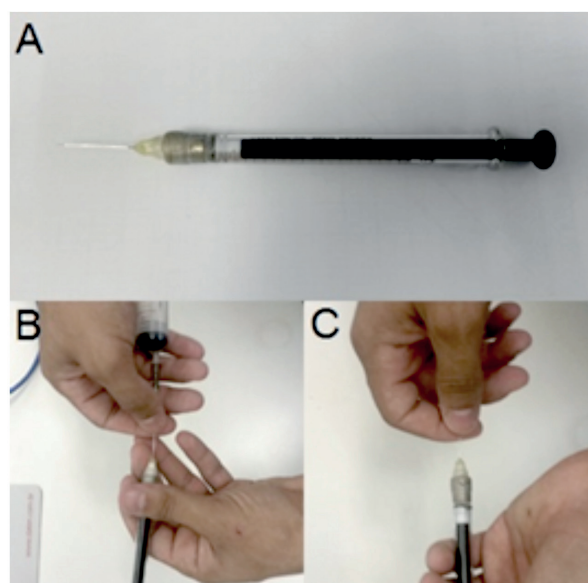
4 ANALYSIS AND DISCUSSION OF RESULTS

4.1 SUBSTRATE CHARACTERIZATION

The *in natura* grass sample (before pre-treatment) had a ST content of 91.7% and a VS content of 88.9%. The preliminary drying process of the samples at 50° C aims to eliminate excess moisture from the grass, however, the high ST content of this type of biomass indicates potential for conversion into biogas, when compared to animal waste—which has high humidity and thus low yield in terms of production, considering the total

Figure 7 – PerkinElmer Chromatograph - Clarus 680

Figure 8 – Gastight glass syringe (A), transfer (B) and air seal of syringe (C)



mass of the substrate. Likewise, the high concentration of VS indicates high amount of organic matter, which—if properly pre-treated—can be converted into biogas by biological processes. It

solution tended to be neutral (13.9 to 7.3) due to the incorporation of water to correct the total volume of the tests (Table 1).

Table 3 – Physical-chemical characterization of residues of raw grass pruning and after the sequence of chemical pre-treatments

PARAMETERS	TS (%)	VS (%TS)	BLACK LIQUOR PH
RGW	91,7±0,1	88,9±0,2	ND
B1	89,4±0,8	93,7±0,1	13,9*
B2	90,6±0,1	93,6±0,1	13,6
B3	90,6±0,6	93,2±0,01	13,9
B4	90,9±0,2	92,2±0,01	11,7
B5	89,5±0,3	92,9±0,01	9,8
B6	89,1±0,1	92,8±0,1	8,0
B7	92,1±0,6	92,4±0,2	7,3

TS: total solids; VS: volatile solids; RGW: raw garden waste; B: batch; ND: non-defined.

*Alkaline KOH solution with a 5% concentration.

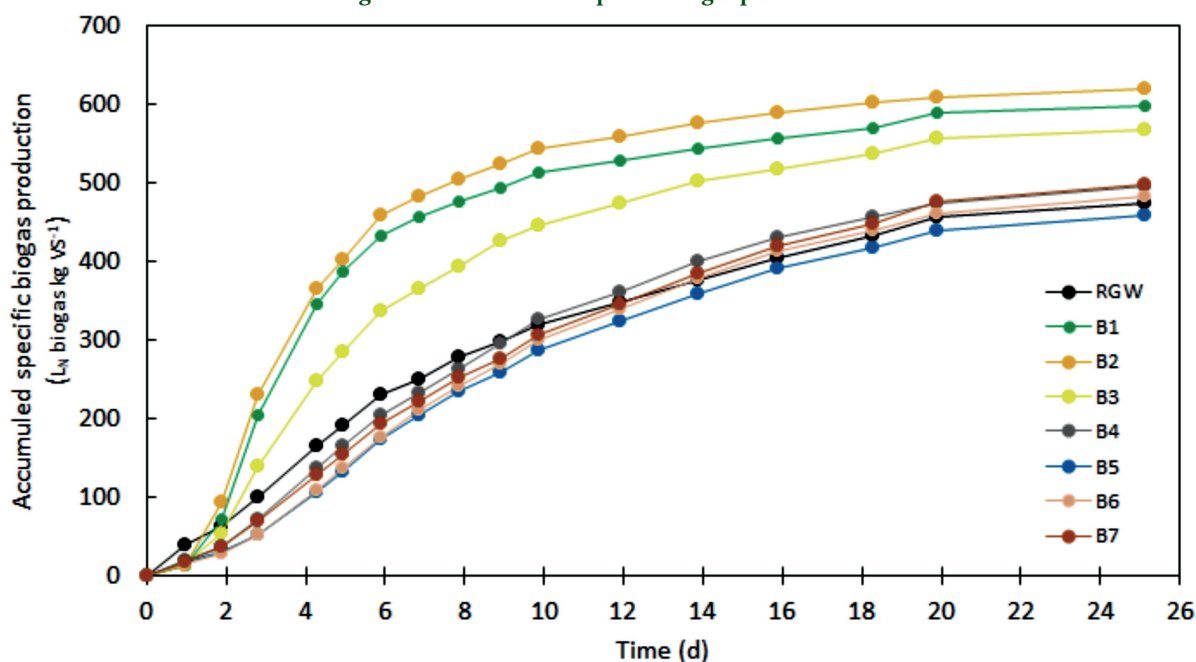
was observed that during the 7 pre-treatment batches the ST content was not altered, and this effect is related to the preliminary drying process, which leaves the substrate sample more homogeneous.

In contrast, the VS values of the pre-treated fractions were, on average, 5% higher than *the in natura* grass, which may be related to the dragging of solids in the course of reusing the black liquor. The pH of the *in natura* grass was not determined by the fact that it is a solid sample. However, the pH of the pre-treatment alkaline

4.2 BIOCHEMICAL METHANE POTENTIAL OF PRE-TREATED SAMPLES

The *in natura* grass sample had a biogas production potential of 474 L_N biogas/VS⁻¹ kg with 58% CH₄ content (274 L_N CH₄/VS⁻¹ kg). This reveals the low availability of easily biodegradable organic matter and, consequently, the low methane production when compared to biomass from other urban sources such as fruit and vegetable residues, which have the potential of 352 L_N CH₄/VS⁻¹ kg (Jiang et al., 2012), due mainly to the high concentration of

Figure 9 – Accumulated specific biogas production



RGW: raw garden waste; B: batches (1 to 7); L_N: Standard Liter; kg: kilogram; VS: volatile solids; d: day.

simple sugars and low lignocellulose content. After the first pre-treatment batch, a 26% higher potential (598 L_N biogas/V_S⁻¹ kg) of biogas production was observed, indicating a positive KOH efficiency in the breakdown of the lignocellulosic structure, thus guaranteeing a greater availability of organic matter after pre-treatment. The most significant efficiency of the alkaline pre-treatment and reuse of black liquor in biogas production was observed from the first to the third batch (Figure 9), when the behavior of the accumulated biogas production curve was indicated by higher velocity and volume when compared with the grass *in natura*.

Batch 2 (B₂) had the highest biogas yield (620 L_N biogas/V_S⁻¹ kg), and after that, batch 1 and 3

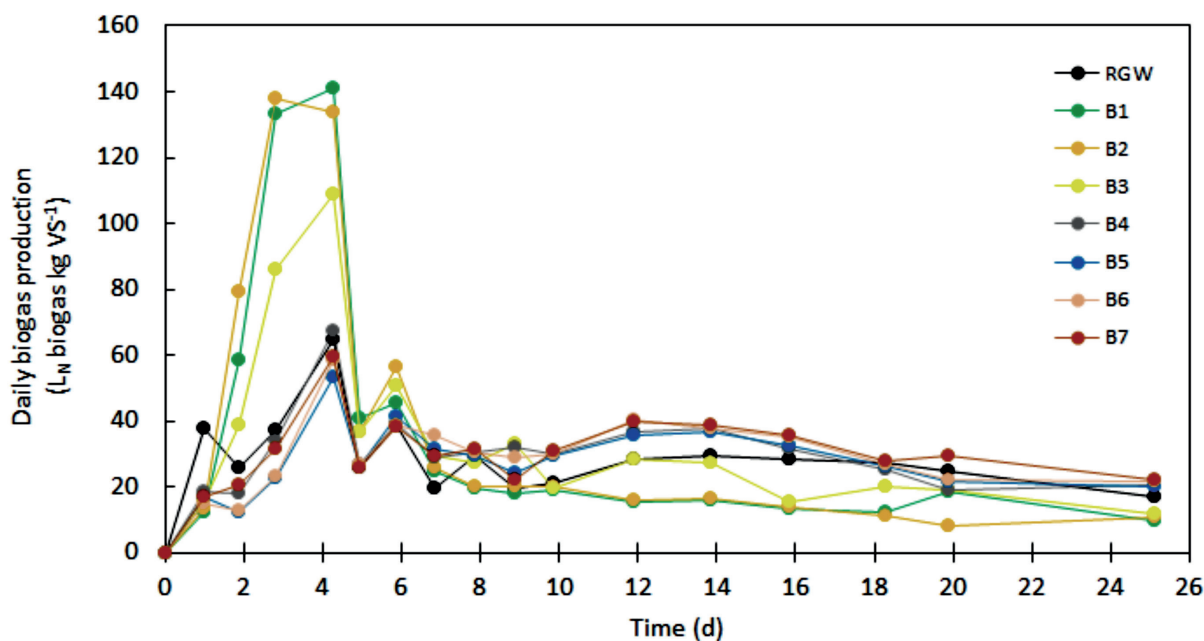
presented lower values (598 and 568 L_N biogas/V_S⁻¹ kg, respectively), however with a 20% higher average than the *in natura* grass (Table 1). Such behavior indicates the efficiency of the alkaline pre-treatment in the lignin removal and the influence of the pH in the process, since the alkaline environment of the first three batches presented values equal to 13. Although the concentration of potassium hydroxide is higher in batch 1, a decrease of 4% in biogas yield was observed when compared to batch 2. This effect can be explained by the presence of biodegradable compounds dragged from the first to the second batch. Even though this effect also occurs in the subsequent batch, there is also, and in a cumulative way, the

Table 4 – Accumulated biogas production, pre-treatment efficiency and methane content

SUBSTRATES	BIOGAS (L _N /V _S ⁻¹ KG)	EFFICIENCY (%)	DRY METHANE (%)
RGW	474±35	ND	58±2,6
B ₁	598±5	+26	56±2,3
B ₂	620±4	+30	57±0,7
B ₃	568±20	+20	57±0,5
B ₄	495±36	+5	57±0,2
B ₆	484±18	+2	57±0,2
B ₇	499±13	+5	57±0,2
B ₇	92,1±0,6	92,4±0,2	7,3

VS: volatile solids; GWR: raw Garden waste; B: batches (1to 7); L_N: Standard Liter; kg: kilogram; ND: non-defined.

Figure 10 – Daily production of biogas



RGW: raw garden waste; B: batches (1 to 7); L_N : Standard Liter; kg: kilogram; VS: volatile solids; d: day.

effect of the black liquor dilution, which, in this case, has shown to be superior to the dragging of organic matter in the liquid solution.

From the third batch, biogas yield values were observed close to the substrate without pre-treatment ($474 L_N \text{ biogas}/\text{VS}^{-1} \text{ kg}$). However, the efficiency of pre-treatment with black liquor reuse was still positive until the seventh batch. Batch 5 was pre-treated with an additional volume of distilled water and, due to this procedural error, the data were subtracted from the BMP analysis. Methane production also showed a behavior similar to biogas, since the dry methane content of all treatments remained in the 56-58% range (Table 2).

The influence of pre-treatment and reuse of black liquor was also observed in daily biogas production (Figure 10). Batches 1, 2 and 3 showed peak production between days 2 and 4, with 141, 138 and 109 $L_N \text{ biogas}/\text{VS}^{-1} \text{ kg}$, respectively. This factor indicates the contribution of pre-treatment in the hydrolysis stage with the removal of lignin and the availability of easily biodegradable compounds, such as sugars present in the lignocellulosic structure (cellulose and hemicellulose). On the other hand, *in natura* grass showed a peak in the same period, but with a considerably lower biogas production ($65 L_N \text{ biogas}/\text{VS}^{-1} \text{ kg}$). The other batches also showed behavior and values similar to the *in natura* grass (Figure 10).

5. CONCLUSION

One of the most important prerequisites for sustainable development is the production of appropriate and sustainable fuels from biomass, which can be used as an alternative to fossil fuels. Biogas produced from waste materials is a favorable renewable source of energy, which is already being used for the production of heat and electricity, and also for car fuel.

This study presented a methodology applied to green grasses that make this material accessible and with adequate degradability to be treated in the anaerobic reactors. Alkaline pre-treatment with KOH and reuse of black liquor from grass pruning proved to be an effective method of pretreatment and bioconversion of organic matter into biogas. The application of a novel chemical compound by replacing a sodium hydroxide solution (NaOH) by a potassium hydroxide (KOH) solution was proved, being less inhibitory and can be reused as a biofertilizer in agriculture.

The pretreatment was efficient until the third batch, with an average yield of 20% in biogas production when compared to *in natura* grass. Even after reducing efficiency after 3 pre-treatment batches, the strategy has advantages, since it reduces the demand for chemical solutions by up to 3 times and, consequently, the need to treat an effluent, which can increase the operation costs in

biogas plants. This methodology, therefore, presents sustainable improvements by reducing the consumption of water and chemicals for pretreatment of lignocellulosic residues, besides allowing the biochemical conversion of a waste into a biofuel, stimulating the search for a more renewable energy matrix.

For future studies, the investigation of different alkaline chemical solutions in the breakdown of the lignocellulosic structure and in the production of biogas is recommended.

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