



## Hydrothermal Pretreatment of Sugarcane Bagasse for Biomethane Production

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### Highlights

- Hydrothermal pretreatment of BCA is a promising way to utilize this residue for methane production.
- The BMP of the hydrolysate was highest with hydrothermal pretreatment using water.
- Energy recovery from BCA co-products offers a viable strategy to diversify the use of this biomass.

### Introduction

The advancement and consolidation of biorefineries represent a promising strategy to add value to the agribusiness production chain, while also contributing significantly to the achievement of the Sustainable Development Goals (SDGs). According to the International Energy Agency (IEA, 2023), Brazil is a global leader in second-generation biofuels, and ethanol supply is expected to reach an average of 660,000 barrels per day by 2026—an increase of 90,000 barrels per day compared to 2020.

With this growing demand, the increase in ethanol production can be linked to the generation of residues from the production process, as sugar-energy plants produce sugarcane bagasse, vinasse, molasses, and filter cake. It is estimated that the ethanol industry generates around 13 liters of vinasse for every liter of ethanol produced, and approximately 30% of the sugarcane mass is considered bagasse (Hiranobe et al., 2024). Currently, sugarcane bagasse (SCB) has gained attention in several studies focused on biofuel production, especially as a feedstock for second-generation ethanol and biomethane (Varella et al., 2024).

However, the recalcitrant lignocellulosic structure of SCB is a limiting factor for fermentation processes, as it contains, on average, 26–47% cellulose, 19–33% hemicellulose, 14–23% lignin, and 1–5% extractives (Hiranobe et al., 2024). To improve the degradability of SCB, pretreatment methods must be applied to break down its complex and resistant structure. Among the various pretreatment options, hydrothermal pretreatment stands out due to its ability to solubilize hemicellulose without the need for catalysts and to produce lower concentrations of inhibitory compounds compared to chemical pretreatments (Bhatia et al., 2021).

This study aimed to evaluate the energy potential of hydrothermal pretreatment of sugarcane bagasse using vinasse and water, with a focus on biomethane production from the resulting hydrolysate. For comparison purposes, different energy scenarios were considered, including direct anaerobic digestion of vinasse, biomethane production from hydrolysates, electricity generation from the combustion of raw and pretreated bagasse, and second-generation (2G) ethanol production.

### Material and Methods

#### Inoculum and Substrate Characterization

The inoculum used in this study was collected from a completely mixed anaerobic reactor, composed of a mixture of swine digestate and raw cattle manure in a 20:80 (v/v) ratio, respectively. The reactor has been operating for 18 months under mesophilic conditions (37 °C), with a volumetric organic loading rate (OLR) of 0.15 g VS·L<sup>-1</sup>·d<sup>-1</sup>. Sugarcane bagasse (SCB) and vinasse were supplied by a sugar and ethanol mill located in the state of Goiás, Brazil, and collected from the plant's waste storage yard. To reduce the moisture content of the SCB, the material was exposed to ambient temperature (27 °C – 32 °C) for 7 days. After this period, it was milled using a knife grinder to obtain a particle size smaller than 10 mm. Vinasse was stored in plastic drums and kept refrigerated at 4 °C until the experiments were conducted.





### Hydrothermal Pretreatment of the Substrate

The hydrothermal pretreatment of sugarcane bagasse (SCB) was carried out in a 1 L stainless steel reactor (Parr model 5100), with manual control of temperature and agitation. Operating conditions were defined based on literature data, with a temperature of 180 °C and a reaction time of 15 minutes. Vinasse or water was used as the liquid medium, and the mixture was adjusted to contain 10% total solids (TS). After the pretreatment process, the solid and liquid fractions were manually separated using a mesh 18 sieve. The liquid fraction (hydrolysate) was used in the fermentation assays, while the solid fraction was dried at 50 °C for 24 hours to quantify the recovery efficiency of the process.

### Biochemical Methane Potential (BMP) Assay

The biochemical methane potential (BMP) assay was conducted following the methodology proposed by Holliger et al. (2016). An inoculum-to-substrate ratio (ISR) of 4:1 was used, and microcrystalline cellulose (Dinâmica brand) was employed as a reference substrate to validate the inoculum quality. The experimental setup consisted of 100 mL penicillin bottles sealed with rubber stoppers and aluminum crimps. The assays were carried out under mesophilic conditions (37 °C) and monitored until the daily biogas volume dropped to 1% of the cumulative volume. Gas volume was measured daily using a glass syringe connected to the penicillin bottle, along with a digital manometer.

### Energy Scenario Analysis

To evaluate different energy valorization routes for sugarcane coproducts, five energy scenarios based on the utilization of sugarcane bagasse (SCB) and vinasse were defined and compared: C1: Use of SCB in boilers for steam generation (combustion); C2: Use of SCB for second-generation (2G) ethanol production; C3: Use of vinasse as substrate for biomethane production; C4: Use of the hydrolysate obtained from hydrothermal pretreatment with water for biomethane production, combined with the use of the pretreated solid fraction for steam generation (combustion); C5: Use of the hydrolysate obtained from hydrothermal pretreatment with vinasse for biomethane production, combined with the use of the pretreated solid fraction for steam generation (combustion).

The scenario calculations were based on mass and energy flow quantification per ton of processed sugarcane, assuming that one ton of sugarcane generates 280 kg of bagasse and 800 L of vinasse per ton of cane (Carpio et al., 2022). Biomethane yields from vinasse and the hydrolysate obtained after hydrothermal pretreatment were experimentally determined, while the 2G ethanol yield was based on literature data (220 L/t of SCB) (Carpio et al., 2022). Energy potentials were converted to kcal per ton of sugarcane, using the respective lower heating values (LHV) of biomethane (8,574 kcal/m<sup>3</sup>), 2G ethanol (6,300 kcal/kg), and SCB (2,130 kcal/kg) (BEN, 2023).

## Results and Discussion

### Influence of Hydrothermal Pretreatment on the Hydrolysate Composition

Hydrothermal pretreatment acts as an autocatalytic process and promotes the depolymerization of biomass polysaccharides, which aids in the recovery of hemicellulose sugars in the liquid fraction. The total sugar content was higher in the hydrolysate treated with water (136.9 mg/L), suggesting greater solubilization of structural sugars from the sugarcane bagasse (SCB), whereas the use of vinasse resulted in lower sugar release (43.9 mg/L), possibly due to the conversion of sugar molecules into organic acids (Table 1).

**Table 1** Composition of the Hydrolysate and Vinasse

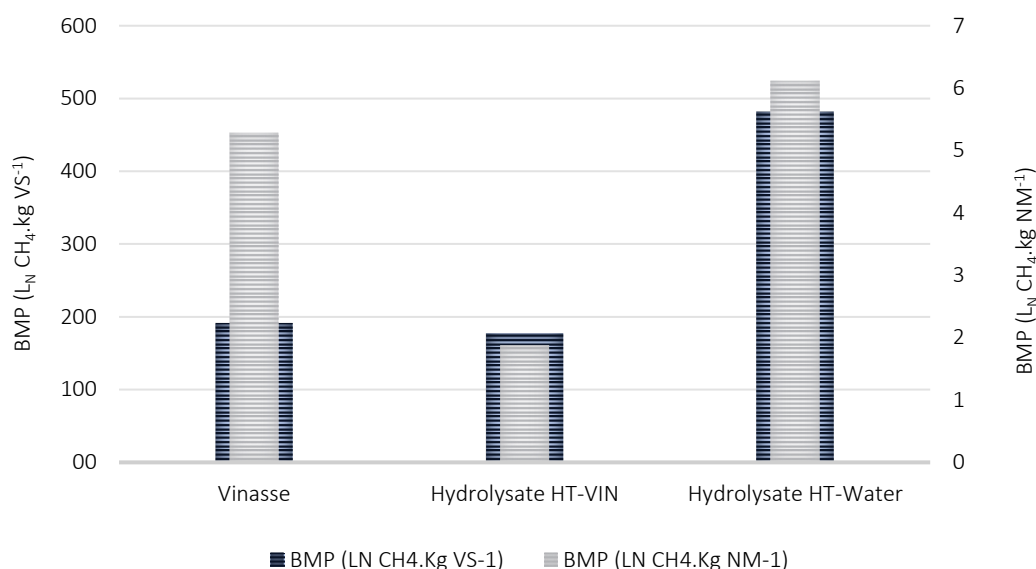
Parameters	Vinasse	Hydrolysate HT - Vinasse	Hydrolysate HT - Water
pH	4.8±0,1	4.6±0,1	3.5±0,1
Total Carbohydrates (mg/L)	n.d	43.9±0,1	136.9±0,1
Volatile Fatty Acids (mg/L)	1,636.9±0,1	7,538.8±0,1	2,703.3±0,1
Hydroxymethylfurfural (mg/L)	n.d	61.3±0,1	164.8±0,1
Furfural (mg/L)	n.d	115.0±0,1	453.3±0,1



Although vinasse is considered an acidic substrate ( $\text{pH} = 4.8$ ), the amount of organic acids present in its composition is 64.2% lower compared to the hydrolysates obtained from the pretreatment (Table 1). However, when used as the liquid medium in the process, it is observed that the pretreatment with vinasse recovered more organic acids (7,538.8 mg/L), whereas the pretreatment with water resulted in 2,703.3 mg/L. This effect can be explained by the initial composition of vinasse, which is rich in organic compounds residual from alcoholic fermentation, potentially generating acid intermediates without intensifying the formation of inhibitory compounds.

### Biochemical Methane Potential Assay

The biochemical methane potential (BMP) was higher for the hydrolysate obtained from the water-based pretreatment (Hydrolysate HT-Water), reaching approximately  $470 \text{ LN CH}_4 \cdot \text{kg VS}^{-1}$  (Figure 1). The higher concentration of readily biodegradable compounds, such as total sugars, contributed to this result and indicates that vinasse, when used as the liquid medium in the pretreatment, did not favor the efficient release of these compounds for methane conversion.



**Figure 1** – Biochemical Methane Potential of Hydrolysates and Vinasse

When considering the BMP on a natural matter basis (NM), a reduction in the absolute methane values is observed (Figure 1). Nevertheless, the hydrolysate obtained from the water-based pretreatment (Hydrolysate HT-Water) maintained the highest performance, with approximately  $6.3 \text{ LN CH}_4 \cdot \text{kg NM}^{-1}$ , demonstrating that the substrate presents high energy efficiency per mass of hydrolysate. The BMP of the hydrolysate from the vinasse-based pretreatment showed the lowest value ( $1.8 \text{ LN CH}_4 \cdot \text{kg NM}^{-1}$ ) and did not demonstrate competitiveness regarding the use of vinasse for hydrothermal pretreatments.

### Energy Scenario Analysis

The analysis of the calculated energy scenarios allowed the identification of different levels of energy utilization of SCB and vinasse (Table 2). The scenario of direct combustion of bagasse in boilers (C1) showed the highest energy potential, with approximately 600,000 kcal per ton of sugarcane, reflecting the high calorific value of bagasse and the consolidated efficiency of this process in sugar and ethanol mills. However, it is known that only 25% of this potential is converted into electricity, while the remainder is used as thermal energy within the industrial process itself, which limits its direct contribution to replacing fossil fuels outside the plant (Carpio et al., 2022). In contrast, 2G ethanol produced from SCB proved to be promising among the analyzed energy scenarios, with a potential of approximately 157,000 kcal per ton of sugarcane.



**Table 2** Energy potential scenarios using coproducts from sugar-energy plants

Scenario	Condition	Yield (Nm <sup>3</sup> CH <sub>4</sub> or m <sup>3</sup> Ethanol/ton cane)	Energy Potential (kcal/ton cane)
C1	SCB: thermal energy*	–	596.400
C2	SCB: 2G Ethanol	30,8	156.978
C3	Vinasse: biomethane	4.353	37.326
C4	Hydrolysate PT-Water: biomethane**	9.316	79.875
	Solid fraction: thermal energy	–	483
C5	Hydrolysate PT-Vinasse: biomethane**	6.131	52.563
	Solid fraction: thermal energy	–	477

\* SCB with 50% moisture. \*\* Does not consider electricity consumption in the pretreatment.

The scenarios involving vinasse (C3) and the hydrolysates from the pretreatments (C4 and C5) were, on average, 75% lower than C2, demonstrating the challenge of making integrated sugar-energy plants viable for biomethane production when considering only the energy potential of the substrates individually. However, when evaluating the combined energy potential of scenarios 3 and 4, it is possible to achieve a total of 117,684 kcal per ton of sugarcane without needing to divert vinasse to a separate process, thus maintaining the initiative to utilize SCB for gaseous biofuel production. Moreover, biofuel production from SCB generates additional environmental benefits (e.g., Decarbonization Credits for Biofuels – CBIO, and Guarantee of Origin Certificate for Biomethane – CGOB) and encourages the use of diverse techniques to enhance the valorisation of this lignocellulosic residue.

## Conclusion

The results obtained so far indicate that hydrothermal pretreatment with water is the best option to utilize SCB as a substrate for methane production. When evaluating the energy potential of different valorisation routes for SCB, its use in boilers for thermal energy production stands out as the best option among the analysed scenarios. However, diversifying the use of bagasse is an important alternative for sugar-energy plants, since biofuel production generates environmental benefits for the company and promotes the advancement of innovative techniques in the sector to optimize SCB utilization.

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