EFFECT OF STEAM EXPLOSION PRE-TREATMENT SEVERITY ON THE ANAEROBIC DIGESTION PERFORMANCE OF BREWERY SPENT GRAIN

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ABSTRACT

Brewery Spent Grain (BSG) is the main by-product of beer production, but there are still challenges for obtaining value-added bioproducts from BSG to reach the industrial scale. Steam explosion (SE) pre-treatment is a potential strategy to increase the BSG digestibility in AD. In this regard, this study aimed to assess the influence of various severity factors on SE pre-treatment on the anaerobic digestion of BSG through Biochemical Methane Potential (BMP) tests. The results revealed that elevating the severity factor from 3.65 to 3.97 led to 11% biogas production increase. However, more extreme pre-treatment conditions resulted in a 20% biogas reduction.

Keywords: Beer Production. Biorefinery Concept. Inhibitory Compounds. Biogas. Methane.

1 INTRODUCTION

The beer sector is one of the largest in the world. The different stages of beer production generate various by-products, and the Brewery Spent Grain (BSG) is the main solid waste from the process, representing 85% of the waste generated [1,2]. However, the complex lignocellulosic biomass of BSG is still a limiting factor for the biological conversion processes to reach full scale. Therefore, it is necessary to apply a pre-treatment to increase the digestibility of BSG during anaerobic digestion (AD).

Among available pre-treatments, steam explosion (SE) stands out as a highly efficient and easily scalable method for industrial applications. This technique has proven successful in treating a variety of lignocellulosic waste materials, with findings indicating its efficacy in breaking down organic matter [3,4]. However, it is important to note that hydrothermal pre-treatments under severe conditions can release compounds at concentrations that hinder microbial activity during AD.

The severity factor is a vital comparative metric for assessing the impact of hydrothermal pre-treatments across various experimental conditions. Thus, this study aims to evaluate how different severity factors influence the SE pre-treatment of BSG to utilize the BSG hydrolyzate in anaerobic digestion.

2 MATERIAL & METHODS

The BSG was collected at the Heineken brewery (Pacatuba, CE, Brazil). After collection, the material was washed and frozen at 4 ºC. The hydrolyzate was obtained after subjecting the material to SE pre-treatment under different operating conditions, which resulted in different severity factors, as shown in Table 1.

Table 1 – Experimental Conditions of Steam Explosion Pre-treatment of BSG.

Experimental Condition	SE-10	SE-15	SE-20
Temperature (°C)	185	201	214
Pressure (bar)	10	15	20
Time (min)	10	10	10
Severity Factor	3.5	3.97	4.36

Severity factor values were calculated using the following equation:

$$\text{Fator de Severidade} = \log[t\,.\exp\,(\frac{T-100}{14{,}75})]$$

Where t and T refer to the test time (min) and temperature (°C), respectively.

Subsequently, Biochemical Methane Potential (BMP) tests were carried out to evaluate the effect of different severity factors used to obtain the hydrolyzate via pre-treatment by SE in anaerobic digestion, especially in the volumetric production of biogas. The experiments were carried out in triplicate in 250 mL borosilicate bottles, with 125 mL being the working volume and 125 mL headspace.

The substrate was of a mixture of 50% raw BSG and 50% hydrolyzed (gVS/gVS). Furthermore, the reaction medium consisted of the inoculum (sludge from an anaerobic brewery wastewater treatment plant) and a solution of macro and micronutrients in a substrate/inoculum ratio (S/I) of 0.5 and total solids content (TS) of 10%. The pH of the medium was corrected to 7.0 using HCl or NaOH. The basal medium was buffered with sodium bicarbonate. In addition to the working reactors, two control groups were also adopted: an endogenous control (inoculum) and a positive control (inoculum and glucose).

The reactors were sealed with butyl rubber stoppers and purged with N_2 for 1 minute. The BMP assay was conducted in a shaker incubator (MA-420, Marconi LTDA, Brazil) at 37 °C with orbital shaking at 150 rpm for 80 days. Biogas production was quantified by measuring the gauge pressure in the reactor. The analysis of biogas composition was conducted utilizing gas chromatography employing a gas chromatograph featuring dielectric barrier ionization discharge detection (GC-BID) (GC BID-2010 Plus, Shimadzu Corporation, Japan). This instrument was equipped with a Select Biodiesel GC Column (15 m x 0.32 mm) manufactured by Agilent Technologies Inc. (USA). Additionally, Total Organic Carbon (TOC) levels were assessed at the initial and conclusion of the experimental period, following the recommendations of standard methods [5].

3 RESULTS & DISCUSSION

In Figure 1, it is observed that the SE-15 condition yielded the highest cumulative biogas production, while the SE-10 reactors attained a cumulative production equivalent to that of the positive control ($p < 0.05$) (Table 2). During the initial five days of operation, SE-10, SE-15, and the positive control exhibited comparable slopes, indicating that steam explosion pre-treatment facilitated the solubilization of the complex organic matter of BSG. This led to a short latency phase, as when using the readily available substrate (glucose) on the positive control.

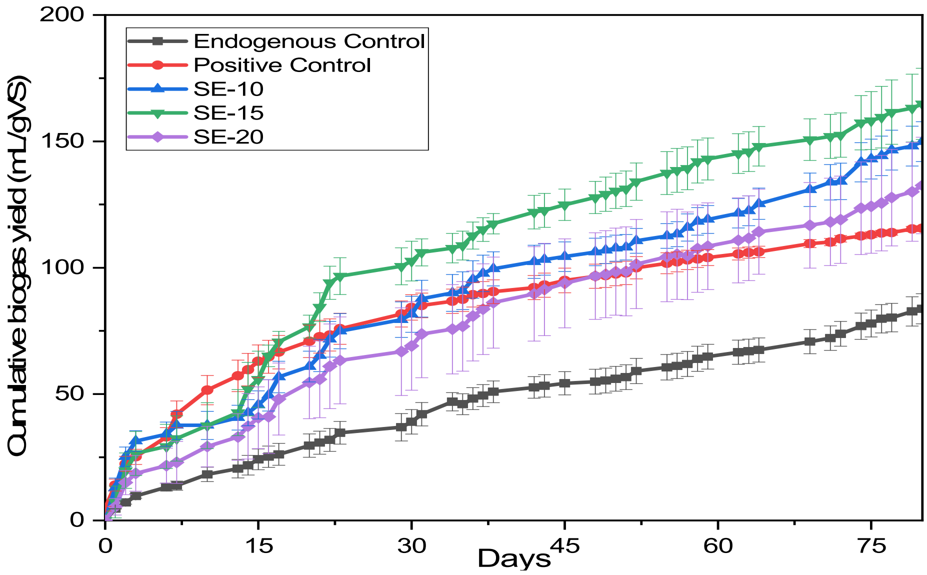

Figure 1 – Cumulative biogas yield throughout BMP assays.

SE pre-treatment results in rapid alterations in the cell wall structure, generating a soluble fraction abundant in sugars originating from hemicellulose [3]. As the pre-treatment severity factor is increased, there is a significant increase in the monomeric sugars produced from polysaccharide molecules degraded in pre-treatment, favoring the bioconversion of organic matter into biogas [6]. In the BMP assays, elevating the severity factor from 3.5 to 3.97 yielded a favorable outcome, resulting in an 11% increase in the final biogas production (Table 2).

Table 2 – Total organic carbon soluble, biogas, and biogas composition.

Reactor	TOC (mg/L)		Biogas (mL/gVS)	Percentage of methane (%)
	Initial	Final		
Endogenous Control	696	5418	83.7a	57a
Positive Control	1,735	729	115.6a,b	56.7a
10bar	1,445	6,871	148.9b,c	60a
15bar	1,653	6,368	164.8c,d	69b
20bar	1,782	9,913	132.5b,c	53a

However, increasing the severity factor from 3.97 to 4.36 resulted in a 20% drop in biogas production. Fibers rupture of the lignocellulosic structure under extreme conditions can also result in the release of furanic compounds and organic acids that can inhibit microbial activity in high concentrations [7,8]. Therefore, the lower biogas production in the condition with the substrate treated with the highest severity factor (SE-20) is possibly linked to the presence of higher concentrations of compounds arising from the breakdown of the lignocellulosic matrix during SE pre-treatment, such as furfural and hydroxymethylfurfural (HMF). These compounds harm bacterial cells and decrease microbial activity during AD [9].

Furthermore, increasing the severity factor can also result in a higher concentration of residual lignin. The compound's presence limits biomass bioconversion since lignin has a complex molecular structure that makes it resistant to microbial attack [4]. Concomitantly, lignin breakdown during AD produces phenolic compounds, such as *p*-cresol, which can also negatively impact methanogenesis and significantly decrease methane production [10].

Regarding organic matter, the positive control group attained a removal efficiency of 58% (Table 2), consistent with the production of biogas rich in methane throughout the experiment. Total Organic Carbon (TOC) removal indicates that the anaerobic digestion process was efficient, as soluble organic matter was converted into methane [11]. In conditions SE-10, SE-15, and SE-20, the increase in final TOC indicates that complex organic matter was converted into soluble organic matter during hydrolysis but not completely transformed into biogas during AD. Soluble organic matter accumulation was even more pronounced in the SE-20 reactor, corroborating the results regarding lower biogas production. In the endogenous control group, the increase in final TOC values is likely related to cell lysis resulting from the absence of substrate available in the medium.

Following the trend of previous results, the biogas composition in the SE-15 condition was superior to the others, while the SE-20 reactors obtained biogas with the lowest concentration of CH_4. Despite the lower values in the condition with the highest severity factor, all results obtained were consistent with what was expected in anaerobic digestion (50-70%) [12].

4 CONCLUSION

Steam explosion pre-treatment promoted an increase in soluble organic matter available for AD in all severity factors used, but the effects were different in methane production. The results showed that increasing the pre-treatment severity factor from 3.65 to 3.97 increased methane production by 11%, but when increasing the factor to 4.36, production fell by 20%. The hydrolyzate produced in the most extreme pre-treatment condition may contain high concentrations of compounds that can inhibit microbial metabolism and impair anaerobic digestion performance.

REFERENCES

[1] MUSSATTO, S. I., DRAGONE, G., ROBERTO, I. C. Brewers' spent grain: Generation, characteristics and potential applications. Journal of Cereal Science vol. 43 1–14 (2006).

[2] ZUPANČIČ, G. D., ŠKRJANEC, I., MARINŠEK LOGAR, R. Anaerobic co-digestion of excess brewery yeast in a granular biomass reactor to enhance the production of biomethane. Bioresour. Technol. 124, 328–337 (2012).

[3] HOANG, A. T. *et al.* Steam explosion as sustainable biomass pre-treatment technique for biofuel production: Characteristics and challenges. Bioresour. Technol. 385, 129398 (2023).

[4] BATISTA, G., SOUZA, R. B. A., PRATTO, B., DOS SANTOS-ROCHA, M. S. R., CRUZ, A. J. G. Effect of severity factor on the hydrothermal pre-treatment of sugarcane straw. *Bioresour. Technol.* 275, 321–327 (2019).

[5] AMERICAN PUBLIC HEALTH ASSOCIATION. Standard methods for the examination of water and wastewater. (American Public Health Association, 2012).

[6] RUIZ, H. A. *et al.* Severity factor kinetic model as a strategic parameter of hydrothermal processing (steam explosion and liquid hot water) for biomass fractionation under biorefinery concept. Bioresour. Technol. 342, 125961 (2021).

[7] PANJIČKO, M. *et al.* Biogas production from brewery spent grain as a mono-substrate in a two-stage process composed of solid-state anaerobic digestion and granular biomass reactors. J. Clean. Prod. 166, 519–529 (2017).

[8] LECH, M., LABUS, K. The methods of brewers' spent grain treatment towards the recovery of valuable ingredients contained therein and comprehensive management of its residues. Chem. Eng. Res. Des. 183, 494–511 (2022).

[9] LIN, R. *et al.* Inhibitory effects of furan derivatives and phenolic compounds on dark hydrogen fermentation. Bioresour. Technol. 196, 250–255 (2015).

[10] SEŽUN, M., GRILC, V., ZUPANČIČ, G. D., LOGAR, R. M. Anaerobic digestion of brewery spent grain in a semi-continuous bioreactor: Inhibition by phenolic degradation products. Acta Chim. Slov. 58, 158–166 (2011).

[11] MEEGODA, J. N., LI, B., PATEL, K. & WANG, L. B. A Review of the Processes, Parameters, and Optimization of Anaerobic Digestion. Int. J. Environ. Res. Public Heal. *2018, Vol. 15, Page 2224* 15, 2224 (2018).

[12] ROMERO, H. I. *et al.* Comparison of the methane potential obtained by anaerobic codigestion of urban solid waste and lignocellulosic biomass. Energy Reports 6, 776–780 (2020).

ACKNOWLEDGEMENTS

The authors thank the support given by the Brazilian institutions: National Council for Scientific and Technological Development – CNPq; Higher Education Personnel Improvement Coordination – CAPES; and National Institute of Science and Technology in Sustainable Sewage Treatment Stations – INCT ETEs Sustentáveis.