ASSESSMENT OF THE BIOGAS PRODUCTION POTENTIAL FROM HOP CROP RESIDUES

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ABSTRACT

The Brazilian energy sector has stood out in the use of renewable sources. Therefore, the search for new biomass or even improvements in the production chains of consolidated biorefineries is of great relevance. Lignocellulosic waste from hop cultivation is considered an environmental problem due to several metabolites that interfere with their biodegradability. In this sense, this work aims to add value to the hop chain by evaluating the Biochemical Methane Potential (BMP) of this residue, in a biorefinery context. Studies of the operational conditions of organosolv pretreatment with crude glycerol, varying time, temperature, and glycerol concentration, were carried out to maximize cellulose production. The black liquor, rich in hemicellulose, lignin, and glycerol was evaluated in anaerobic digestion (AD) studies. The experiments were carried out before and after a detoxification step to assess the inhibitory effect on the process, aiming at the lignin removal. The results showed that detoxified liquors greater CH_4 production when compared to non-detoxified liquors, in all conditions evaluated.

Keywords: pretreatment. methane. detoxification. inhibitors.

1 INTRODUCTION

The valorization of agro-industrial residues and by-products in biorefineries has garnered global attention[1]. Among lignocellulosic biomasses, hop (*Humulus lupulus L.*) crop residue demonstrates attractiveness in the country since Brazil is the third-largest beer producer in the world, with the brewing industry accounting for 2% of the national GDP[2]. Hop crop residues have no commercial value and, due to improper and inefficient disposal, have become an environmental problem, as they are not effective in soil coverage[3]. For the application of this biomass in a biofuel biorefinery, a pretreatment step is necessary to fractionate the macromolecular components, reducing the material's recalcitrance and facilitating its use in biofuel production. The crude glycerol organosolv pretreatment have demonstrated great attractiveness because they promote such effects on biomass at a low cost. Additionally, the polar structure of glycerol allows for rapid penetration into the lignocellulosic biomass fiber, providing effective conditions for delignification and hemicellulose solubilization[4]. A potential application for the hemicellulosic fraction of the biomass, rich in pentoses and glycerol, is the production of biogas, a process that treats organic substrates through a diverse microbial community under anaerobic conditions, primarily generating methane and carbon dioxide, along with other trace gases. Besides biogas, this process produces biofertilizers, which can be used to enhance agricultural productivity[5]. However, during pretreatment, potential fermentative inhibitors may be produced due to the breakdown of the lignocellulosic structure, which can negatively affect microbial activity and viability[6]. To mitigate this issue, a detoxification step can be applied to the liquor to reduce the concentration of these inhibitors and thereby improve biogas production. In this context, aiming to contribute to the study of hop crop residue valorization, this work seeks to evaluate the effect of the detoxification step on the hemicellulosic liquor obtained post-crude glycerol organosolv pretreatment of hop crop residues on the Biochemical Methane Potential (BMP).

2 MATERIAL & METHODS

The hop residues were provided by LUPAM (Research Group – Hops: Applications and Management) located at Unesp, Botucatu Campus. The mature Cascade variety plants were obtained after 3 years of cultivation in a certified nursery. After harvesting, the material was air-dried at room temperature and the biomass was ground in a hammer mill (MA600/CF, Marconi) to obtain a material with a particle size of less than 6 mesh (1.230 mm). The material was stored in plastic bags to be used in experimental assays and for chemical characterization according to the methodology of Sluiter et al[7]. The inoculum used in the biodigestion processes was sourced from a Canadian model biodigester constructed with PVC canvas, located at Fazenda Campestre (São Pedro – SP), which utilizes waste from confined cattle.

The pretreatment assays were conducted using the organosolv process with crude glycerol, considering three operational conditions as presented in Table 1. These conditions were derived from a process optimization study conducted by the research group, selecting distinct conditions for delignification and hemicellulose solubilization. The reactions were carried out in 500 mL 316L stainless steel reactors with 10% solids (w/w) under the conditions described in Table 1, in triplicate. The reactors were immersed in a heating bath (MA 159/BB, Marconi), using glycerin as the thermal fluid, according to each pretreatment condition. After the reaction time, the reactors were cooled in an ice bath, opened, and the slurry (solid + liquid mixture) was separated by filtration. The liquors were stored under refrigeration for detoxification assays, anaerobic biodigestion (AD), and chemical characterization according to the methodology described by Nakasu et al.[8].

Tabela 1 Pretreatment conditions.

Assay	Time (min)	Temperature (°C)	Glycerol:water ratio
1	15	140	30:70
2	30	155	50:50
3	45	170	70:30

The AD assays were evaluated using both detoxified and non-detoxified liquor. For the detoxification step, the liquor was diluted with water at a 1:1 ratio to promote lignin precipitation in the medium. Afterward, the samples were centrifuged (NT 820, Nova Técnica) for 15 min at 2500 rpm to remove the precipitate. AD assays were conducted in quintuplicate, using the German methodology VDI 4630[9]. The experiments were carried out in 100 mL penicillin bottles, maintaining a 40% headspace. Inoculum and substrate were added to ensure a 2:1 ratio of VS (volatile solids) of inoculum to substrate. To achieve this, a series of analyses were performed for total solids (TS), volatile solids (VS), and t fixed solids (FS), following the methodology described by APHA[10]. In addition to the liquors, microcrystalline cellulose was used as a positive control, and the inoculum was used as a negative control. Biogas production was monitored daily to calculate the accumulated volume of biogas produced and methane concentration was determined using gas chromatography (PerkinElmer model Clarus SQ8T) [11].

A modified stacked sigmoidal function (Equation 1), based on Boltzmann double sigmoid[11], was used for modeling CH_4 volumetric production in time. The mathematical adjustment was proposed to better predict the behavior of the residues about microbial community in the production of biogas since a co-digestion process was carried out and a residue that has not been reported in the literature is being used AD systems. Based on the parameters that the model provided, it was possible to obtain better conclusions regarding the BMP of the residues.

$$V_{CH4}^{STP}(t) = V_{CH4}^{max} * \left[\frac{p}{1 + e\left(\frac{4r_1 * (t_1 - t)}{V_{CH4}^{max} * p}\right)} + \frac{1 - p}{1 + e\left(\frac{4r_2 * (t_2 - t)}{V_{CH4}^{max} * (1 - p)}\right)} \right] \quad (1)$$

where V_{CH4}^{STP} is the specific CH_4 production in time (NmLCH_4 g/VS), V_{CH4}^{max} is the maximum specific volumetric production reached in the experiment (NmLCH_4/g VS), *p* is the proportion between ordinate values of the first and second stacked sigmoid, t_1 and t_2 are the time which the production of the first and second sigmoidal pattern reaches the maximum rate (d), and *r1* and *r2* are the maximum rate of CH_4 production for the first and second sigmoidal pattern, respectively (NmLCH_4/g VS.d).

3 RESULTS & DISCUSSION

Table 2 presents the chemical composition of the liquor obtained under each pretreatment condition, both before and after the detoxification step. Since the detoxified liquor was diluted once during the detoxification step, for the purpose of comparing the data regarding the extraction of compounds, the concentrations of the detoxified liquor, determined chromatographically, were adjusted to account for this dilution.

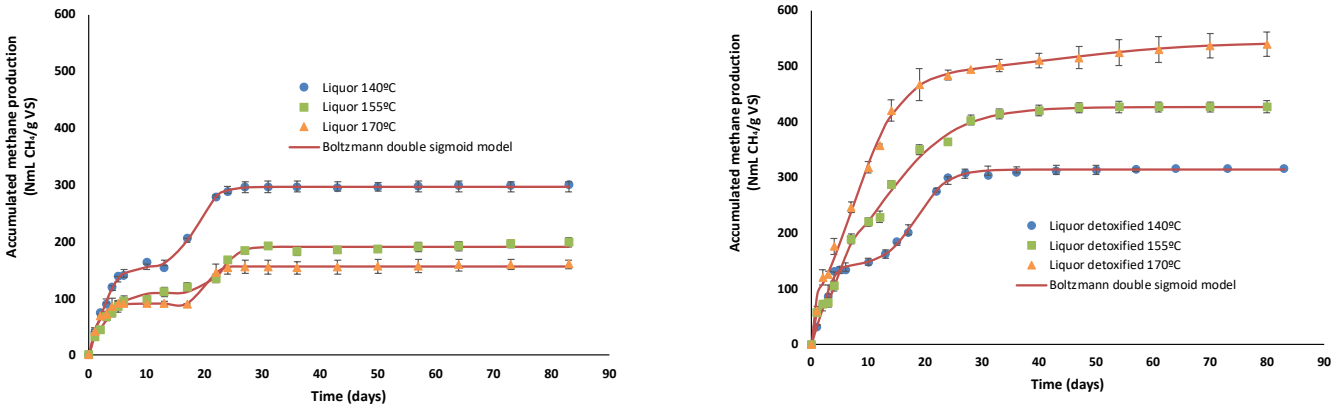
Table 2 Chemical composition of the liquor before and after the detoxification step.

Concentration (g/L)	Liquor					
	140ºC, 15 min, 30:70		155ºC, 30 min, 50:50		170ºC, 45 min, 70:30	
	Non-detoxified	Detoxified	Non-detoxified	Detoxified	Non-detoxified	Detoxified
Xylose	5.63	4.94	3.61	3.30	2.00	1.90
Arabinoxylan	0	0	0	0	1.24	2.44
Glucose	5.29	4.8	4.52	4.51	2.87	2.61
Glucan	7.45	5.73	8.99	4.66	9.60	7.10
Arabinose	0.24	0.16	0.43	0.26	0	0
Formic acid	0	0	0	0	0	0
Acetic acid	2.17	1.86	4.23	4.12	6.95	7.16
Levulinic acid	0	0	0	0	0	0
Furfural	0.008	0.008	0.012	0.012	0.018	0.016
HMF	0.001	0.001	0.002	0.002	0.006	0.005
Phenolic compounds	6.45	6.14	9.92	6.32	14.51	7.60
Glycerol	23.78	21.38	33.08	33.5	48.79	48.91

Analyzing Table 2, it is possible to observe decreasing concentrations of monomeric sugars (glucose and xylose) from the mildest pretreatment condition (140ºC, 15 min, and 30:70 glycerol:water) to the severest (170ºC, 45 min, and 70:30 glycerol:water). This is justified by the fact that the severity of pretreatment promotes the degradation of these compounds and the formation of other products such as HMF and furfural. The more severe the pretreatment condition, the higher the concentration of phenolic compounds in the medium. Regarding the detoxification step, there is a similarity in the concentration of sugars and glycerol present in the medium, indicating that there was no significant precipitation/removal of these compounds during detoxification. On the other hand, there is a significant reduction in the concentration of phenolic compounds in the medium, especially in the detoxified liquor from the severest condition, which resulted in a 47.6% reduction of phenolic compounds in the medium.

Figure 1 shows the accumulated methane production for each of the trials, with the process monitored over 83 days.

Figure 1 Methane production in the anaerobic digestion of non-detoxified (a) and detoxified (b) liquors.



(a) (b)

After detoxification, a significant methanogenic potential was observed in the liquor. For the most severe pretreatment condition (170ºC, 45 min, and 70:30 glycerol:water), methane production reached 540.3 ± 31.3 NmL CH_4/g VS. Detoxification led to an increase in BMP compared to non-detoxified liquor, with methane production 70% lower in the raw state, highlighting the substantial negative influence of phenolic compounds on the AD process. Mathematical modeling using the Boltzmann double sigmoid model showed a good fit for all studied conditions, with an R^2 of 0.99 in all cases. This model, typically applied in anaerobic co-digestion processes, suggests that although the substrate was singular (liquor), its different components (glycerol, sugars, and phenolic compounds) were consumed at different stages of the process.

4 CONCLUSION

The detoxification of the liquor, with the addition of water as an anti-solvent, resulted in a removal of 47.6% of phenolic compounds under the most severe pretreatment condition (170ºC, 45 min, and 70:30 glycerol:water). In this same pretreatment condition, methane production of 159.9 ± 8.1 NmL CH_4/g VS was observed using the non-detoxified liquor after 83 days of fermentation. Upon detoxification of the same liquor, methane production increased to 540.3 ± 31.3 NmL CH_4/g VS, which is 3.3 times higher, demonstrating the positive effect of phenolic compound removal on the AD process.

REFERENCES

1. Rabelo SC, Brenelli LB, Pin TC, Scopel E, da Costa AC. Pretreatments as a key for enzymatic hydrolysis of lignocellulosic biomass. In: Polysaccharide Degrading Biocatalysts: Foundations and Frontiers in Enzymology. Academic Press; 2023:109–137. doi:10.1016/B978-0-323-99986-1.00003-X
2. MAPA. LÚPULO NO BRASIL: PERSPECTIVAS E REALIDADES. Vol 1. Ministério da Agricultura, Pecuária e Abastecimento; 2022.
3. Kanai N, Nishimura K, Umetani S, et al. Upcycling of Waste Hop Stems into Cellulose Nanofibers: Isolation and Structural Characterization. ACS Agric Sci Technol. Published online 11 de junho de 2021:acsagscitech.1c00041. doi:10.1021/acsagscitech.1c00041
4. Novo LP, Gurgel LVA, Marabezi K, Curvelo AAS. Delignification of sugarcane bagasse using glycerol-water mixtures to produce pulps for saccharification. Bioresour Technol. 2011;102(21):10040–10046. doi:10.1016/j.biortech.2011.08.050
5. Gao Z, Alshehri K, Li Y, et al. Advances in biological techniques for sustainable lignocellulosic waste utilization in biogas production. Renew Sustain Energy Rev. 2022;170:112995. doi:10.1016/J.RSER.2022.112995
6. Camargo FP, Rabelo CABS, Duarte ICS, Silva EL, Varesche MBA. Biogas from lignocellulosic feedstock: A review on the main pretreatments, inocula and operational variables involved in anaerobic reactor efficiency. Int J Hydrogen Energy. 2023;48(54):20613–20632. doi:10.1016/J.IJHYDENE.2023.02.073
7. Sluiter JB, Chum H, Gomes AC, et al. Evaluation of Brazilian sugarcane bagasse characterization: An interlaboratory comparison study. J AOAC Int. 2016;99(3):579–585. doi:10.5740/jaoacint.15-0063
8. Nakasu PYS, Ienczak LJ, Costa AC, Rabelo SC. Acid post-hydrolysis of xylooligosaccharides from hydrothermal pretreatment for pentose ethanol production. Fuel. 2016;185:73–84. doi:10.1016/j.fuel.2016.07.069
9. VDI 4630. Fermentation of organic materials - Characterization of the substrate, sampling, collection of material data, fermentation tests.; 2016. Acessado dezembro 1, 2022. https://www.vdi.de/richtlinien/details/vdi-4630-fermentation-of-organic-materials-characterization-of-the-substrate-sampling-collection-of-material-data-fermentation-tests
10. APHA. Standard Methods for the Examination of Water and Wastewater,. 23rd Editi.; 2017. Acessado abril 14, 2022. https://engage.awwa.org/PersonifyEbusiness/Store/Product-Details/productId/65266295
11. Volpi MPC, Brenelli LB, Mockaitis G, Rabelo SC, Franco TT, Moraes BS. Use of lignocellulosic residues from second-generatiton ethanol production to enhance methane production through co-digestion. Bioenergy Res. Published online 2021. doi:https://doi.org/10.1007/s12155-021-10293-1

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