Understanding Sugarcane Bagasse Hemicellulosic Hydrolysate: Enhancing Biogas and Biomethane Production

Araújo, S.C.*, Alcântara, L.F.Q.S.A.L.*, Veras, S.T.S.*, Kato, M.T.*, Florêncio, L.*
*Federal University of Pernambuco, Department of Civil and Environmental Engineering.
sayonara.araujo@ufpe.br; flor@ufpe.br

Highlights:

- Furfural enhances biogas and $BioCH_4$ yields by up to 53.9% and 72.6%, respectively, in the presence of xylose.
- Optimal furfural concentrations (0.6 and 1.2 $g.L^{-1}$) achieve close to theoretical BioCH4 production potential; higher concentrations (2.4 $g.L^{-1}$) inhibit production.
- Conversion of furfural to acetic acid boosts biogas and $BioCH_4$ yields; extended microbial adaptation improves tolerance and efficiency.

Keywords: Xylose; furfural; batch test.

INTRODUCTION

Brazil is the world's second-largest producer of biofuels, especially ethanol, and its energy matrix is largely composed of renewable sources Currently, the country is home to the only global company operating E2G plants on an industrial scale, playing a crucial role in the energy transition. This company operates two plants at full capacity: the first, located in the Costa Pinto Biopark in Piracicaba (SP), which reached a production of 30,000 m³ in the 2023/2024 harvest; and the second, situated in the Guariba Biopark, which began operations in 2024 as the largest E2G plant in the world (Raízen, 2024).

Despite advancements in E2G production, significant challenges remain, particularly with the utilization of the C5 fraction, such as xylose (Xyl), and the presence of inhibitors like furfural (FF) in sugarcane bagasse hemicellulosic hydrolysate (SBHH) generated during the pre-treatment of bagasse or straw for E2G production (Oliveira Pereira *et al.*, 2024; Nascimento *et al.*, 2023). These challenges affect biotechnological processes. These issues hinder biotechnological processes such as fermentation, anaerobic digestion. To address these challenges, plants have implemented specific inhibitor mitigation strategies, including detoxification methods like physical separation, chemical treatments, and biological approaches involving adapted microorganisms (Oliveira Pereira *et al.*, 2024)

In this context, several studies have focused on evaluating the effects of inhibitors and using C5 sugars for E2G production (de Oliveira Pereira *et al.*, 2024; Nascimento *et al.*, 2023), and more recently for biohydrogen production (Siqueira & Reginatto, 2015; Sá et al., 2013). However, biogas and biomethane production have been underexplored so far. So far, only the studies Barakat *et al.*, (2012) and Sun *et al.*, (2019) have been reported in the literature.

Therefore, further research is needed to elucidate these aspects and advance significantly in this field. It is essential to understand the impacts of SBHH components, such as Xyl and FF, to optimize biogas and biomethane ($BioCH_4$) production. This requires a more detailed analysis of the level of inhibition, synergistic or antagonistic effects, microbial tolerance mechanisms, and the environmental and operational factors involved. This study aims to investigate the impacts of Xyl and FF present in SBHH on the production of biogas and $BioCH_4$. Specifically, the study seeks to understand the level of inhibition caused by these compounds and identify potential synergistic or antagonistic effects.

METHODOLOGY

Inoculum: Anaerobic granular sludge (AGS) is sourced from an internal circulation anaerobic reactor used for treating brewery wastewater. The AGS underwent elutriation, and characterization, and was then added to the reactors at an initial concentration of 1.0 g $VS.L^{-1}$.

Biodigestion tests: Batch tests were conducted in triplicate, using varying concentrations of FF (0.0, 0.6, 1.2, and 2.4 $g.L^{-1}$ as controls) and the same concentrations in combination with 5 $g.L^{-1}$ of Xyl. Glass 320 mL flasks with a 215 mL working volume were used as reactors. The initial pH was adjusted 7.0 by adding 40% (m/m) NaOH or 1 M HCl. Sodium bicarbonate (1g $NaHCO_3.g^{-1}$ added), macronutrients (Siqueira & Reginatto, 2015) and micronutrients (Sá *et al.*, 2013) were added, along with yeast extract and resazurin as well as yeast extract and resazurin, according to (Florencio *et al.*, 1993). The flasks were sealed with a lid lined with isobutylene-isoprene rubber, and the headspace was purged with nitrogen gas for 2 min to ensure anaerobic conditions. The reactors were incubated in a 30 ± 1°C room with a shaker at 130 rpm.

Analytical methods: High-performance liquid chromatography (HPLC) equipment (Shimadzu, Agilent 1100 series) was utilized to quantify Xyl, employing a refractive index detector (RID-20A, Shimadzu). FF was identified and quantified using gas chromatography with ethyl ether extraction. Gas chromatography with flame ionization detection (GC-FID) was performed using model 7890A from Agilent Technologies.

The analytical composition of the biogas (H_2, CH_4, and CO_2) was determined using a gas chromatograph (GC-2014, Shimadzu Scientific). The volume of biogas was measured using a glass syringe (Luer Metal Arti Glass) (Owen *et al.*, 1979) The cumulative volume produced was determined by (Logan *et al.*, 2002) converted to Normal Temperature and Pressure Conditions (NTPC), i.e. 0 °C or 273 K and 1 atm. The digestion was stopped when the daily Biogas production fell below 1% of the accumulated Biogas volume.

RESULTS AND CONCLUSIONS

The inhibitory effect of furfural is well documented. However, in this study, with 5 $g.L^{-1}$ of Xyl, adding furfural (FF) at concentrations of 0.6, 1.2 and 2.4 $g.L^{-1}$ increased the biogas yield by 31.0%, 48, 7%, and 53.9% (Figure 1a). Compared to the control (Xyl without FF), the $BioCH_4$ yield increased by 55.3%, 61.1%, and 72.6% (Figure 1b). It is noteworthy that the accumulated $BioCH_4$ production ($BioCH_4.g_{substrate}^{-1}$) observed (Table 1) in the controls (5 Xyl, 0.6 FF, 1.2 FF, and 2.4 FF) represented 72.8%, 84.1%, 97.9%, and 19.2% of the theoretical potential, respectively. In the combinations of xylose with furfural (5.0 $g.L^{-1}$ Xyl + 0.6, 1.2, and 2.4 $g.L^{-1}$ FF), the production represented 94.9%, 82.9%, and 69.1% of the theoretical $BioCH_4$ potential, in that order. It can be observed that furfural at concentrations of 0.6 and 1.2 $g.L^{-1}$ can be used as the sole carbon source and, when combined with Xyl, results in an accumulated production relatively close to the theoretical potential. Conversely, furfural at high concentration (2.6 g.L^{-1}) resulted in lower production for both the control and the mixture, which may indicate partial inhibition of BioCH$_4$ production.

Figure 1. Accumulated Biogas (a) and BioCH$_4$ (b) Yield.

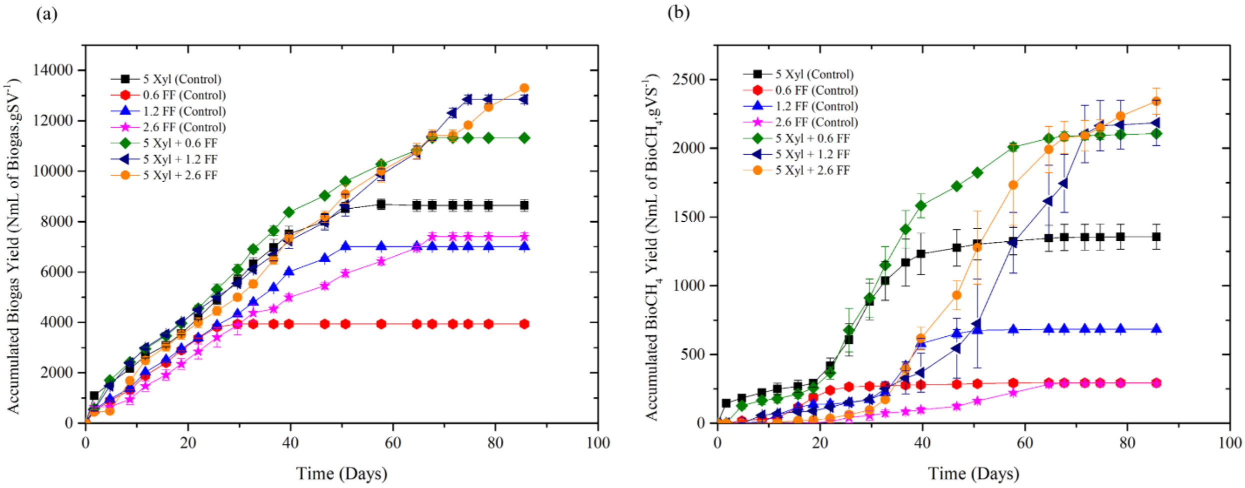

Table 1. Accumulated BioCH$_4$ production relative to theoretical potential.

Essay	**Theoretical biomethane potential (NmL BioCH$_4$.g$_{substrate}$$^{-1}$)**	**Accumulated BioCH$_4$ (NmL BioCH$_4$.g$_{substrate}$$^{-1}$)**	**Percentage of Theoretical (%)**
5 Xyl (Control)	400.9	292.1	72.8
0.6 FF (Control)	75.2	63.2	84.1
1.2 FF (Control)	150.4	147.2	97.9
2.6 FF (Control)	325.8	62.6	19.2
5 Xyl + 0.6 FF	476.1	452.1	94.9
5 Xyl + 1.2 FF	551.3	457.5	82.9
5 Xyl + 2.6 FF	726.8	502.7	69.1

The observed increase in this study may be linked to two main factors: first, the fact that furfural can be completely converted into furfuryl alcohol, which can subsequently be transformed into furoic acid and, finally, into acetic acid (Sun *et al.*, 2019). It is known that acetic acid is a key intermediate in the production of biogas and BioCH$_4$. Second, the duration of the experiment (85 days) may have allowed for a more prolonged adaptation of the microbial community, resulting in increased tolerance to FF and, consequently, its conversion into biogas and BioCH$_4$.

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