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Combined fungal and chemical pretreatment of lignocellulosic biomass for biogas production: effect of pretreatment order and fungal strains

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Abstract: The recalcitrance of lignocellulosic poses a challenge for conversion to biogas and therefore, pretreatment is an essential step in addressing this. Combining fungal pretreatment with a biomimetic system, a mild Fenton reaction, was studied to improve the methane production from straw. The pretreatment conditions studied were able to reduce lignin content and cause an increase in the relative polysaccharides content. Interestingly, variations among the strains of the same species were observed in their ability to improve the biomethane yield. The order of pretreatment combination also played an important role as it affects the mechanism of action of subsequent pretreatment on the biomass, which indirectly affects the biogas yield.

Keywords: *Pleurotus ostreatus*; Fenton pretreatment; Straw

Introduction

An important research topic in biogas production from lignocellulosic biomass has been pretreatment because of the recalcitrant nature of biomass and technological bottlenecks. Lignin hampers the valorization of hemicellulose and cellulose, thereby preventing the carbon recovery of lignocellulosic biomass. The digestibility is improved by pretreating the biomass with physical, chemical, thermal, and biological methods. Although these pretreatment methods have successful implementations, they are either energy/chemical-intensive processes and/or require corrosion-resistant high-pressure reactors that increase the costs. Additionally, no pretreatment works for all biomass or at various scales. Therefore, there is a need to constantly improve current pretreatment methods or develop new technologies (Chen and Davaritouchaee, 2023; Van Der Made et al., 2023).

Combining fungal and chemical pretreatment has been shown to reduce the drawbacks of sole pretreatment while improving biofuel yields (Shirkavand et al., 2016). White rot fungi are well known for their unique oxidative enzyme system which is capable of mineralizing lignin. However, their uncertainty of performance and long reaction time make them limited in their application. Therefore, combining fungal with Fenton pretreatment which is a type of reaction used naturally by the fungi to oxidize the biomass, could help to improve the efficiency of the fungal pretreatment (Xie et al., 2017). Therefore, in this project, the efficiency of combined white rot fungal and Fenton pretreatment to improve the methane production from wheat straw is assessed against sole fungal pretreatment. The efficiency of two different strains of white rot fungi and the order of the sequential pretreatment is also evaluated.

Material and Methods

Sole fungal pretreatment (FP) was studied using two strains of *Pleurotus ostreatus*, namely PO3 and HK35. The efficiency of combined pretreatment was evaluated for both orders of sequential pretreatment, namely fungal followed by chemical pretreatment (FP+CP) and chemical followed by fungal pretreatment (CP+FP). The chemical pretreatment was carried out using low-level Fe^{2+} and H_2O_2 at pH 3 for the Fenton reaction to occur. The biomethane potential (BMP) test was carried out in an Automatic Methane Potential Test System-II (AMPTS) as described in Meenakshisundaram et al. (2023). A systematic study of physicochemical changes before and after pretreatment was carried out using techniques such as scanning electron microscopy (SEM), Simons' staining, elemental analysis (CHNSO), attenuated total reflectance (ATR), wide-angle X-ray diffraction (WAXD), Nuclear magnetic resonance spectroscopy (NMR), and thermogravimetric analysis (TGA).

Results and Conclusions

Biomethane Potential (BMP) Test

From Figure 1, it can be observed that sole fungal pretreatment (FP) with PO3 was seen to improve the biomethane yield by 27.5%, whereas there was no improvement when treated with the HK35 strain. Therefore, even though PO3 and HK35 are two different strains of the same species, *Pleurotus ostreatus*, there is a difference in their efficiency in degrading biomass and improving the BMP. However, subsequent chemical pretreatment (FP+CP) helped to improve the BMP for the HK35 strain by 22.6%. For both strains, the chemical followed by fungal pretreatment (CP+FP) was less efficient than the converse order of pretreatments. This is because when Fenton pretreatment (CP+FP) is carried out first, carbohydrates is made more bioavailable for subsequent fungal pretreatment. Fungi use the readily available carbohydrates as carbon source for their growth and fruiting and therefore, a lesser substrate quantity is available for conversion to biogas. This resulted in the formation of a fruiting body in CP+FP samples. Therefore, the order of pretreatments in combined pretreatment and the strain of fungi used play an important role in the improvement of methane yield.

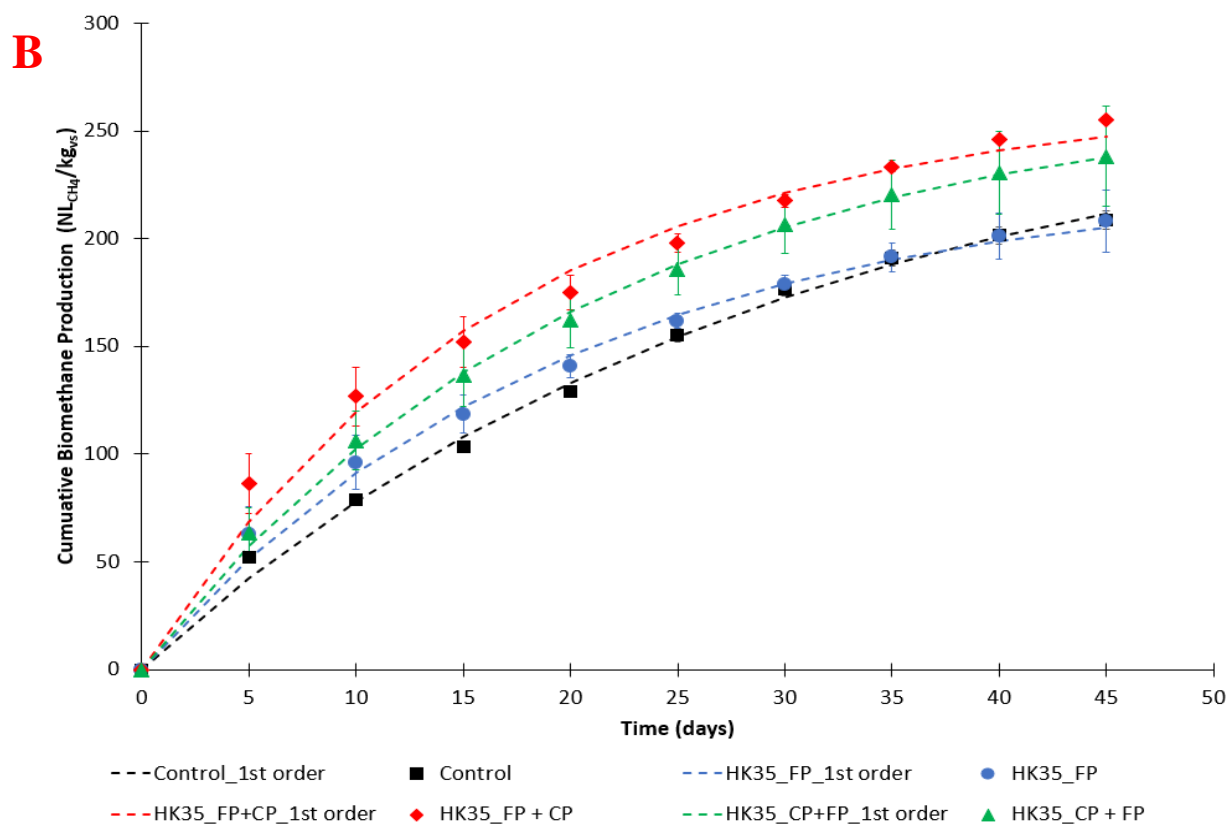
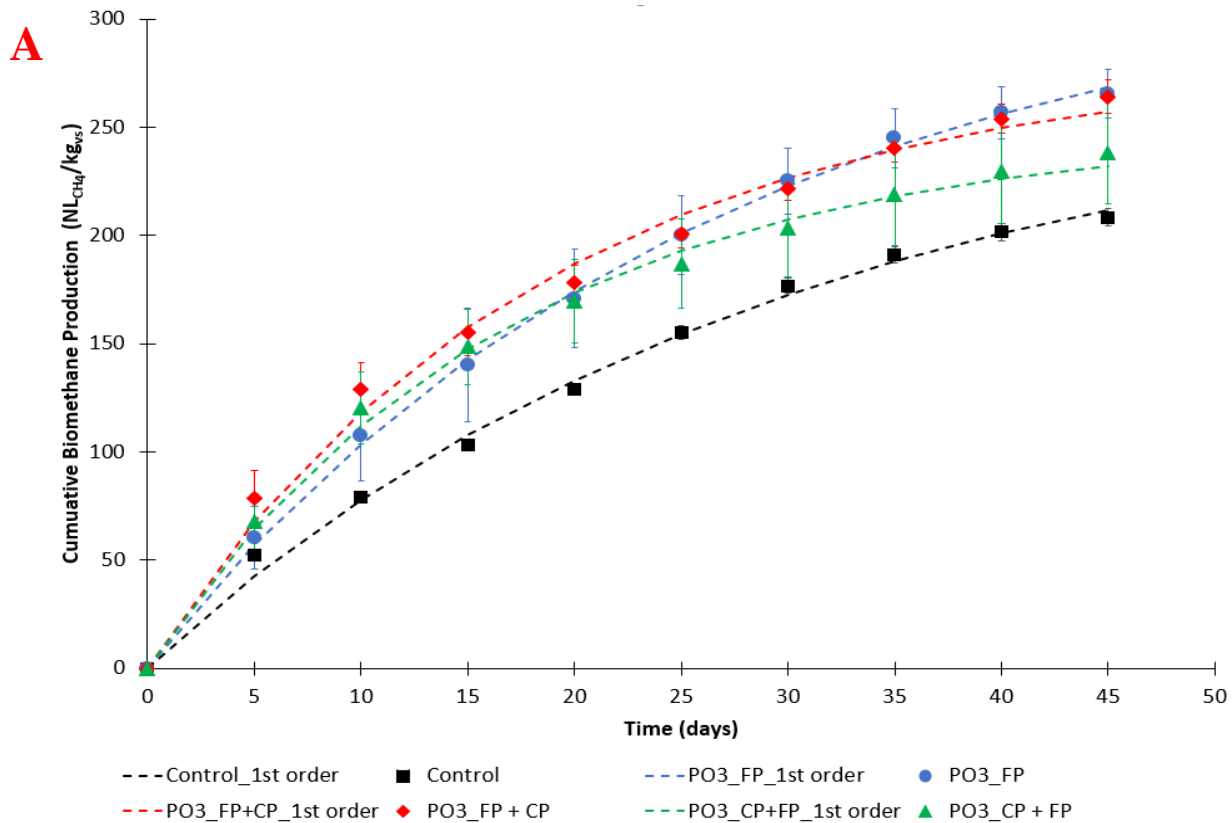


Figure 1 Cumulative biomethane production over time and its modelling for sole fungal pretreatment and combined fungal and chemical pretreatment of straw for the (A) PO3 and (B) HK35 strain.

Derivative thermogravimetry (DTG)

The first derivative of the thermogravimetric analysis (TGA) curve over temperature can be used to obtain the biomass composition (as shown in Table 1) by deconvolution using an asymmetrical Gaussian function. The relative lignin content is seen to decrease after pretreatments, while hemicellulose content increases. Overall, the relative percentage of polysaccharides (cellulose and hemicellulose) is seen to be higher for the PO3 strain, which could explain the higher BMP (Figure 1) obtained as compared to HK35.

Table 1: Composition of control and pretreated straw obtained using DTG deconvolution

| Sample name | % Moisture | % Hemicellulose | % Cellulose | % Lignin | % Residual Weight |
|-------------|------------|-----------------|-------------|----------|-------------------|
| Control | 3.69 | 19.66 | 31.54 | 28.44 | 16.67 |
| PO3_FP | 3.29 | 25.72 | 32.21 | 21.95 | 16.82 |
| PO3_FP+CP | 2.28 | 31.11 | 34.48 | 21.80 | 10.34 |
| PO3_CP+FP | 2.15 | 30.31 | 30.36 | 19.80 | 17.37 |
| HK35_FP | 3.93 | 22.40 | 31.29 | 20.61 | 21.77 |
| HK35_FP+CP | 3.26 | 31.91 | 30.76 | 18.76 | 15.32 |
| HK35_CP+FP | 2.74 | 28.85 | 27.78 | 20.70 | 19.92 |

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