Detoxification strategy of wheat straw hemicellulosic hydrolysate: an approach for cultivating *Trichoderma reesei.*

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Introduction

With a significant share in cereals and residues, wheat is one of the leading agricultural drivers for the European Union. In 2019, the production of 131.7 million tonnes of wheat species (common wheat and spelt) was reported, representing approximately 44 % of the total production of cereals in the EU (Eurostat, 2021). In consequence, wheat straw residues are produced during the harvesting stage, representing approximately 50% of total straws produced in Europe (Helin et al., 2012). Despite the several applications implemented for wheat straw (i.e., animal bedding and energy production), there is still a surplus of this residue that can be used for biorefining purposes.

The lignocellulosic nature of wheat straw makes it an interesting feedstock for obtaining several products, including C6 and C5 mono and oligosaccharides based-products (Serna-Loaiza et al., 2021). Hemicellulose hydrolysis is favoured because of its low molecular weight multibranched and amorphous structure. These characteristics make glycosidic bonds an easy target for pretreatment strategies. Liquid hot water (LHW) treatment is a hydrothermal treatment using water at high temperatures to deconstruct biomass. LHW releases monomeric and oligomeric sugars mainly from xylan fractions and in low amounts from glucan fractions. The obtained hydrolysates can be used as substrate for microorganisms cultivation (Ruiz et al., 2021; Serna-Loaiza et al., 2022; Tian et al., 2022). However, inhibitory compounds such as acetic acid, furfural and hydroxymethylfurfural (HMF) are produced during the pretreatment affecting the growth and metabolism of microorganisms.

In this regard, the definition of operational conditions and detoxification strategies are essential for process upstreaming in the valorization of the hemicellulosic fraction as a step for building a conceptual wheat straw biorefinery. This work aimed to evaluate a complete pretreatment strategy for producing a sugar solution, which is a suitable substrate for microorganism cultivation, using wheat straw as feedstock. The pretreatment strategy assessed a LHW extraction followed by a detoxification of the extract to remove acetic acid and furfural. Finally, as a proof of concept, the detoxified hydrolysate was used for the cultivation of *Trichoderma reesei*, a fungus used for the industrial production of enzymes, in particular cellulases (Shen et al., 2022; Siamphan et al., 2022).

Material and methods

Wheat straw hydrolysis and detoxification

Wheat straw was treated through LHW at 160°C for 90 min to produce a C5-sugars-rich hemicellulosic hydrolysate. Then, a detoxification strategy aimed to remove furfural and acetic acid was evaluated. The removal of these compounds was addressed by evaporation using the volatility differences as the driving force for the separation. The operational conditions, namely pressure and temperature, were selected based on simulation (ASPEN Plus V10.0), with a targeted furfural removal > 90%. To improve acetic acid removal, the influence of two parameters was evaluated over the detoxification performance. The first parameter was the evaporated fraction, considering 60% and 90% of volume evaporated. The second parameter was the modulation of pH through NaOH and H_2SO_4 addition to modify the distribution of acetic acid species in synthetic solutions.

Toxicity assays

Preliminary toxicity assays were performed to determine the maximum concentration of furfural, acetic acid, and HMF tolerated by *T. reesei*. Therefore, different concentration of the mentioned inhibitory compounds

were added to a synthetic medium using commercial glucose as sole carbon source for cultivation of *T. reesei*. The dry weight of biomass was measured after 72 h of cultivation.

T. reesei *cultivation on detoxified hydrolysate*

After the detoxification, *T. reesei* was grwon on seven differently composed media. The tested media included a control using glucose as sole carbon source, four synthetic and two hydrolysate solutions treated under different conditions. Detoxified synthetic and hydrolysate solutions were used as carbon source for *T. reesei* cultivation at 30°C for 96 h at pH 5, minor and macronutrients were added.

Analytics

Monomeric and oligomeric arabinose, xylose, glucose, galactose, mannose and fructose sugars were determined using high-performance anion-exchange chromatography with a pulsed amperometric detection (HPAEC-PAD). Degradation products (furfural, HMF and acetic acid) were determined by high-performance liquid chromatography with a refractive index detector (HPLC-RID). Biomass was determined by dry weight.

Results and Discussion

Wheat straw was submitted to LHW treatment producing a hydrolysate with 12 g/L total sugars (mono and oligomeric sugars), 1.7 g/L acetic acid, 409.5 mg/L furfural, and 26.6 mg/L HMF. The evaporation was performed at 55°C and 140 mbar. Through these conditions, 100% of the initial furfural mass was removed in all the cases and between 6.5 % and 44.8 % removal of the initial mass of acetic acid. The improvement in the reduction of acetic acid was reached by the modification of pH in the synthetic and hydrolysate solution.

According to the dry biomass and compared to the control, the solution which presented the better results was the real hydrolysate detoxified under acidified conditions with a higher evaporation rate (90%). This hydrolysate led to the highest fungal biomass, equivalent to 77% of the biomass produced in the control, compared to the 9 % of equivalent biomass obtained for the hydrolysate without treatment. The biomass obtained indicated the effectiveness of the detoxification strategy for wheat straw hydrolysate under the evaluated conditions.

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