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Ethanol production from glucose and xylose obtained from steam exploded water-extracted olive tree pruning using phosphoric acid as catalyst

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HIGHLIGHTS

- Steam explosion of olive tree pruning using H_3PO_4 was evaluated for the first time.
- High enzymatic hydrolysis and SSF yields were obtained with pretreated material.
- Glucose and xylose from olive tree pruning are jointly fermented for the first time.
- Ion-exchange resins resulted in ethanol yield similar to xylose control fermentations.
- About 160 g of ethanol from kg of olive tree pruning could be obtained.

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ABSTRACT

In this work, the effect of phosphoric acid (1% w/w) in steam explosion pretreatment of water extracted olive tree pruning at 175 °C and 195 °C was evaluated. The objective is to produce ethanol from all sugars (mainly glucose and xylose) contained in the pretreated material. The water insoluble fraction obtained after pretreatment was used as substrate in a simultaneous saccharification and fermentation (SSF) process by a commercial strain of *Saccharomyces cerevisiae*. The liquid fraction, containing mainly xylose, was detoxified by alkali and ion-exchange resin and then fermented by the xylose fermenting yeast *Scheffersomyces stipitis*. Ethanol yields reached in a SSF process were close to 80% when using 15% (w/w) substrate consistency and about 70% of theoretical when using prehydrolysates detoxified by ion-exchange resins. Considering sugars recovery and ethanol yields about 160 g of ethanol from kg of water extracted olive tree pruning could be obtained.

1. Introduction

Among the different lignocellulosic materials generated in the Mediterranean countries, olive tree pruning is one of the most abundant. Besides, the culture of olive tree has grown worldwide in different countries such as Argentina, Australia and USA reaching a culture surface of more than 8.6 million ha (Ballesteros et al., 2011). In olive tree cultivation, the pruning is done every two year in order to prepare the trees for the next crop. This process generates a variable amount of olive tree pruning (up to 3 ton/ha) depending on the culture condition (Cara et al., 2008). Disposal of pruning residues is necessary to keep fields clean and to prevent propagation of vegetable diseases. Usually they are eliminated by either burning or grinding and scattering on fields, causing economic cost and environmental concerns (Romero et al., 2010). Considering the large amount of this yearly generated residue, its need of disposal, low cost and its sugars content, this renewable material has been recently proposed as a source for bioethanol production (Cara et al., 2008; Romero et al., 2007, 2008). So, olive tree pruning has been pretreated using different pretreatments such as steam explosion, liquid hot water even using sulfuric acid as catalyst (Ballesteros et al., 2011; Cara et al., 2008; Díaz-Villanueva et al., 2012; Fonseca et al., 2013; Romero et al., 2008, 2010).

However, to our best knowledge, phosphoric acid has never been used as catalyst in steam explosion pretreatment of olive tree pruning. In spite of its higher cost compared to sulfuric acid, phosphoric acid has several advantages like being less corrosive and toxic, representing a reduction in the cost of plant construction (Geddes et al., 2011). Furthermore, the use of this acid could also

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provide a co-product opportunity as a valuable component of plant fertilizers (Geddes et al., 2010; López-Linares et al., 2013a,b). As well, at low concentrations, phosphoric acid presents a high capacity of solubilizing the hemicellulose fraction with the advantage of being a source of phosphorus, a nutrient for the microorganism in the fermentation of prehydrolysates (Vasconcelos et al., 2013).

So, considering the relative high amount of xylose that could be contained in the liquid fraction after pretreatment, it would be necessary to ferment this sugar in order to make the process economically feasible. Among the xylose-fermenting yeasts *Scheffersomyces stipitis* (*Pichia stipitis*) has shown promising results for industrial application, because it ferments xylose with high ethanol yield (Krahulec et al., 2012), does not require vitamin addition and it is also capable of fermenting a wide range of sugars (Agbogdo and Wenger, 2006). Besides, two of the most strains used in the literature (*P. stipitis* CBS5773 and CBS 6054) are furthermore of interest because the xylose pathway from these two strains was the preferred point of departure for construction of xylose fermenting strain of *Saccharomyces cerevisiae* (Krahulec et al., 2012). So, all these features make that *S. stipitis* should be regarded as a promising candidate for use in xylose conversion (Agbogdo and Coward-Kelly, 2008; Li et al., 2011). On the other hand, this yeast has been employed to ferment hydrolysates from different pretreated biomasses such as: wheat straw (Bellido et al., 2011, 2013), yellow poplar (Cho et al., 2010), hazelnut shell (Arslan and Eken-Saraçoğlu, 2010), corn stover (Zhu et al., 2011), corncob (Lee et al., 2011), sugar maple (Shupe and Liu, 2012) and olive tree pruning (Díaz et al., 2009; Díaz-Villanueva et al., 2012).

However, during pretreatment not only sugars (mainly xylose) are solubilized but also some compounds coming from sugar and lignin degradation. These compounds have been reported to inhibit fermentation of hydrolysates from olive tree pruning by *P. stipitis* (Díaz et al., 2009; Díaz-Villanueva et al., 2012; Fonseca et al., 2013) and its presence should be taken into account when the liquid fraction is considered for fermentation. So, in order to eliminate such compounds and increase the fermentability of the liquids fractions a detoxification step prior to fermentation is required. Biological, physical and chemical methods have been employed for detoxification lignocellulosic hydrolysates (Palmqvist and Hahn-Hägerdal, 2000). The choice of detoxification method more adequate to pretreat the liquid fraction obtained after steam explosion in order to improve fermentability depends on the composition of the hydrolysates, type of raw material and fermenting microorganism. Anyway, the selected detoxification method should selectively remove inhibitors and be cheap and easy to integrate in the ethanol production process (Palmqvist and Hahn-Hägerdal, 2000).

In this work, water extracted olive tree pruning was subjected to steam explosion pretreatment using phosphoric acid as catalyst for the first time. The objective is to ferment all the sugars (mainly glucose and xylose) contained in the pretreated material. So, the slurry obtained was filtered and two different fractions were produced: water insoluble solid (WIS) and liquid fraction or prehydrolysate. The WIS fraction, containing mainly glucose and lignin was used as medium in a simultaneous saccharification and fermentation process (SSF) by a commercial strain of *S. cerevisiae*. The prehydrolysate containing mainly xylose was detoxified (by alkali treatment and ion-exchange resin) and then fermented by the xylose fermenting yeast *S. stipitis*.

2. Methods

2.1. Raw material

Olive tree pruning (collected in the province of Jaén, Spain) was comminuted (4 mm) and subjected to water extraction in autoclave at 121 °C for 60 min. Extraction was carried out in Sovirel 1 L containing 50 g (dry matter) of olive tree pruning (10% w/w). After the extraction process the material was filtered and two fractions were obtained: a liquid and a solid fraction. Both fractions were analyzed as described in analytical methods. The solid fraction was used as substrate for pretreatment.

2.2. Pretreatment

Material obtained after extraction (dried at 40 °C) was pretreated in a steam explosion unit equipped with 2 L reactor designed to reach a maximum operating pressure of 4.12 MPa which has been described elsewhere (Ballesteros et al., 2000). The reactor was filled with 300 g (dry basis) impregnated with 500 mL of phosphoric acid solution 1% (w/w). Pretreatment conditions were 175 °C and 195 °C for 10 min. After the explosion, the slurry was recovered in a cyclone, cooled to about 40 °C and then filtered for solid and liquid recovery. The water insoluble fraction (WIS) was washout thoroughly with water and characterized in terms of the main components (glucans, hemicellulose, lignin, and ash) as describe in Section 2.7. The liquid fraction or prehydrolysate was analyzed for sugars and degradation compounds.

2.3. Microorganisms and growth conditions

S. cerevisiae (Fermentis Ethanol Red, Marcq en Baroeul, Cedex, France) and *S. stipitis* CBS 6054 were used in SSF and prehydrolysates fermentation, respectively. Active cultures for inoculation were obtained in 100 mL Erlenmeyer flasks with 50 mL of growth medium containing (in g/L):, yeast extract (2), NH_4Cl (1), KH_2PO_4 (1), $MgSO_4 \cdot 7H_2O$ (0.3) and glucose or xylose (30) for *S. cerevisiae* and *S. stipitis*, respectively. After 16 h on a rotatory shaker at 35 °C and 150 rpm, the preculture was centrifuged at 10,000*g* for 10 min. Supernatant was discarded and cells were washed with saline solution and then diluted to obtain an inoculum level of 1 g/L.

2.4. Enzymatic hydrolysis

The washed WIS obtained after pretreatment was used as substrate for enzymatic hydrolysis. Experiments were performed in 100 mL Erlenmeyer flasks, each containing 25 g of 0.05 M sodium citrate buffer (pH 4.8) at 10% and 15% (w/w) WIS consistency at 50 °C for 72 h. Cellulose-hydrolyzing enzymes, Novozyme 50013 with an activity of 65 filter paper units (FPU)/g, and Novozyme 50010 with a β-glucosidase activity of 590 IU/g, were used in all experiments. Enzymes were kindly provided by Novozymes A/S (Denmark). Enzyme loading of 15 FPU/g of dry WIS of cellulase and 15 IU/g WIS of β-glucosidase was used. After enzymatic hydrolysis completion, glucose and xylose content was analyzed by HPLC.

2.5. Simultaneous saccharification and fermentation

The SSF experiments were carried out in 250 mL Erlenmeyer flasks, each containing 100 g of 0.05 M sodium citrate buffer (pH 4.8) at 10% and 15% (w/w) WIS consistency. Experiments were conducted at 35 °C for 72 h and agitated at 150 rpm. Enzymes were added in the same loading as in enzymatic hydrolysis tests. Flasks were inoculated with 1 g/L of *S. cerevisiae*. Samples were withdrawn after 24, 48 and 72 h and analysed for ethanol and glucose. All tests were carried out in triplicate.

2.6. Fermentation of prehydrolysates

The prehydrolysate obtained after filtering the slurry was subjected to two detoxification (alkali and ion-exchange resin)