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# Pretreatment of olive tree biomass with $FeCl_3$ prior enzymatic hydrolysis

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## HIGHLIGHTS

- ▶ Olive tree biomass is a potential feedstock for the production of bioethanol.
- ▶ Pretreatment of olive tree biomass with $FeCl_3$ was assessed.
- ▶ Yields are better than those reported for this feedstock using other pretreatment methods.

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## ABSTRACT

Olive tree biomass (OTB) is an agricultural residue which can be used as raw material for bioethanol production. OTB was pretreated with 0.05–0.275 M $FeCl_3$ solutions at 120–180 °C for 0–30 min. Enzymatic hydrolysis yields were used for assessing pretreatment performance. Optimum $FeCl_3$ pretreatment conditions were found to be 152.6 °C, 0.26 M $FeCl_3$ for 30 min. Under such conditions, 100% of hemicellulose was removed, and enzymatic hydrolysis of pretreated solids resulted in a yield of 36.6 g glucose/100 g of glucose in the raw material. Hemicellulosic sugar recovery in the prehydrolysate was 63.2%. Results compare well with those obtained by other pretreatment strategies on the same raw material, confirming $FeCl_3$ solutions as a new, feasible approach for bioethanol production.

## 1. Introduction

Lignocellulose materials are considered the most promising feedstock that can be transformed into renewable fuels and partially replace fossil fuels. Olive tree biomass (OTB) is one of the most abundant lignocellulose materials in Mediterranean countries, and olive tree cultivation is spreading all over the world (FAOSTAT, 2012). The conversion of OTB into ethanol has been proposed by Cara et al. (2008a) and Requejo et al. (2012a). Pruning of olive trees is performed every two years to eliminate unproductive branches and to increase fruit production.

This operation generates a huge amount of cheap and renewable lignocellulose material, which must be eliminated to prevent spread of diseases. From an environmental point of view, the transformation of OTB into bioethanol represents an alternative to other disposal methods such as direct burning.

The conversion process includes pretreatment, enzymatic hydrolysis to release fermentable sugars, fermentation, and distillation.

Pretreatment has been recognized as the key step for bioethanol production. The main objectives of pretreatment are removal of lignin and hemicellulose, reduction of the crystallinity of cellulose and increase in the porosity of the materials. The goal of pretreatment is to attain maximal fermentation yields and rates by improving the accessibility of enzymes to the cellulose structure without the formation of inhibitors for subsequent hydrolysis and fermentation processes (Gírio et al., 2010). It has been estimated that pretreatment is the most expensive single step of the conversion process, accounting for up to 20% of the total cost (Yang and Wyman, 2008).

Pretreatment of OTB has been carried out with dilute acid (Cara et al., 2008b), liquid hot water (Cara et al., 2007), organosolvolysis (Díaz et al., 2011), and steam explosion (Cara et al., 2008a). Dilute acid pretreatment produced relatively good results in terms of sugar yields after enzymatic hydrolysis of up to 75% (Cara et al., 2008b); however, the combination of dilute sulfuric acid solutions (less than 2% w/v) with temperatures of around 180–200 °C, re-

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*Abbreviations:* OTB, olive tree biomass; WIS, water-insoluble solid; SR, solid recovery; $CS_0$, combined severity; $CR_0$, combined severity factor; $R_0$, severity factor; $GR_S$, glucose recovery in solid fraction; $HSR_S$, hemicellulosic sugars recovery in solid fraction; $GR_L$, glucose recovery in liquid fraction; $HSR_L$, hemicellulosic sugars recovery in liquid fraction; $Y_{WIS}$, enzymatic hydrolysis yield referred to glucose in the WIS; $Y_{EH}$, enzymatic hydrolysis yield referred to glucose in raw material.

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quired a special reactor material due to corrosion concerns. In addition, the pH of the resulting hydrolysates must be neutralized before enzyme addition, generating gypsum that must be adequately handled. In an attempt to overcome these drawbacks, the use of metal salt solutions instead of acid has been proposed (Liu et al., 2009a).

Metal salts such as $FeCl_3$, especially when used in combination with dilute acid pretreatment, can improve results of enzymatic hydrolysis yields (Liu et al., 2009a) by altering the structure of the lignocellulose matrix. The mechanisms by which these salts affect hemicellulose degradation and improve enzyme access are not completely understood (Zhao et al., 2011).

The main objective of the present study r was to evaluate the effects of $FeCl_3$ solutions for pretreatment method of OTB. The effects were evaluated in terms of enzymatic hydrolysis of pretreated solids. In addition, the pretreatment process was optimized with respect to salt concentration, temperature, and time and enzymatic digestibility and recovery of the monomeric and oligomeric sugars in the liquid fraction obtained after pretreatment (prehydrolysates) was investigated. The formation of inhibitors such as furfural, 5-hydroxymethylfurfural (HMF), and formic acid was also evaluated.

## 2. Methods

### 2.1. Raw material

OTB was collected in Jaén (southern Spain) after fruit-harvesting and chopped in the field. Once in the laboratory, OTB was air-dried to equilibrium (moisture content of about 10%) and milled using a laboratory hammer mill (Retsch) to a particle size smaller than 1 cm and stored at room temperature.

### 2.2. Pretreatment with $FeCl_3$

Dry feedstock (120 g) was loaded into a laboratory-scale stirred Parr reactor with a total volume of 1 L, $FeCl_3$ solution was added at a 1:5 (w/v) solid/liquid ratio, and both were directly mixed for 1 h before heating (Liu et al., 2009a). The reactor was heated at a rate of 5 °C/min. The temperature profiles in the heating and cooling stages corresponded to standard operational conditions (Díaz et al., 2010). Agitation was set at 350 rpm and the process time was measured once the selected pretreatment temperature was reached. After the desired process time was completed, cooling water was charged through the serpentine coil. The reactor vessel was kept sealed and introduced in an ice bath with the slurry agitated until the reactor was cooled to about 40 °C. The wet material was filtered through stainless steel sieve for solid and liquid fraction recovery.

The water-insoluble solids (WIS) were washed with deionized water and analyzed for hemicellulosic sugars, glucose, and acid-insoluble lignin content, and used as substrate in enzymatic hydrolysis tests. The liquid fraction obtained after pretreatment (prehydrolysate) was analyzed for sugars, acetic acid and sugar-degradation products (furfural, HMF, and formic acid). Recoveries of glucose and hemicellulosic sugars were determined as a percentage of the sugar content in the raw material,

$$\text{Sugar Recovery} = \left(\frac{\text{g sugars in WIS or prehydrolysate}}{100\,\text{g sugars in OTB}}\right) \tag{1}$$

### 2.3. Enzymatic hydrolysis

The washed WIS of OTB were hydrolysed with a cellulolytic complex (Celluclast 1.5 L), kindly provided by Novozymes A/S (Denmark). The cellulose enzyme loading was 45 filter paper units (FPU)/g WIS. Fungal ß-glucosidase (Novozym 188, Novozymes A/S) was used to supplement the ß-glucosidase activity with an enzyme loading of 15 International Units (IU)/g WIS. The pH was adjusted to 4.8 with 0.05 M sodium citrate buffer and enzymes were added to the pretreated substrate (5% dry basis) for a total working volume of 25 mL. The flasks were incubated at 50 °C in an orbital shaker (Certomat-R, B-Braun, Germany) agitated at 150 rpm for 48 h. Sample aliquots (2 mL) were taken at 6 and 24 h, centrifuged at 9.000 g for 10 min, and analyzed for glucose concentration. The enzymatic hydrolysis yields were calculated as follows:

$$Y_{\text{WIS}} = \left(\frac{\text{g glucose EH}}{100\,\text{g glucose WIS}}\right) \tag{2}$$

$$Y_{\text{EH}} = \left(\frac{\text{g glucose EH}}{100\,\text{g glucose OTB}}\right) \tag{3}$$

All experiments were carried out in duplicate and showed standard deviations of <2%.

### 2.4. Severity factors

The harshness of pretreatment was measured in terms of the severity. To take into account the effect of varying $FeCl_3$ concentration, the combined severity concept has been proposed as follows:

$$\text{Combined Severity} = \text{CS}_0 = \log \text{CR}_0 \tag{4}$$

The combined severity factor ($CR_0$), adapted from Overend and Chornet (1987) by Chum et al. (1990) and Silverstein et al. (2007), was applied following this equation:

$$\begin{aligned} \text{CR}_0 &= C^n \cdot R_0 = C^n \int_0^t \exp\left(\frac{T(t) - T_b}{\omega}\right) dt \\ &= C^{2.1} \int_0^t \exp\left(\frac{T(t) - 100}{14.75}\right) dt \end{aligned} \tag{5}$$

where '$t$' is time (min); '$C$' is the chemical concentration (M); '$T$' is the reaction temperature (°C); '$T_b$' is the base temperature (usually set to 100 °C); '$\omega$' is a fit parameter, which in this and most other studies is assigned the value of 14.75, and '$n$' is an arbitrary constant that indicates the influence order of the catalyst concentration on the kinetics of the process.

The constant '$n$' in Eq. (5) was determined from a previously calculated severity factor ($R_0$) as defined by the above integral, adjusting solid recovery (SR, solids remaining after pretreatment divided by original oven-dried weight, %) as a function of $R_0$, the best result being $n = 2.1$ ($R^2 = 0.984$; Fig. 1). Thus, $CR_0$ (Table 1) combines the effects of time, temperature and $FeCl_3$ concentration on the different responses.

### 2.5. Experimental design

OTB was pretreated at 15 different operational conditions according to a Box–Behnken experimental design, including one point and two replicates at the center of the domain selected for each factor under study, as shown in Table 1. Center values and intervals were chosen based on previous experience with agricultural residues (Liu and Wyman 2006; Liu et al., 2009a; Marcotullio et al., 2011) to ensure a broad range of responses. Once the responses from the experimental design were analyzed, five additional assays were performed, corresponding to more severe operational conditions, in order to obtain more information for optimization purposes. As reported in Table 1, the total number of runs was 20.

To obtain high enzymatic digestibility of OTB, $FeCl_3$ concentration, pretreatment time, and temperature were optimized by statistical methodology with a solid-to-liquid ratio of 1:5. This consistency is, for the most part, higher than that used with most lignocellulosic materials. The use of this to-liquid ratio provides an