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Optimization of the steam explosion and enzymatic hydrolysis for sugars production from oak woods

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HIGHLIGHTS

- Three different oak woods were investigated for ethanol production.
- EH yield and overall sugar yield were optimized by RSM.
- Severity factor, total solid and enzyme loading were used as variables in the DoE.
- Steam explosion pretreatment is a good process for oak wood conversion.

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ABSTRACT

Fermentable sugars production from three kind of steam-exploded oak wood was optimized by Response Surface Methodology (RSM), using the severity factor (R_0), the pretreated total solids (TS%) and the enzyme loading (EL%) as variables of a central composite design. A total of 17 experiments for each biomass were carried out. The optimal conditions established with RSM were: severity, 4.46 for holm, 4.03 for turkey oak and 3.92 for downey oak; total solids, 5.4% for holm, 5.0% for turkey oak and 12.7% for downey oak; and enzyme concentration, 9.6% for holm, 15.0% for turkey oak and 15.0% for downey oak. Under these conditions, the model predicted an overall sugar yield of 67.1% for holm, 79.9% for turkey oak and 68.4% for downey oak. The results of the confirmation experiments under optimal conditions agreed well with model predictions. Oak wood species may be a good feedstock for the production of reducing sugars.

1. Introduction

Second generation ethanol is one of the most promising biofuel and its diffusion is increasing constantly (Gupta and Verma, 2015); it can be obtained from different lignocellulosic materials as agricultural waste (Ekman et al., 2013; Cotana et al., 2015) and forest harvest residues (Yamamoto et al., 2014).

Lignocellulosic biomass is recalcitrance and its bioprocessing requires a pretreatment step before efficient biochemical conversion can take place. Carbohydrates in woody biomass are not readily accessible to fermentation, then pretreatment step is crucial in order to deconstruct biomass matrix and make available polysaccharides for subsequent enzymatic hydrolysis and fermentation to ethanol (Chiaramonti et al., 2012; Santos et al., 2012b). Steam explosion is one of the most effective pretreatments for breaking the crystalline structure of biomass through chemical effects and mechanical input induced by sudden explosive decompression and the most useful, due to low energy consumption and environmental impact, thanks to no chemical agents involved (Jacquet et al., 2015; Singh et al., 2015). It is well known that acid soaking of biomass prior to steam explosion can improve the treatment but, however, water, when super-heated, acts like an acid (López-Linares et al., 2015). In particular, the highly acetylated nature of some biomass (such as hardwood) allows for uncatalyzed steam pretreatment due to the liberation of acetyl groups present in hemicellulose and formation of acetic acid (Alvira et al., 2010). Furthermore, partial redistribution or removal of lignin also occurs (Chen and Qiu, 2010). In particular, removal of hemicelluloses is useful for exposing the cellulose surface and increasing enzyme accessibility to the cellulose microfibrils (Chen and Li, 2002).

Thus, pretreatment and enzymatic hydrolysis are related each other and are responsible for the main economic outlay in the production of cellulosic ethanol (Kumar and Murthy, 2011). An efficient pretreatment is the one, which gives cellulosic polymers rich solid residue simply processing by low concentration of

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enzymes in the saccharification, minimizing the formation of fermentation step inhibitor products.

Therefore, it is important to perform a process optimization considering both pretreatment and saccharification process parameters. In particular the major factors affecting the efficiency of steam explosion are particle size, temperature, residence time (Alvira et al., 2010) while the critical process parameters of the enzymatic saccharification are biomass loading, enzyme loading, surfactant concentration and incubation time (Maurya et al., 2013).

Oak wood is a widespread biomass across northern hemisphere, extending to the temperate zone of tropical America, Europe, North Africa, and Asia. In Italy forest land covers 35% of total national area and oak wood is one of the most widespread species, with 26% filled surface (INFC, 2005); forestry operations determine a large amount of woody residue causing an expensive and polluting disposal.

Oak wood residue have been used for different purposes in the bioenergy field, recently for biochar production (Jung and Kim, 2014; Mohan et al., 2014), while its use as cellulosic material for ethanol production is not spread in the Literature.

Furthermore, several studies investigated pretreatment and enzymatic hydrolysis stages separately, optimizing the operating variables of each process (Zhang and Chen, 2012; Pereira Ramos et al., 2015), while no earlier studies are reported in Literature on the simultaneous optimization of the two stages.

Thus, the aim of the paper is the optimization of fermentable sugars production for ethanol production from different oak wood residues. Pretreatment and enzymatic hydrolysis operating parameters were used as process variables in a Response Surface Methodology design, assessing the response in terms of glucose yield from saccharification and the best overall fermentable sugars yield is also calculated.

In addition, the understanding of the influence of different species of the same feedstock on fermentable sugars yield was also targeted.

2. Methods

2.1. Raw material

Holm (*Quercus ilex*), turkey oak (*Quercus cerris*), downey oak (*Quercus pubescens*) were collected from the forests around the city of Perugia in Italy. They were chipped to particle size of 2–3 cm for pretreatment and stored at room temperature, reaching the equilibrium moisture below 20%.

A part of each variety was carried out to a mechanical size reduction to 0.5 mm with a centrifugal mill (Retsch ZM200) to analyze the chemical composition.

2.2. Steam explosion pretreatment and enzymatic digestion

The pretreatment of the chipping raw material was carried out in a batch pilot unit with a nominal capacity of 10 L, a maximum operating pressure of 28 bar and a temperature of 230 °C.

The operating procedure was already described in a previous work (Cotana et al., 2014).

The pretreatment conditions were defined by a semi-empirical parameter called the severity parameter, log R_0, combining treatment time and temperature, according to Eq. (1):

$$R_0 = t \cdot e^{[(T-100)/14.75]} \quad (1)$$

where t (min) is the residence time, T (°C) is the explosion temperature.

The reactor was charged with 400 g of feedstock per batch and five operating conditions were tested.

The solid (SF) and liquid (LF) fraction from pretreatment were previously characterized in order to determine sugars and organic acids content.

Sugars recoveries in the SF and LF were calculated as ratio of the sugar content in the pretreated material and in the raw material one.

The SF was then submitted to enzymatic saccharification in 250 mL Erlenmeyer flasks, added a 0.1 M citrate buffer (pH 6) to reach a pH 5.0 in an orbital shaker incubator at 50 °C for 48 h, using a commercially available cellulase cocktail, Cellic CTec2 (Novozymes, Denmark) for glucan digestion. The activity of CTec2 cellulase (120 FPU/mL) was determined to be according to a standard method (Adney and Baker, 2008).

The hydrolysate was filtered by vacuum system and analyzed by HPLC.

The enzymatic hydrolysis (EH) yield was calculated as follows:

$$\text{EH yield } (\%) = \frac{[\text{GH}]}{g_p \times [\text{BP}] \times 1.111} \times 100 \quad (2)$$

where:

[GH]: glucose concentration in enzyme hydrolysis supernatant (g/L); [BP]: dry pretreated biomass weight concentration at the beginning of enzymatic hydrolysis (g/L); g_p: glucan content of dry pretreated biomass (%); 1.111: converts glucan to equivalent glucose.

Hydrolysis conditions in terms of total solid and enzyme loading (EL) were reported in Table 1 as part of central composite design of experiments.

2.3. Response Surface Methodology design (RSM)

A three variable central composite design (CCD) was carried out in order to optimize the fermentable sugars' production from each raw material: the chosen variables are severity factor for pretreatment R_0, total solid (% dry matter) TS, and enzyme loading (% enzyme/dry matter) EL in enzymatic hydrolysis. The variables were defined in five levels in the following ranges: 3.92–4.70 (R_0), 5–15% (TS), and 5–15% (EL). Levels of variables were determined according to preliminary tests and on the basis of literature reports (Cotana et al., 2015; Buratti et al., 2015; Rana et al., 2014; Pereira Ramos et al., 2015).

Therefore, a total of 17 experiments with two replicates in central point (CP) were carried out in a random order to minimize the effects of external factors not taking in account in the experimental design (Table 1).

Table 1
Experiments performed by the central composite design. Coded values of the variables are reported in parenthesis.

Experiment	R_0	TS (% d.m.)	EL (% g enzyme/g d.m.)
E1 (CP)	4.31 (0)	10 (0)	10 (0)
E2	4.08 (−1)	7 (−1)	7 (−1)
E3	4.31 (0)	15 (+1.68)	10 (0)
E4	4.70 (+1.68)	10 (0)	10 (0)
E5	4.54 (+1)	13 (+1)	7 (−1)
E6	4.54 (+1)	7 (−1)	13 (+1)
E7	4.08 (−1)	7 (−1)	13 (+1)
E8	4.54 (+1)	7 (−1)	7 (−1)
E9	4.31 (0)	5 (−1.68)	10 (0)
E10 (CP)	4.31 (0)	10 (0)	10 (0)
E11	4.54 (+1)	13 (+1)	13 (+1)
E12	4.31 (0)	10 (0)	15 (+1.68)
E13 (CP)	4.31 (0)	10 (0)	10 (0)
E14	3.92 (−1.68)	10 (0)	10 (0)
E15	4.08 (−1)	13 (+1)	13 (+1)
E16	4.08 (−1)	13 (+1)	7 (−1)
E17	4.31 (0)	10 (0)	5 (−1.68)