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Optimization of acid hydrolysis of olive tree pruning residue. Fermentation with *Candida guilliermondii*

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ABSTRACT

Olive tree pruning biomass may become an interesting lignocellulosic material to produce simple sugars by one stage dilute acid hydrolysis, whenever the conditions have been optimized to take full advantage of this process step. In this situation, the hydrolyzates could be directly fermented without using a previous detoxification or additional hydrolytic treatments. A central composite face design for temperature (100–120 °C), hydrolysis time (30–90 min) and acid concentration (ω = 1.0–2.0%) with three replicates, has been carried out using olive tree pruning with and without leaves. Fermentability of the hydrolyzates (concentrated and non-concentrated) by *Candida guilliermondii*, obtained in accordance with the optimal operating conditions according to the experimental design, was investigated for ethanol and xylitol biogeneration. Considering D-xylose, D-glucose and total sugars yields, the most suitable results have been achieved at the higher temperature (120 °C), sulfuric acid with a mass fraction of 2% and the longer time (90 min). With this yeast, ethanol yields of 430 g kg^{-1} and 460 g kg^{-1} of D-glucose for concentrated hydrolyzates from whole and leaves free respectively, were obtained. On the other hand, the fermentation of hydrolyzates from leaf free olive pruning generated xylitol at 240 g kg^{-1} of D-xylose.

1. Introduction

Olive tree pruning residue (OTPR) is the most available residual biomass source in Andalusian autonomous region (Spain) and important through other Mediterranean countries [1]. The possibility of using this biomass to foster the process economy and produce high added value products by fermentation allows acid hydrolysis can be located as a real alternative for generation of simple sugars (D-glucose and D-xylose) derived primarily from hemicellulosic fraction. The subsequent fermentation of acid hydrolyzates can generate products for use both as biofuel (ethanol) as products with important properties from the pharmacologic standpoint and food industry (xylitol) [2].

Hardwood hemicelluloses are mostly composed of highly acetylated heteroxylans, classified as 4-O-methyl glucuronoxylans. Hexosans are also present, but in low amounts as glucomannans [3].

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The acid-based treatment of biomass is gaining its position as a viable saccharification process [4]. One problem associated with acid hydrolysis is the generation of inhibitors or toxic compounds that can be considered as a limiting factor in the subsequent fermentation process [5]. In this direction, to find those effective conditions to generate large amounts of sugars is needed but not producing concentrations of these compounds above their toxicity threshold [6]. Probably, two of the most harmful inhibitors were phenolic compounds [7], because they cause loss of integrity of biological membranes [8], or acetic acid; this one can cross the cell membrane, in undissociated form, while decreasing the intracellular pH; so that death occurs [9]. Furans are serious toxic compounds for the microorganisms and they are in large available quantities in fermentation media.

If treatment conditions are selected carefully, low-concentration acid hydrolysis could offer some advantages compared to other process of obtaining sugars: low acidity minimizes the gypsum production, if any, low corrosion, minimal equipment cost and limited environmental effect [4]. Sulfuric acid could be a good choice for this hydrolytic treatment because it is fairly well understood [10] and dilute acid hydrolysis appears to be more economical than other techniques [11].

The main role of sulphuric acid is to alter the structural matrix of the polysaccharides (cellulose, hemicellulose and lignin), making it possible to obtain from the fibers some oligomers or simple sugars [12]. Among the treatments tried with other lignocellulosic materials, dilute acid hydrolysis appears to be in the best position in economic terms [13], specially using sulfuric acid that is low in cost itself although there are some aspects that could be improve, especially when working with high acid concentrations, such as the requirements for hydrolyzate conditioning (related to sugar loss, base requirement and filtration) and the resistant construction materials that must be used [14].

Moreover, several researches have employed *Candida guilliermondii* yeast to carry out fermentation processes, but the studies have been focused to xylitol production as this is a microorganism which owns specific biotechnological routes for this type of pentose. It would be interesting to analyze the behavior of this yeast on OTPR hydrolyzates where it is predicted that xylose-glucose ratios are much lower than those found in other hemicellulosic hydrolyzates.

One of the purposes of our research is to work with acid hydrolysis conditions that could allow to break hemicellulose fibers and get some information on what happens to cellulose fraction. In order to optimize sugars production, taking into account the amount of the main inhibitors that are generated in the process, a 2^3 experimental design with three central points has been carried out (using OTPR just as it is and the same kind of residue but devoid of their leaves) to find the best conditions in respect to acid concentration, reaction time and temperature of the acid hydrolysis treatment. The hydrolyzate obtained under the best conditions tested, will be fermented to bioalcohol production using a yeast which is able to ferment both, pentoses and hexoses, with high yields. *C. guilliermondii* behavior, in the fermentation process, will be studied both as non-concentrated and concentrated hydrolyzates.

2. Materials and methods

2.1. Raw material characterization

OTPR (*Olea europaea*) was collected from 15 to 20 years old trees (located between 411 730–411 740 m EW and 4 196 882–4 196 893 m NS relative to UTM coordinates) after the fruit harvest; the samples were taken from fresh branches located around 1.5 m above the ground [15]. Once the pruning operation was done, the branches were windrow to be chopped in situ (Hawk chopper model PH320 1) and then collected in plastic holed boxes of 50 dm^3 (to allow the air passageway) and taken to the laboratory. Subsequently, the material was air-dried at room temperature to equilibrium moisture content ($\omega \simeq 8\%$) and milled using a laboratory hammer mill (Retsch); fraction graded to a particle size between 0.6 and 0.425 mm, according to ASTM guidelines, was stored into jars glass seals until used. This fraction consists of leaves (45%) and wood chips (55%).

On the other hand, a part of this material has been taken off their leaves previously to be chopped and the wooden branches of olive pruning were treated in the same way above mentioned.

For chemical characterization (of original residues without extracts), the modified methodology proposed by [16] was used. All determinations were carried out in triplicate. It consisted of samples hydrolysis with H_2SO_4 (10 cm^3 $\omega = 72\%$) and vigorous agitation at 50 °C for 7 min. Distilled water (50 cm^3) interrupted the hydrolysis reactions and each sample was immediately transferred by addition of distilled water (225 cm^3) into 500 cm^3 Erlenmeyer flasks and autoclaved at 1.11 atm for 45 min. The material was cooled to room temperature and bulked to 500 cm^3, followed by its homogenization and filtration using previously weighed filter. After filtration of the hydrolyzate, a solution is obtained which will allow the determination of the acid soluble lignin, monomeric carbohydrates, acetic acid, furfural and hydroxymethylfurfural (HMF) by HPLC.

The lignin percentages were determined gravimetrically, ash contents were calculated by weight difference before and after incineration of the pruning samples in a muffle furnace at 550 °C for 4 h.

2.2. Acid hydrolysis procedure

The acid hydrolysis treatment was carried out in 250 cm^3 glass flasks, using H_2SO_4 1:10 solid/liquid ratio (w/w). The material was mixed with 100 cm^3 of sulfuric acid with the desired concentration at designed hydrolysis time and temperature. The liquid fractions were separated by filtration and the resulting solids were washed with ultrapure water (30 cm^3) at room temperature for residual sugars elimination that could have been retained. The filtrates were analyzed and D-xylose, D-glucose, L-arabinose, total sugars, acetic acid and total phenols concentrations were determined.

Once optimized hydrolysis conditions, we proceeded to carry out the sulfuric acid treatment on a larger scale, in a stainless steel reactor of 50 dm^3 capacity, with a useful volume of 40 dm^3 provided with a system of electrical resistance heating and agitation by rotation about its own axis. The solid and liquid