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Comparison of fermentation strategies for ethanol production from olive tree pruning biomass

Felipe Fernandes-Klajn, Juan Miguel Romero-García, Manuel J. Díaz, Eulogio Castro*

Dpt. Chemical, Environmental and Materials Engineering, Center for Advanced Studies in Energy and Environment (CEAEMA), Universidad de Jaén, Campus las Lagunillas, 23071, Jaén, Spain

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ABSTRACT

Olive tree pruning is a widely available and low cost lignocellulosic residue generated every year, being a potential source for bioproducts and renewable fuels production. In this context, this work has as main objective to propose an efficient scheme for the production of second generation ethanol from olive tree pruning biomass, focusing on the evaluation of different detoxification strategies (activated carbon, ammonium hydroxide and overliming) of the liquid fraction obtained after pretreatment, as well as different configurations of enzymatic hydrolysis and fermentation (including hydrolysis and sequential or simultaneous fermentation, pre-saccharification or co-fermentation) of the pretreated olive tree pruning solid. The best results of each fraction were tested in slurry, at varying initial solid loadings and aeration levels. The use of NH_4OH 5N as a detoxification method and the pre-saccharification and co-fermentation configuration without aeration are proposed, resulting in 13.86 g ethanol/100 g raw material after 46 h of process.

1. Introduction

Biomass from pruning of olive trees is one of the most widely available lignocellulosic materials particularly in the Mediterranean countries. Pruning, performed after fruit harvest, produced every year a huge amount of biomass that must be adequately handled from both economic and environmental viewpoint. Olive tree pruning biomass (OTPB) has been reported to be a suitable raw material for the production of bioethanol as well as number of bioproducts (Romero-García et al., 2014). OTPB is composed of leaves, thin branches and thick branches in different proportions, depending on several factors such as the tree age, cultivation techniques applied or olive production (Romero-García et al., 2014). OTPB contains structural sugars as cellulose (26.1–36.6% by weight), hemicellulose (20.0–25.0%), lignin (17.9–25.0%) and extractives (14.4–31.4%) (Romero-García et al., 2014).

The bioconversion of OTPB into biofuels and other compounds requires a pretreatment step to modify its complex and recalcitrant lignocellulosic structure thus facilitating access to the enzymes in charge of releasing monomeric sugars (Sebayang et al., 2017a,b). As a parallel result of the pretreatment, a number of compounds such as furan derivatives, phenolics and weak organic acids are also produced and encountered in the liquid phase issued from pretreatment. These compounds may exert an inhibitory effect on both the enzymes and the fermentative microorganisms. Some detoxification methods can be applied to reduce the concentration of the inhibitors, based on chemical, physical or biological treatments (Mussatto and Roberto, 2004; Vallejos et al., 2016).

Among chemical detoxification methods, examples can be found using $Ca(OH)_2$ or NaOH (Martínez et al., 2000), activated carbon (Vallejos et al., 2016), NH_4OH (Geddes et al., 2013) or organic solvents (Mateo et al., 2013).

For the production process of ethanol from lignocellulose to be efficient, a complete hydrolysis of the sugars, both from cellulose and hemicelluloses, and an efficient fermentation of the sugars to ethanol is required. In addition, the process must be done with a low energy demand. To achieve these objectives, high concentrations of initial solids in the fermentation medium are necessary to obtain high final concentrations of ethanol, together with an efficient integration of the stages of the process that allows achieving high yields and low investment in equipment. This integration will have benefits in terms of higher yields, production rate and higher ethanol concentrations, which will translate into lower capital and operating costs.

There are several options for fermenting the sugars contained in the lignocellulosic materials (Manzanares et al., 2011). For example, sugars contained in the liquid fractions can be fermented separately of the glucose released by enzymatic hydrolysis from pretreated solids or the whole slurry can be fermented without previous separation. Other

* Corresponding author.
E-mail address: ecastro@ujaen.es (E. Castro).

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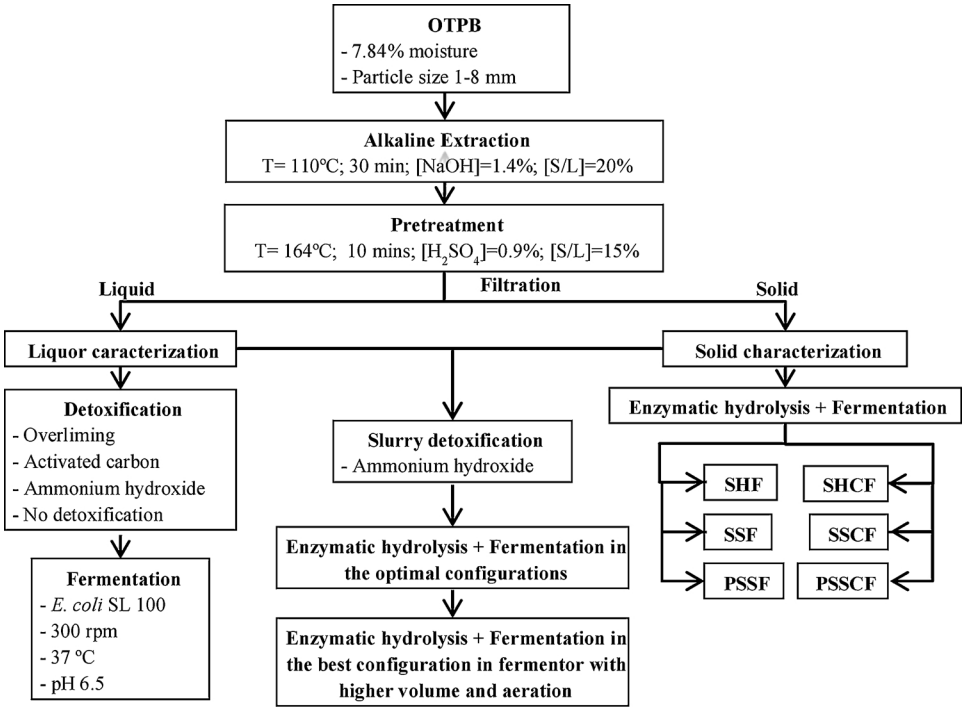

Fig. 1. Work flow for ethanol production from OTPB.

options include simultaneous or separate saccharification and fermentation of the pretreated solid; and the same configurations preceded by an additional and previous saccharification (Tomás-Pejó, 2011). This work compares the production of ethanol from OTPB under six different fermentation configurations, which are briefly described next.

The so called Sequential Hydrolysis and Fermentation (SHF) and the Sequential Hydrolysis and CoFermentation (SHCF) configurations include two main steps, i.e., first the enzymatic hydrolysis of the pretreated solid and, second, the fermentation to ethanol. While both steps are conducted under the best options for each of them (i.e., higher temperature for the enzymatic hydrolysis and then lower temperature for the fermentation), the main drawback is the glucose accumulation produced by the hydrolysis that can result in enzyme inhibition and reduced ethanol yield (Vohra et al., 2014). The main difference between both configurations is that the SHCF considers the fermentation of all sugars present, that is, glucose and hemicellulosic sugars (while SHF is directed to just glucose). Cofermentation requires a microorganism able to ferment both types of sugars or the use of several microorganisms. Several advantages have been reported for the single microorganism approach, e.g., lower cost and operation time and reduced inhibitory effects and contamination risk (Rastogi and Shrivastava, 2017).

In Simultaneous Saccharification and Fermentation (SSF) and Simultaneous Saccharification and CoFermentation (SSCF) sugars are fermented as they are released, in a single step. This eliminates the inhibition issues due to sugar accumulation. On the negative side, enzymes and microorganisms do not present the same optimal operation temperatures (around 50 °C for enzymatic hydrolysis versus 28–35 °C for the fermentation).

Variations of the SSF and SSCF are the so called Presaccharification and Simultaneous Saccharification and Fermentation (PSSF) and the Pressacharification and Simultaneous Saccharification and CoFermentation (PSSCF), in which a rapid liquefaction step is included, previous to the hydrolysis and fermentation steps. This allows reducing the viscosity of the slurry, resulting in an increase of ethanol yields. This procedure is specially indicated for high solid loadings (Moreno et al., 2017).

As far as the use of the whole slurry is concerned, solid loading and aeration level have been revealed as relevant factors whose influence on the ethanol yields from OTPB has rarely been addressed.

The objective of this work is to compare the main configurations for the fermentation of sugars released from OTPB, including detoxification of the liquid issued from pretreatment. The study of solid loading and aeration level when using the whole slurry as fermentation medium is also addressed.

2. Materials and methods

2.1. Raw material and pretreatment

The OTPB was collected in an olive orchard after fruit harvesting, in March 2016. Once in the laboratory, this material was dried at room temperature, reaching 7.8% moisture. Subsequently, the pruning was grinded using a Retsch SM 100 blade mill until obtaining a particle size between 1 and 8 millimeters, which was stored until its use. The high extractives content in olive tree pruning are mainly derived from its amount of leaves. Olive tree leaves are a significant source of bioactive phenolic compounds such as iridoids, flavonoids, triterpenes and phenolic compounds (Xynos et al., 2014). On the other hand, during the pretreatment, extractives can be condensed or precipitated and produce condensation reactions between lignin and degraded sugars (Ballesteros et al., 2011). For these reasons, in this work an alkaline extraction stage was carried out previous to pretreatment (Burkhardt et al., 2013). In order to minimize the presence of extracts in the raw material, after milling, the OTPB was subjected to an alkaline extraction with NaOH. The treatment with alkaline compounds, such as NaOH, favors the access of the enzymes in the subsequent hydrolysis stage (Umagiliyage et al., 2015). The conditions to carry out this extraction were selected in a previous assay as 110 °C, 30 min, 1.4% NaOH and 20% S/L ratio. In these conditions the presence of non-structural compounds in olive pruning was reduced by 75%. The characterization of the raw material and extracted material was made based on the technical report of the National Renewable Energy Laboratory of the United States (NREL, 2012).

The extracted olive pruning was subjected to a combined thermal + acid pretreatment, at a temperature of 164 °C, for 10 min, in

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