## USE OF OLIVE-PRUNING DEBRIS. HYDROLYSIS AND FERMENTATION WITH Candida tropicalis

Sebastián Sánchez<sup>1</sup>, Vicente Bravo<sup>2</sup>, Nicolás Cruz<sup>1</sup>, Juan F. García, Manuel Cuevas<sup>1</sup> <sup>1</sup> Dept. Chemical, Environmental and Materials Engineering,, University of Jaén, 23071 Jaén, Spain <sup>2</sup> Dept. Chemical Engineering, University of Granada, 18071 Granada, Spain Tel.: 34 953 212219, E-mail: <u>ssanchez@ujaen.es</u>

For the debris from olive pruning, as with many wastes, no technologically or economically viable applications have been found. In most cases, the olive debris is left over the land to be incinerated, with the disadvantages that this may involve (air pollution, soil mineralization, increased risks of fires, propagation of pests).

One way of making use of this debris is fractioning, by hydrolysis, of its main components: cellulose, hemicellulose, and lignin. The hydrolysis (using acids or enzymes) of these agricultural wastes provides a solution of the sugars coming from the hemicellulose and cellulose fractions, which, by fermentation with yeasts and fungi, can provide products of industrial interest. The most commonly used yeasts, of the genera *Saccharomyces* and *Schizosaccharomyces*, ferment mainly hexoses and are incapable of transforming pentoses. One of the yeasts recommended for fermentation of pentoses and hexoses is *Candida tropicalis* (Oh and Kim, 1998). To achieve integral use of the lignocellulose residues, in which D-xylose is the main pentose of the hemicellulose fraction, an alternative that provides complete conversion is needed, as it should be taken into account that hemicelluloses can constitute 15 to 35% of the waste in dry base (Clausen and Gaddy, 1983).

In the present work, olive-pruning debris were hydrolysed and afterwards fermented to xylitol with one of the non-traditional yeasts, *Candida tropicalis* (IFO-0618).

The pruning debris, after being ground and screened to a particle size of 0.300 to 0.425 mm, was placed in a stirred tank batch reactor of stainless-steel in experiments in which hydrolysis was carried out under pressure. The load consisted of 50 g of pruning debris and a variable volume of water. The system was heated to 200°C and, immediately afterwards, cooled to room temperature. After pretreatment, the hydrolysate was transferred to a stirred tank batch reactor of glass to complete the volume to 0.5 L. The operating conditions during all the enzymatic-hydrolysis experiments were: T of 90°C and a stirring rate of 250 rpm. In this reactor, solid/liquid relationships of 1/5 and 1/6 were used. The hydrolytic agent used was sulphuric acid at a concentration of 1.0 N. Afterwards, the solid residue was separated by filtration and the hemi cellulose, cellulose, and lignin fractions were determined, and the hydrolysate samples collected at different times were analysed for D-glucose, total reducing sugars, and acetic acid. The fermentation experiments were made using a stirred tank batch bioreactor at a temperature of 30°C and a stirring rate of 500 rpm. In all cases, microaerobic conditions were used. The pH of the culture was adjusted to the range 5.0 to 6.5.

From the results, the following parameters were determined: specific growth rates, substrate consumption, and ethanol and xylitol production. In addition, the overall yields in biomass, ethanol and xylitol were calculated. It was found that the hydrolysates, which were subjected to hydrolysis pretreatment under pressure presented at the fermentation stage a high level of inhibition of the yeast in its stages of biomass-formation, substrate consumption, and bioproduct formation. A good indicator of this inhibition was the acetic acid concentration during the stages of pressurized hydrolysis and/or acid hydrolysis. Under the operating conditions tested, the highest overall yields in xylitol and ethanol were determined with the hydrolysates that came from the pressurized hydrolysis and that was afterwards fermented at pH 5.0, with an aeration level equivalent to an air flow that could enter through the stirring vortex. Under these conditions, the volumetric productivity of xylitol was 0011 kg  $m^{-3}h^{-1}$ .

<sup>-</sup> Clausen, E. C., Gaddy, J. L. Annals New York Acad. of Sciences, 435-447, 413 (1983)

<sup>-</sup> Oh, D.-K., Kim, S.-Y. Increase of xilitol yield by feeding xylose and glucose in *Candida tropicalis*. *Appl. Microbiol. Biotechnol.* 419-425, **50** (1998)