Valorization Olive Mill Solid Waste to Ethanol by Microwave Pretreatment and Enzymatic Saccharification

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Liquid transportation fuel replacements are of global importance, first among them is ethanol that can be used as drop-in fuel additive, resulting in lower emission. To-date ethanol is produced mainly from especially grown crops such as sugar cane, corn and sorghum, but interest in ethanol production from lignocellulose waste (second generation) is growing rapidly with some factories already working. This pursue is of especial importance for many Mediterranean countries where there is not enough land and water to grow specialized energy crops.

Growing olive trees is a long tradition in the Mediterranean basin, with ~700,000 trees in the Mediterranean alone. Unlike many other crop types, the olive trees are long term crops, a recent survey (2014) found that more than 50% of the Mediterranean trees were 50 years old or more, producing crop every year. About half of the olives are used for olive oil production, being one of the most important economic agro-food sectors in the Mediterranean. Each year ~2,000,000 tons of olive oil is produced, resulting in large amount of solid wastes, among them ~1,350,000 tons of Olive Mill Solid Waste (OMSW). This waste is considered as environmental pollution and phytotoxic, and no economically viable use for its valorization is available.

The OMSW is a mixture of skin, pulp, and seeds. It is rich in cellulose, hemicellulose and in lignin (In our samples 18.4±0.2, 15.9±0.1% and 40.2±5.1% respectively but could vary by cultivar and extraction process). This composition suggest it could be valorized to ethanol, and indeed such conversion was previously demonstrated, using thermochemical treatment in the presence of weak organic acids (Abu Tayeh *et al*, 2014, 2016). Nevertheless, the efficiency of the conversion was low, resulting in ~30g ethanol for each dry kg of OMSW, representing conversion of only ~40% of the cellulose fraction (Abu Tayeh *et al*, 2014).

Here we demonstrate the efficient conversion of the cellulosic fraction of OMSW to ethanol. We have compared two different pre-treatments (microwave and autoclave), different additives (water, sulphuric- and formic acid), and two commercial saccharification enzyme. Parameters tested were sugar loss, formation of fermentation inhibitors, saccharification efficiencies and ethanol production by fermentation with commercial yeast. The results suggest that microwave treatment combined with formic acid resulted in the best results - low loss of glucose in the pre-treatment stage, high scarification rates and good fermentation yields, up to 90% and 100% of the cellulose fraction for Dupont's Accellerase® 1500 and Novozyme's Cellic® CTec2, respectively. Microwave pre-treatment also produced lower levels of fermentation inhibitors such as Furfural and HMF in comparison to autoclave pre-treatments. When fermentability was tested the microwave-formic acid-CTec2 combination was found to give the highest ethanol concentration (12.8 g/l), equivalent to 90% of the theoretical yield, and comparable to more traditional feedstocks. The microwave-formic acid-Accelerase followed, resulting in 12g/l ethanol (~80% of theoretical). Interestingly the sulphuric acid addition, a common pre-treatments additive, gave the worst results in almost all cases (Table 1).

Table 1: Comparison of ethanol production after the different pretreatments-additive-enzyme combinations

Pre-treatment	Enzyme	Additive		
		Water	**FA**	**SA**
Microwave	**Cellic CTec2**	10 g/L 67% (of theoretical)	**13.5** **90%**	2.5 g/L 16.6%
	Accellerase 1500	5 33%	**12** **80%**	2 13.3%
Autoclave	**Cellic CTec2**	0.5 3.3%	5.8 39%	2.5 16.6%
	Accellerase 1500	0.5 3.3%	2.5 16.6%	2.2 15%
$160\left({}^{g}/_{l}\right) \times 18.4\ (\%) \times 0.51\ \left({}^{g}/_{g}\right) = \ 15\ {}^{g}/_{l}$ teoretical EtOH : 18.4% w/w cellulose content in the OMSW; 160 g/L – pretreated solid content in the enzymatic hydrolysis; 0.51 g/g – theoretical ethanol yield from glucose.				

The results presented here suggest that carful choice of pre-treatment conditions (heating method and additives) could result in effective valorization of this complex lignin rich agricultural waste to ethanol.

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