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Effect of steam explosion pretreatment on sugar production by enzymatic hydrolysis of olive tree pruning

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Abstract

Steam explosion pretreatment of olive tree pruning was investigated in pilot-scale, in order to maximize the overall glucose yield. Raw material was firstly characterized in order to determine the content of sugars, ash, extractives, acid insoluble lignin and acetyl groups. Biomass samples were then pretreated at seven different severities and, after pretreatment, solid residues and liquid fractions were separated by filtration. Pretreated solids were submitted to enzymatic hydrolysis for glucose release, using a commercial enzyme preparation. An overall highest theoretical ethanol yield of 14.41 g/100 g dry raw material was achieved at the severity of 4.41.

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Keywords: Steam explosion; Severity; Enzymatic hydrolysis; Olive tree pruning; Ethanol

1. Introduction

Lignocellulosic materials are considered the most promising feedstock that can be transformed into renewable fuels and partially replace fossil fuels. Their use is particularly interesting, because the disposal of solid wastes usually does not have any economic alternative.

Olive tree pruning is one of the most abundant agricultural residue in the Mediterranean countries. In Italy the surface cultivated with olive orchards covers about 970,000 ha and the annual production of olive tree pruning amounts to about 1.6 million t of dry matter [1]. The disposal of pruning residues is necessary to keep fields clean and to prevent propagation of vegetable diseases.

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Nomenclature

RM	Raw Material	XMG	Xylan-Mannan-Galactan
WIS	Water Insoluble Solid	xmg	Xylose-Mannose-Galactose
LF	Liquid fraction	AIL	Acid insoluble lignin

Usually they are eliminated by either burning or grinding and scattering on fields, causing economic cost and environmental concerns [2].Therefore they could be a low-cost source of sugars, convertible into valuable products, such as ethanol.

The bioconversion process for ethanol production from lignocellulosic biomass typically involves the three steps of pretreatment, hydrolysis, and fermentation. A pretreatment step is essential to overcome the natural recalcitrance of lignocellulosic biomass to enzymatic hydrolysis through opening up the lignocellulosic complex and making highest sugar yields as possible.

The main objectives of the pretreatment are removal of lignin and hemicellulose, reduction of the crystallinity of cellulose, and increase in the porosity of the materials. The goal of pretreatment is to attain maximal fermentation yields and rates, by improving the accessibility of enzymes to the cellulose structure without the formation of inhibitors for subsequent hydrolysis and fermentation processes [3].

Pretreatment of olive tree pruning was carried out with steam explosion or liquid hot water even using sulfuric acid as catalyst [2, 4, 5, 6]. However, only one study [5] examined the steam explosion pretreatment without acid impregnation, in which only the temperature of the process was varied, maintaining a constant duration.

The main scope of the present study was to evaluate the effects of steam explosion pretreatment of olive tree pruning by varying the severity of the process in terms of enzymatic hydrolysis of pretreated solids. In particular, in this study Cellic CTec2 was used for the enzymatic hydrolysis, while Celluclast was applied in a previous study by Cara et al. [5]; the recovery of the monomeric sugars in the liquid fraction obtained after pretreatment and the formation of inhibitors were also evaluated.

2. Materials and methods

2.1. Raw material

The olive tree pruning were obtained from an orchard located in Umbria, Italy, from the Moraiolo and Leccino olive tree varieties during the harvest of 2013. The material was air-dried at room temperature in order to reach the equilibrium moisture content (42.2%) and then it was reduced by chipping to an average length of 2 cm.

After steam explosion pretreatment, the raw material (RM) fractionates into two substrates: a water insoluble solid (WIS) and a liquid fraction (LF). Only the first one was subjected to enzymatic hydrolysis.

2.2. Steam explosion pretreatment

The pretreatment of the RM was performed in a batch pilot unit at the Biomass Research Centre, equipped with a thermal insulated stainless steel reactor with a nominal capacity of 10 l and designed to reach maximum operating conditions of 28 bar and 230 °C. A pneumatic valve connects the cylindrical reactor to a 650 l cyclone, equipped with a cooling jacket.

The feedstock is loaded and the steam is added to the vessel through an air-actuated valve, which also automatically maintains the pressure in the vessel at the chosen set point. A manual opening valve at the bottom of the cyclone is used to collect the pretreated material into a removable vessel; it is provided with a 1 mm sieve, in order to obtain the separation between the WIS and the liquid fraction.

The reactor was charged with 700 g of feedstock per batch. Saturated steam from the boiler was then allowed to enter the reactor and to heat the RM to the selected conditions. Seven pretreatment tests were carried out, by increasing the severity of the process (Tab. 1).

Table 1. Experimental conditions for steam explosion pretreatment of olive tree pruning.

Test	Temperature (°C)	Pressure (bar)	Time (min)	Severity
1	190	13	15	3.83
2	200	16	10	3.94
3	200	16	15	4.12
4	210	20	10	4.24
5	210	20	15	4.41
6	220	23.5	10	4.53
7	220	23.5	15	4.71

The treatment severity was calculated by a semi-empirical parameter called the severity parameter, log R_0, according to Eq. (1) [7]:

$$\log(R_0) = \log\left(t \times e^{\frac{(T-T_r)}{14.75}}\right) \qquad (1)$$

where t (min) is the residence time, T (°C) is the reaction temperature, T_r is the base temperature (100 °C), and the number 14.75 is the constant assuming that the overall process is hydrolytic and obeys a first order kinetic law.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of the WIS fraction was performed in Erlenmeyer flasks, using 0.05 M sodium citrate buffer (pH 5.7) and 5% dry matter (w/w) at 50°C, on a bench-top orbital shaker (IKA KS4000 I control) at 100 rpm for 48 hours. After reaching thermal equilibrium, the Cellic®CTec2 enzyme (Novozymes, USA) was added to the flasks at 0.22 g per gram of dry solid pretreated substrate. A 2 ml liquid sample was withdrawn from each flask at 1, 3, 16, 20, 24, 40 and 48 h and analyzed by HPLC for evaluating glucose and sugar degradation products concentration.

The enzyme loading and enzymatic hydrolysis operating conditions were chosen in order to maximize the overall yield of the process, based on preliminary experimental tests; only the influence of the pretreatment conditions was in fact considered in this study.

2.4. Analytical methods

The RM composition was determined according to the National Renewable Energy Laboratory (NREL, Golden, CO) analytical methods for biomass [8]. Extractives content was obtained as the solubilised material after a two-step Soxhlet extraction.

The contents of moisture and ash were determined with the Thermogravimetric Analyzer TGA-701 LECO, according to the UNI methods [9] and [10], respectively.

Dried RM and WIS samples were submitted to a two-step acid hydrolysis: the first step was carried out with 72% (weight basis) sulfuric acid at 30 °C in a water bath for 1 h, followed by dilute acid (4%, weight basis) hydrolysis at 121 °C by autoclaving [8]; the AIL content was taken as the ash free residue after acid hydrolysis.

Monomeric sugars, acetic acid, and formic acid were determined analyzing the post-hydrolysis liquor by high performance liquid chromatography (HPLC-YoungLin 9100) with refractive index detector, equipped with Aminex HPX – 87H column (BioRad, Hercules, CA); the column works at 50°C with water – 0.005 M sulfuric acid as mobile phase, running at 0.6 ml/min.

The efficiency of the enzymatic hydrolysis was calculated using the equation provided by NREL's LAP TP-510-43630 [8], as described below:

$$\text{glucose yield (\%)} = ([\text{glucose}] + 1.053\ [\text{cellobiose}])/(1.111 \times f\ [\text{biomass}]) \qquad (2)$$

where [glucose] and [cellobiose] are respectively glucose and cellobiose concentrations (g/L) in the enzymatic hydrolysis liquor, 1.053 is a multiplication factor that converts cellobiose to equivalent glucose, [biomass] represents dry biomass concentration at the beginning of the enzymatic hydrolysis (g/L), f is the glucan fraction in dry biomass (g/g) and 1.111 is a factor that converts glucan to equivalent glucose.

All analytical determinations were performed in duplicate and average results are shown.

3. Results and discussion

3.1. Raw material composition

The olive tree pruning chemical composition is reported in Table 2. Glucan and AIL content (31.8% and 27.8%) is consistent with data reported by other authors for the same biomass [11].

XMG (xylan, mannan, galactan) account only for 13.5%, while extractives amount to 14.9% of the raw material. However, the cultivar and the production area significantly influence these parameters [12]; this occurrence is evident comparing lignin content to the study by Cara et al. [5] on olive tree pruning obtained in Spain (16.6%).

Table 2. Chemical composition of olive tree pruning (raw material).

Component	% dry matter
Glucan	31.8
XMG	13.5
Arabinan	1.8
Acid insoluble lignin (AIL)	27.8
Extractives	14.9
Ash	0.4
Acetyl groups	2.9
Other	7.0

3.2. Water insoluble solid

Pretreatment of olive tree pruning was carried out using seven different steam explosion conditions, with severities ranging from 3.83 to 4.71.

The composition of the WIS fraction is shown in Table 3. As it can be observed, the solid recovery was in the range between 49% and 66%. The solubilisation of hemicellulose and extractives during the pretreatment led to an increase of glucan concentration in the WIS fraction.

Table 3. Solid recovery (%) and WIS composition (% on dry weight basis) of olive tree pruning pretreated at different severity conditions.

Severity	Solid recovery	Glucan	XMG	Arabinan
3.83	52.95	36.25	13.91	1.14
3.94	49.11	39.43	9.81	0.67
4.12	51.92	40.67	9.63	0.27
4.24	65.85	39.79	7.88	0.50
4.41	57.17	42.19	5.86	0.00
4.53	58.50	38.68	7.84	0.19
4.71	49.40	36.42	9.72	0.71

The oligomeric sugars recovery was calculated as the amount of the sugars in WIS fraction divided by the sugars content in the raw material; results are shown in Figure 1.

Glucan recoveries mean values range from 51% to 70%, showing that some partial glucan solubilisation occurs at any pretreatment conditions.

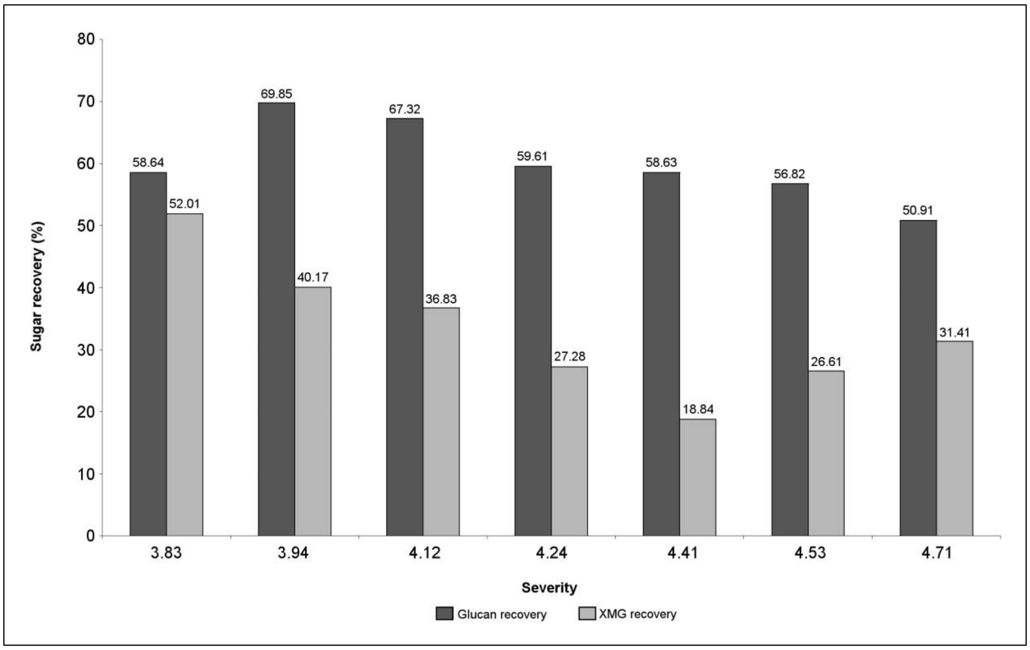

Figure 1. Oligomeric sugars recovery in the WIS fraction at different pretreatment conditions.

The effect of pretreatment conditions on XMG solubility is also displayed in Figure 1. In a steam explosion process, part of xylan, mannan, and galactan is removed from the solid fraction and solubilised into the liquid one.

In the experiments carried out for this study, at the lowest severity of 3.83, the content of XMG in WIS fraction accounted for 13.91%, which corresponded to a recovery rate of 52.01%; then, the recovery yield decreased as long as the severity increased until the minimum value of 18.84% at the severity of 4.41. Glucan content in the WIS fractions is in accordance to ones obtained by Cara et al. [5] at the same pretreatment conditions; moreover, glucan recovery is lower and this fact is probably related to much higher lignin content in the raw material that doesn’t allow the deconstruction of cellulosic matrix.

3.3. Liquid fraction from pretreatment

Monomeric sugars recovery is shown in Figure 2, as the amount of the sugars in liquid fraction divided by the sugars content in the raw material.

Xmg recovery in the liquid fraction followed a similar trend when compared to glucan recovery in WIS fraction: maximum xmg recovery rate of 35.61% was obtained at severity of 4.12 while, at the others conditions, values stay constant around 23-25%.

However, at all conditions, xmg recovery in the liquid fractions is low when compared to other agricultural residues like sunflower stalks [13], for which the values range between 30% and 40%, but the trend is similar. It can be explained either by xmg retention in the WIS fraction, degradation of hemicellulose sugars, and xmg loss as volatile compounds in the outlet steam [14].

In addition, glucose recovery yields amounted in all cases below 15% (Figure 2), according to low solubility of glucose in liquid fraction.

The composition of the liquid fraction resulting from pretreatment in terms of monomeric sugars and organic acids content in 100 g of raw material is reported in Table 4.

Liquid fractions also contained non-sugars components as acetic acid and formic acid, that constitute inhibitors for yeast growth under selected conditions [15].

In general, their content grows with the pretreatment severity, with an highest value obtained at a severity of 4.12; these values remain lower than the critical values.

These degradation products were previously identified in a similar range in liquid fractions from steam pretreated sunflower stalks [13].

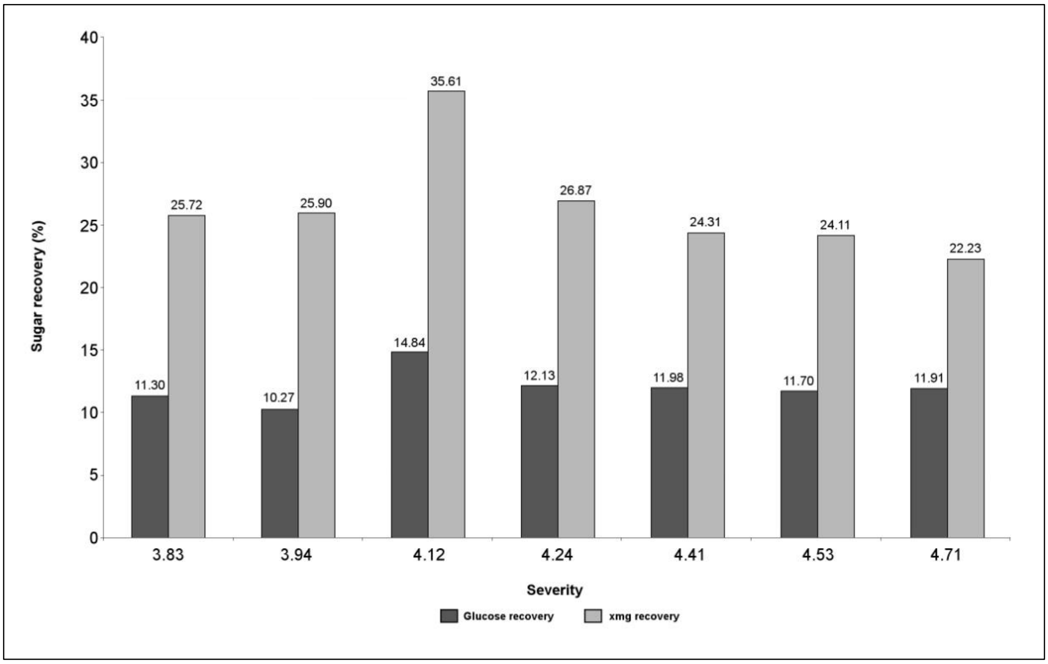

Figure 2. Monomeric sugars recovery in the liquid fraction at different pretreatment conditions.

Table 4. Composition (g/100 g dry raw material) of the liquid fraction resulting from steam explosion pretreatment at different severity conditions.

Severity	Glucose	xmg	Arabinose	Formic acid	Acetic acid
3.83	3.99	3.93	0.62	0.32	1.08
3.94	3.63	3.96	0.59	0.27	1.09
4.12	5.24	5.44	0.76	0.82	1.97
4.24	4.29	4.11	0.60	0.49	1.47
4.41	4.23	3.72	0.55	0.67	1.87
4.53	4.13	3.69	0.54	0.63	1.73
4.71	4.21	3.40	0.57	0.70	1.72

3.4. Glucose production from enzymatic hydrolysis

WIS fractions were submitted to enzymatic hydrolysis, in order to evaluate the effectiveness of the steam explosion pretreatment for olive tree pruning substrate.

Figure 3 shows glucose yield after 48 hours at different pretreatment conditions, calculated with equation (2).

The maximum glucose yield (86%) is obtained at severity of 4.41; this value decreases to 50-60% at lower severity factors, while for stricter conditions is quite lower than highest obtained value.

In particular, lowest glucose EH yields occur at severity of 3.83, that is characterized by higher glucan recovery in WIS fraction from pretreatment, but also XMG sugars (see Figure 1).

It seems clear that pretreatment conditions are crucial for accessibility of enzymes to lignocellulosic matrix; this occurrence should be explained with the higher solubility of hemicellulosic sugars at severity conditions exceeding 4.12, that enhances enzyme digestibility of the substrate.

The enzymatic hydrolysis yields are better than hydrolysis yields obtained by Cara et al. [5], due to another commercial enzyme and different enzyme loading used in the tests.

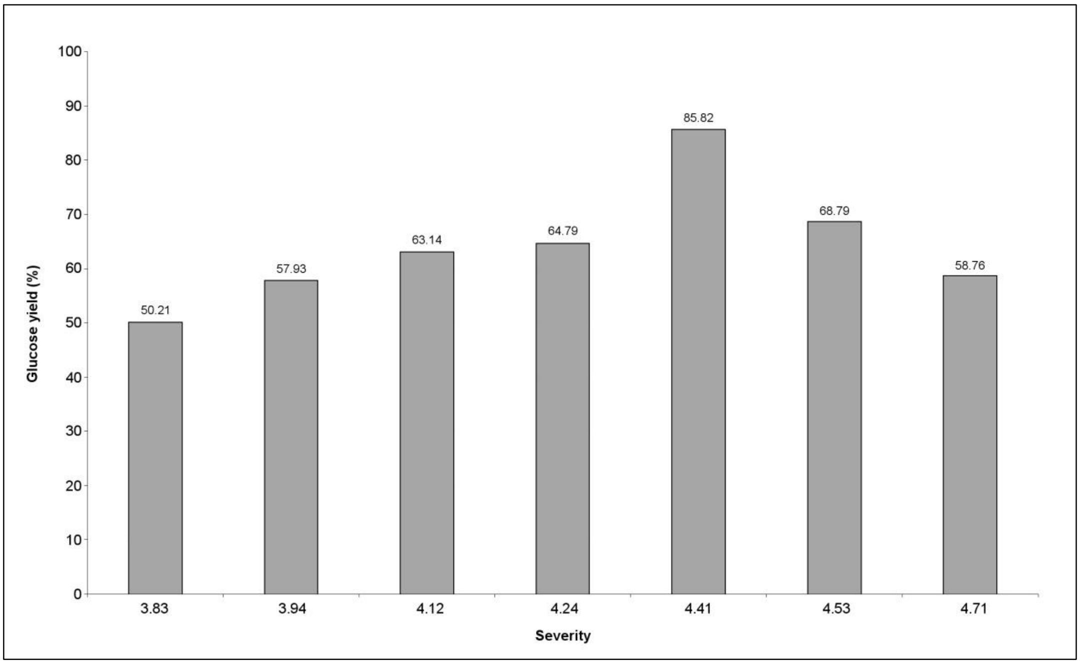

Figure 3. Glucose yields from enzymatic hydrolysis of WIS after 48 hours at different pretreatment conditions

3.5. Potential ethanol production

In order to assess theoretical overall ethanol yield (ethanol yield considering all sugar equally converted) from olive tree pruning processed by steam explosion and subsequent enzymatic hydrolysis, the overall sugars yield is calculated as the sum of sugars in liquid fraction from pretreatment and glucose released from enzymatic hydrolysis.

In Table 5 sugars in liquid fraction, glucose from hydrolysis, overall sugar yield, and theoretical ethanol yield are reported for each pretreatment condition.

Results show that pretreatment conditions play a key role on overall sugar and ethanol yields: the best performance of sugars recovery is obtained at severity of 4.41, equal to 53.7% of total sugars content in raw material (52.6g/100 g).

Therefore, a maximum of 144.1 g of ethanol should be obtained from 1 kg of raw material at the severity of 4.41; it was calculated by applying a stoichiometric conversion factor of 0.51 from glucose to ethanol.

Comparing potential ethanol yields to ones obtained from olive tree pruning in the study of Cara et al. [5] at the same severity conditions, these are quite higher probably due to different enzymatic hydrolysis conditions performed.

Table 5. Sugars (g sugars/100 g dry raw material) and ethanol yields (g ethanol/100 g dry raw material) from olive tree pruning at varying pretreatment conditions

Severity	Sugars in liquid	Glucose from EH	Overall sugars yield	Overall potential ethanol yield
3.83	8.55	11.56	20.11	10.26
3.94	8.17	15.89	24.06	12.27
4.12	11.44	16.69	28.14	14.35
4.24	9.00	15.17	24.16	12.32
4.41	8.50	19.76	28.25	14.41
4.53	8.36	15.35	23.71	12.09
4.71	8.18	11.75	19.93	10.16

4. Conclusion

Steam explosion pretreatment is an interesting strategy for second generation ethanol production from olive tree pruning.

The main purpose of this study was to define the optimal steam explosion pretreatment conditions, in order to maximize the sugar yield from enzymatic hydrolysis.

Taking in account glucan recovery and enzymatic hydrolysis yield, the best glucose yield (86%) was obtained at the severity of 4.41 (T = 210 °C, residence time = 15 min), corresponding to 19.76 g glucose/100 g dry raw material.

In terms of overall sugars yield and considering sugars from liquid fraction from pretreatment, the best pretreatment condition gives a value of 28.25 g sugars/100 g dry raw material, that allows to produce theoretically 144.1 g ethanol from 1 kg of dry olive tree pruning.

Future improvements of this work are the enhancement of hemicellulosic sugars recovery, the delignification of raw material and the enhancement of the enzymatic hydrolysis.

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