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Ethanol Production From Pretreated Olive Tree Wood and Sunflower Stalks by an SSF Process

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Abstract

Olive tree wood and sunflower stalks are agricultural residues largely available at low cost in Mediterranean countries. As renewable lignocellulosic materials, their bioconversion may allow both obtaining a value-added product, for fuel ethanol, and facilitating their elimination. In this work, the ethanol production from olive tree wood and sunflower stalks by a simultaneous saccharification and fermentation (SSF) process is studied. As a pretreatment, steam explosion at different temperatures was applied. The water insoluble fractions of steam-pretreated sunflower stalks and steamed, delignified olive tree wood were used as substrates at 10% w/v concentration for an SSF process by a cellulolytic commercial complex and *Saccharomyces cerevisiae*. After 72-h fermentation, ethanol concentrations up to 30 g/L were obtained in delignified steam-pretreated olive tree wood at 230°C and 5 min. Sunflower stalks pretretated at 220°C and 5 min gave maximum ethanol concentrations of 21 g/L in SSF experiments.

Index Entries: Ethanol; olive tree wood; sunflower stalks; SSF; pretreatment.

Introduction

Olive tree wood and sunflower stalks are among the main agricultural residues found in Mediterranean countries whose conversion into fuel ethanol has considerable advantages. On the one side, these materials must be eliminated, thus preventing propagation of vegetal diseases and keeping fields clear; till date, disposal methods include burning or grinding and scattering, with associated costs and no economical alternatives. On the other side, the huge amount annually generated and their lignocellulosic

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nature make both residues a major low-cost renewable source of sugars that may be converted into valuable products, for example, fuel ethanol.

More than 8×10^6 ha of olive trees are cultivated in the world, most of them in Mediterranean countries *(1)*. Pruning is an essential operation in olive tree cultivation to eliminate less productive branches and prepare trees for the next crop. This action generates an annual volume of lignocellulosic residues estimated at 3000 kg/ha *(2)*. Olive tree pruning is composed of leaves, thin branches, and wood in different proportions, depending on culture conditions, production, and local uses. A typical olive tree pruning lot includes 30% of wood that is separated and put to domestic use as firewood. Sunflower is the fourth largest source of oil-seeds worldwide, representing around 23×10^6 ha of cultivated land *(1)*. Stalks, heads, and leaves account for 3–7 t of dry matter/ha *(3)* and are left in the fields after harvesting for elimination.

The utilization of lignocellulosic residues of these types in a bioconversion process requires pretreatment of the raw material to improve the release of sugars from both the hemicellulose and the cellulose fractions. In steam-explosion pretreatment, biomass is exposed to pressurized steam followed by rapid reduction in pressure. The treatment results in substantial breakdown of the lignocellulosic structure, hydrolysis of the hemicellulosic fraction, depolymerization of the lignin components, and defibration. Therefore, the accessibility of the cellulose components to degradation by enzymes is greatly increased *(4)*.

The production of ethanol from pretreated material may be accomplished either by sequential hydrolysis and fermentation or by a simultaneous saccharification and fermentation (SSF) process. In the SSF process, end product inhibition can be overcome as glucose is fermented as it is formed; another advantage is that a single reactor is used for both saccharification and fermentation. On the contrary, the enzymatic reaction in SSF process is operated at a temperature lower than its optimum level owing to the mismatch in optimum temperatures for hydrolysis (approx 50°C) and fermentation (approx 30°C) *(5)*.

In previous works *(6,7)*, we analyzed the influence of steam-explosion pretreatment on sugar recoveries in both solids and liquids and the enzymatic hydrolysis of solids from sunflower stalks and olive tree wood, as a first step in the residue-to-ethanol bioconversion process. The objective of this work is to determine the feasibility of the production of ethanol by the SSF of steam-exploded sunflower stalks and olive tree wood.

Methods

Raw Materials

Olive tree wood and sunflower stalks were collected locally after crop harvesting, air-dried at room temperature to equilibrium moisture content (approx 10% for olive tree wood and approx 8.2% for sunflower stalks),

milled using a laboratory hammer mill (Retsch, Germany) to a particle size smaller than 10 mm, homogenized in a single lot, and stored at room temperature until used.

Steam Explosion Pretreatment

Steam explosion of raw material was carried out in a batch pilot unit based on Masonite technology using a 2-L reaction vessel designed to reach a maximum operating pressure of 4.12 MPa *(8)*. The reactor was charged with 200 (olive tree wood chips) or 150 (sunflower stalks) g of feedstock (dry matter) per batch and heated to the desired temperature with saturated steam for 5 min. Assayed steam temperatures were 190, 210, 230, and 240°C for olive tree wood and 180, 190, 200, 210, 220, and 230°C in the case of sunflower stalks. The exploded material was recovered in a cyclone and after cooling to about 40°C, filtered for liquid and solid recovery. Dried solids were weighed, analyzed for sugars and lignin composition, and used in enzymatic hydrolysis and SSF tests.

Alkaline-Peroxide Delignification

The water-insoluble fiber from steam-explosion pretreatment of olive tree wood was delignified using a hot alkaline peroxide treatment protocol adapted from Yang et al. *(9)* in 1% (w/v) H_2O_2 solution in 4% (w/v) solids concentration. The pH was adjusted to 11.5 using 4 *M* NaOH. The treatment lasted for 45 min at 80°C, and then the suspension was filtered and water-washed until neutral pH. Delignified, dried solid was weighed and analyzed for carbohydrates and lignin composition.

Enzymatic Hydrolysis

The washed water-insoluble residues from steam-pretreated sunflower stalks and those from steam-pretreated, delignified olive tree wood were enzymatically hydrolyzed by a cellulolytic complex (Celluclast 1.5 L) kindly provided Novozymes A/S (Bagsvaerd, Denmark). Cellulase enzyme loading was 15 FPU/g substrate. Fungal β-glucosidase (Novozyme 188, Novozymes A/S) was used to supplement the β-glucosidase activity with an enzyme loading of 12.6 international unit (IU)/g substrate. Enzymatic hydrolysis was performed in 0.05*M* sodium citrate buffer (pH 4.8) at 50°C on a rotary shaker (Certomat-R, B-Braun, Germany) at 150 rpm for 72 h and at 10% (w/v) pretreated material concentration. Samples were taken every 24 h for glucose concentration determination. All enzymatic hydrolysis experiments were performed in duplicate and average results are given.

Simultaneous Saccharification and Fermentation

The washed water-insoluble residues from steam-pretreated sunflower stalks and that from steam-pretreated, delignified olive tree wood were submitted to an SSF process. The same cellulolytic complex (15 FPU/g

substrate enzyme loading) supplemented by β-glucosidase (12.6 IU/g substrate activity) as in the enzymatic hydrolysis was used for saccharification. Fermentation was performed by *Saccharomyces cerevisiae* (DER-CIEMAT culture collection no. 1701).

SSF experiments were carried out in 100-mL Erlenmeyer flasks, each containing 25 mL of fermentation medium in 0.05*M* sodium citrate buffer (pH 4.8) at 35°C on a rotary shaker (Certomat-R, B-Braun, Germany) at 150 rpm for 72 h and at 10% (w/v) pretreated material concentration. The fermentation medium contained 5 g/L yeast extract, 2 g/L NH_4Cl, 1 g/L KH_2PO_4, and 0.3 g/L $MgSO_4 \cdot 7H_2O$. Flasks were inoculated with 4% (v/v) *S. cerevisiae* culture obtained by growing the organism on a rotary shaker at 150 rpm for 20 h at 35°C in the same growth medium with glucose (30 g/L). Samples were taken every 24 h and analyzed for glucose and ethanol production. Average results of duplicate experiments are shown.

Analytical Methods

The composition of solid materials was determined according to the National Renewable Energy Laboratory (NREL, Golden, CO) analytical methods for biomass *(10)*. Cellulose and hemicellulose content were determined by HPLC with a Waters 2695 liquid chromatograph with refractive index detector. An AMINEX HPX-87P carbohydrate analysis column (Bio-Rad, Hercules, CA) operating at 85°C with deionized water as the mobile-phase (0.6 mL/min) was used. Glucose concentration from enzymatic hydrolysis samples was measured by an enzymatic determination glucose assay kit (Sigma GAHK-20).

Ethanol was measured by gas chromatography, using a HP 5890 Series II apparatus equipped with an Agilent 6890 series injector, a flame ionization detector, and a column of Carbowax 20 *M* at 85°C. The injector and detector temperature was maintained at 150°C. All analytical determinations were performed in duplicate and average results are shown. Relative standard deviations were in all cases below 5%.

Results and Discussion

Raw Material Composition

Table 1 summarizes the composition of raw materials. Although olive tree wood and sunflower stalks are quite different, the cellulose and hemicellulose contents are similar. Xylose is the main sugar of the hemicellulosic fraction in both materials (approx 80%). Lignin content is smaller for sunflower stalks. The main differences are extractives and ash contents.

Pretreatments and Enzymatic Hydrolysis Assays

Both raw materials were subjected to steam-explosion pretreatment for 5 min at different temperatures. In addition, the water-insoluble fiber obtained from steam explosion of olive tree wood was further submitted to

Table 1
Raw Material Composition (% Dry Matter)

Composition	Olive tree wood	Sunflower stalks
Cellulose as glucose	34.4	33.8
Hemicellulosic sugars	20.3	20.2
Xylose	16	16.1
Mannose	1.4	1.7
Galactose	1	1.4
Arabinose	1.9	1
AIL	18	14.6
Acid-soluble lignin (ASL)	2.4	2.7
Acetyl groups	1.8	2.5
Extractives	15.4	6.9
Ash	1.7	9.6

Table 2
Composition of Water-Insoluble Fiber (%) Resulting From Steam Explosion of Sunflower Stalks and Olive Tree Wood at Different Pretreatment Temperatures and Composition of Exploded, Delignified Olive Tree Wood (%)

Steam-explosion temperature (°C)	Olive tree wood				Sunflower stalks					
	190	210	230	240	180	190	200	210	220	230
Steamed water-insoluble fiber										
Total gravimetric recovery	54.2	44.1	44.3	40.4	65.1	55.3	45.8	43.8	38.3	35.5
Glucose	50.5	55.1	64.4	56.2	45.5	52	56.8	58.1	60.6	60.1
Hemicellulosic sugars	13.8	6.8	2.9	2.6	21.5	18.1	11.6	6.4	5.9	2.7
Acid-insoluble lignin	31.4	34.4	35.5	38.2	22.1	24.7	27.6	30.1	34	35.4
Steamed, delignified solid										
Delignified solid recovery	69.1	66.9	57.8	58.9						
Glucose	64	77.6	90.3	85.8						
Hemicellulosic sugars	11.4	6.9	2.8	3.6						
Acid-insoluble lignin	20.7	16.7	11.1	12.5						

an alkaline peroxide delignification step. The complete studies of the influence of pretreatments on sugar recoveries in both solids and liquids and the enzymatic hydrolysis of solids are reported elsewhere *(6,7)*. As a starting point for the research on ethanol production from both residues, the main results of enzymatic hydrolysis performance are briefly presented here.

Table 2 shows, as a function of pretreatment temperature, the composition of water-insoluble fibers obtained after steam explosion of sunflower stalks and olive tree wood. For both lignocellulosic residues, a decrease

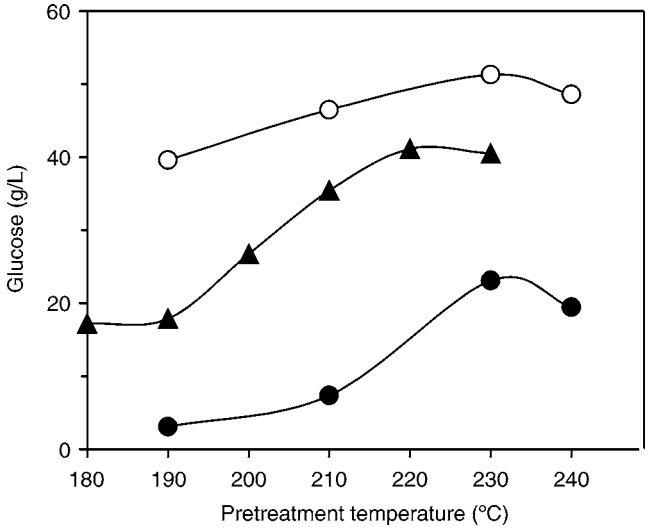

Fig. 1. Glucose concentration (g/L) obtained by enzymatic hydrolysis (72 h, 10% substrate loading) of steam-exploded sunflower stalks (▲), steam-exploded olive tree wood (●), and steam-exploded and delignified olive tree wood (○) at different temperature conditions for steam explosion.

of total gravimetric recovery (solids remaining after pretreatment divided by original oven-dried weight) was detected as the pretreatment temperature increased. This is mainly attributed to the solubilization of hemicellulosic fraction; the hemicellulosic-derived sugars recovery in the water-insoluble fiber decreased as long as the steaming temperature increased. The contents in acid-insoluble lignin (AIL) of the pretreated residue showed slight solubilization increasing, in general, with pretreatment temperature. The cellulose content in the solid increased with pretreatment temperature except for the highest one. The maximum cellulose content for olive tree wood (64.4%) was attained at 230°C and for sunflower stalks (60.6%) at 220°C. Partial solubilization of cellulose was detected for both materials, and hence the cellulose recovery diminished in general as pretreatment temperature was increased.

Enzymatic hydrolysis assays were performed on steam-pretreated residues with a cellulase complex (Celluclast 1.5L) supplemented with β-glucosidase (Novozyme 188). Figure 1 shows glucose concentration obtained after a 72-h period of enzymatic action. Enzymatic hydrolysis yields (determined from glucose obtained in the enzymatic hydrolysis divided by the potential glucose in the pretreated material) depended on the temperature of pretreatment. For sunflower stalks, yields increased as the temperature was raised up to 220°C (67.8%) with a light decrease at 230°C. Regarding steam-pretreated olive tree wood, the enzymatic hydrolysis results were quite poor. The maximum enzymatic yield was just 35.9% for a steam-pretreatment temperature of 230°C. In order to improve the enzymatic hydrolysis of olive tree wood, a delignification step on the steamed solid residue was done. The composition of the

insoluble fiber resulting after alkaline-peroxide delignification is shown in Table 2.

As can be observed in Fig. 1, results corresponding to enzymatic hydrolysis on exploded and delignified olive tree wood were much better than those obtained with just steam pretreatment. For all steaming temperatures, considerable improvements in the susceptibility to enzymatic hydrolysis were evidenced. Nevertheless, glucose inhibition was detected in solids pretreated at the highest temperatures in which cellulose content was elevated.

Simultaneous Saccharification and Fermentation

Solid residues from steam-exploded sunflower stalks and steam-exploded, delignified olive tree wood were further submitted to an SSF process by *S. cerevisiae* at a temperature of 35°C and 10% substrate concentration. In the SSF process, the glucose released by the enzymatic attack is simultaneously converted into ethanol by the yeast, thus reducing enzyme inhibition from glucose. Figure 2 shows ethanol concentrations determined every 24 h in a 72-h SSF period for both lignocellulose materials. Culture samples were also analyzed for glucose content (data not shown); in all cases very low glucose concentrations were obtained, showing a good yeast fermentation performance.

In both residues, the concentration of ethanol increased with time, regardless the pretreatment temperature. At a given time, the concentration of ethanol generally increased with pretreatment temperature until the maximum is reached. Maximum ethanol concentrations of 21 and 29.4 g/L were obtained for sunflower stalks pretreated at 220°C and olive tree wood pretreated at 230°C and delignified, respectively. The higher ethanol concentrations obtained in olive tree wood agreed with the higher glucose content of this solid remaining after the delignification step. This fact can also be confirmed in Table 3, in which ethanol productivity values determined at each sample time are shown. A maximum ethanol productivity at 72 h of 0.291 g/L·h was calculated for SSF-sunflower stalks experiment from 220°C pretreatment temperature. In the case of olive tree wood, ethanol productivities were higher, the maximum value (0.409 g/L·h) being found for the SSF experiment performed with the solid pretreated at 230°C. Comparison of the ethanol productivities calculated after 72-h fermentation time shows a decrease to the half of those obtained after 24 h. Kádár et al. *(11)* found ethanol productivities after 72 h of 0.197 and 0.125 g/L·h when applying SSF process by *S. cerevisiae* to paper-industry wastes. Our results compare with the work of Alfani et al. (*12*), who reported 0.837 g ethanol/L·h after 30 h SSF time using steam-exploded wheat straw as substrate; these authors remark that SSF ethanol productivities are much higher than ethanol productivities obtained by separate hydrolysis and fermentation, making SSF process more favorable from an industrial point of view.

Although the highest ethanol productivities are obtained after 24 h, high ethanol yields are also essential in order to get a more efficient use

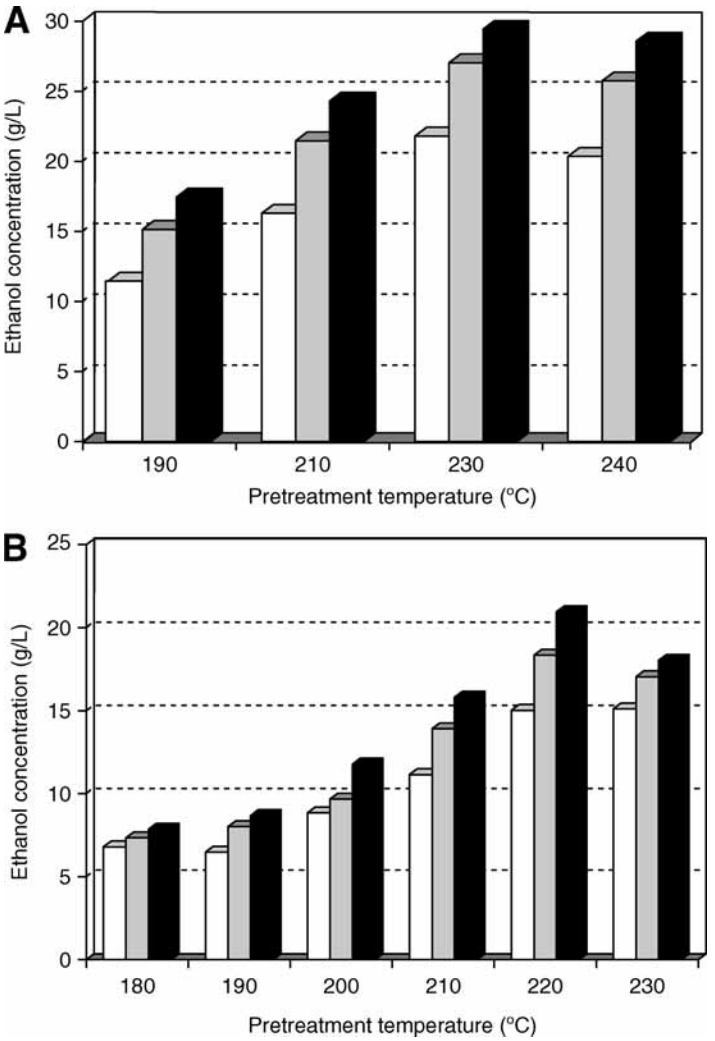

Fig. 2. Concentration of ethanol solutions (g/L) obtained in the SSF process after 24 (white bar), 48 (gray bar), and 72 h (black bar). **(A)** Steam-exploded, delignified olive tree wood. **(B)** Steam-exploded sunflower stalks.

of raw material. Ethanol yields obtained in the SSF process, calculated from ethanol concentration data, are shown in Tables 4 and 5. $Y_{p/s}$ stands for mass of ethanol obtained at different times divided by 100 g of cellulose in pretreated raw material. SSF-ethanol yields were calculated as a percentage of the maximum theoretical ethanol yield by assuming that all the potential glucose in the starting material is available for fermentation. Maximum values of 65.2% and 67.7% were reached for olive tree wood and sunflower stalks, respectively. These results are in the order of those reported by Alfani et al. *(12)*; SSF–ethanol yields close to 68% of theoretical were achieved with steam-exploded (217°C, 3 min) wheat straw washed with NaOH solutions. Stenberg et al. *(13)* in a study on the SSF

Table 3
Ethanol Productivities (g/L·h) at Different Times for SSF Experiments With Steam-Pretreated Sunflower Stalks and Steam-Pretreated, Delignified Olive Tree Wood

Pretreatment Temperature (°C)	Sunflower stalks		
	24 h	48 h	72 h
180	0.283	0.153	0.109
190	0.270	0.167	0.121
200	0.368	0.201	0.164
210	0.465	0.290	0.220
220	0.626	0.382	0.291
230	0.630	0.355	0.250
	Olive tree wood		
	24 h	48 h	72 h
190	0.478	0.315	0.242
210	0.679	0.447	0.338
230	0.908	0.563	0.409
240	0.848	0.536	0.397

Table 4
SSF-Ethanol and Enzymatic Hydrolysis Yields After 24, 48, and 72 h for Steam-Pretreated, Delignified Olive Tree Wood at Different Pretreatment Temperatures

Pretreatment temperature (°C)	Y p/s[a]			SSF-ethanol yield[b]			Enzymatic hydrolysis yield[c]		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
190	17.9	23.7	27.3	35	46.4	53.4	41.2	52	61.8
210	21	27.7	31.3	41.1	54.2	61.2	42.9	52.8	60
230	24.1	29.9	32.6	47.2	58.5	63.8	36.1	50.5	56.8
240	23.7	30	33.3	46.4	58.7	65.2	38.5	51.3	56.6

[a]Yield product/substrate, g ethanol/100 g cellulose (expressed as potential glucose) in pretreated raw material.
[b]As percentage of theoretical ethanol yield (0.511 g ethanol/g glucose).
[c]As percentage of glucose obtained by enzymatic hydrolysis at 50°C from total potential glucose in the pretreated material.

of steam-pretreated softwood, obtained, as a maximum ethanol yield in the SSF step by *S. cerevisiae*, 82% of theoretical using an enzyme loading of 32 FPU/g substrate and 5% (w/v) substrate concentration, instead of 15 FPU/g and 10% (w/v) substrate concentration used in our work.

The feasibility of using 10% (w/v) substrate concentration in SSF is considered to be relevant, since earlier studies on this process have reported the limiting effect of elevated substrate concentrations owing to

Table 5
SSF–Ethanol and Enzymatic Hydrolysis Yields After 24, 48, and 72 h for Steam-Pretreated Sunflower Stalks at Different Pretreatment Temperatures

Pretreatment temperature (°C)	Y p/s[a]			SSF-ethanol yield[b]			Enzymatic hydrolysis yield[c]		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
180	14.9	16.2	17.3	29.2	31.7	33.8	33.3	35.6	37.9
190	12.5	15.4	16.7	24.5	30.1	32.7	31.4	33	34.5
200	15.5	17	20.7	30.3	33.3	40.5	38.9	43.8	46.9
210	19.2	24	27.2	37.6	47	53.2	49.7	57.6	60.9
220	24.8	30.2	34.6	48.5	59.1	67.7	55	60.4	67.8
230	25.1	28.4	30	49.1	55.6	58.7	57.1	64.7	67.3

[a]Yield product/substrate, g ethanol/g cellulose (expressed as potential glucose) in pretreated raw material.
[b]As percentage of theoretical ethanol yield (0.511 g ethanol/g glucose).
[c]As percentage of glucose obtained by enzymatic hydrolysis at 50°C from total potential glucose in the pretreated material.

difficulties in stirring the material, mass transfer problems, or high ethanol inhibiting concentration *(11,14)*. In fact, in most SSF experiments reported in the literature, substrate concentrations lower than 10% (w/v) are employed. On the other hand, low concentrations of substrate would increase the capital cost of equipment and would yield low concentrations of ethanol for distillation *(15)*. As far as the enzyme loading is concerned, high enzyme concentrations can increase conversion yields *(13)*; increasing cellulase loading from 15 to 45 FPU/g substrate in SSF process for ethanol production from paper material resulted in improved ethanol yields coming up from 56.4% to 70.4% *(15)*. Nevertheless, the economic implications of higher cellulase concentrations must be carefully considered.

In an attempt to overcome the problem of the difference in optimum temperature of the cellulases and the fermenting microorganisms, thermotolerant yeast strains, such as *Kluyveromyces marxianus*, have been assayed for ethanol production by SSF process. Nevertheless, Kádár et al. *(11)* found that there was no significant difference between *S. cerevisiae* and *K. marxianus* when comparing ethanol yields in SSF conversion of industrial paper wastes. The SSF–ethanol yields were in the range of 0.31–0.34 g/g for both strains used. Ballesteros et al. *(8)* studied the steam explosion of several herbaceous residues and woods and the subsequent SSF-ethanol production by *K. marxianus* reporting maximum ethanol yields in the range 0.31–0.36 g/g (60.9–71.2% of the theoretical) with maximum ethanol contents from 16 to 19 g/L in fermentation media. Our maximum ethanol yields reported here, obtained at the same conditions of enzyme loading and substrate concentration, are in the same range, but we attained higher ethanol concentrations (21 and 29.4 g/L for sunflower stalks and olive tree wood, respectively) because of a higher cellulose content in pretreated materials.

For comparison purposes, Tables 4 and 5 show enzymatic hydrolysis yields, determined from glucose concentration values (Fig. 1) and the potential glucose concentration in the pretreated material (Table 2) for olive tree wood and sunflower stalks, respectively. Results for olive tree wood indicate that cellulose conversion yields in SSF are higher than enzymatic hydrolysis yields for all pretreatment temperatures except the lowest one. The improvement in SSF-cellulose conversion is greater for 230 and 240°C pretreatment temperatures at which glucose inhibition in the enzymatic hydrolysis for a 10% substrate concentration had been detected. Enzymatic hydrolysis yields at 2% substrate loading (data not shown) resulted in similar values as those in SSF, confirming that the consumption of glucose by the yeast as it is formed alleviates the inhibiting effect of cellulase activity *(16)*. In comparison to enzymatic hydrolysis, pretreated sunflower stalks submitted to SSF led to lower cellulose conversion except at the pretreatment temperature of 220°C, in which cellulose conversion was similar by both procedures. It must be pointed out that, even in the case of equivalent yields, the SSF process is preferable to the separate hydrolysis and fermentation process (SHF) because using a single bioreactor reduces investment and operation costs *(12)*. Another advantage is that the SSF process leads to a significantly increased ethanol productivity *(18)*. Low sugar is also available for invasing organisms.

Overall Process Considerations

The main objective of the present study was to evaluate ethanol production from pretreated sunflower stalks and olive tree wood by an SSF process. According to the compositions shown in Table 1, the maximum ethanol yield attainable if all sugars present in the raw material were converted into ethanol is 17.6 g/100 g olive tree wood and 17.3 g/100 g sunflower stalks, equivalent to 223.1 L/t and 219.3 L/t, respectively. In order to determine the amount of ethanol actually obtainable under the assayed operational conditions, first, the glucose recovery must be taken into account in the steam-explosion-pretreated solid; next, and only in the case of olive tree wood, the glucose material recovery after delignification; and, finally, the ethanol yield attained in the SSF process. From data in Table 2, the values of glucose content in the pretreated material are multiplied by the total gravimetric recovery of pretreatment step (and by the delignified solid recovery in the case of olive tree wood) in order to refer results to initial raw material; finally, the obtained values are multiplied by SSF yields (Yp/s in Tables 4 and 5), leading to the overall ethanol yield that may be obtained from 100 g of each raw material. The results are shown in Fig. 3. From 100 g of sunflower stalks, submitted to steam-explosion pretreatment at 220°C and further SSF by *S. cerevisiae* on the solid-pretreated residue, a maximum of 8 g of ethanol is obtained, representing 46% of the theoretical referred to raw material. Increasing the temperature of steam pretreatment

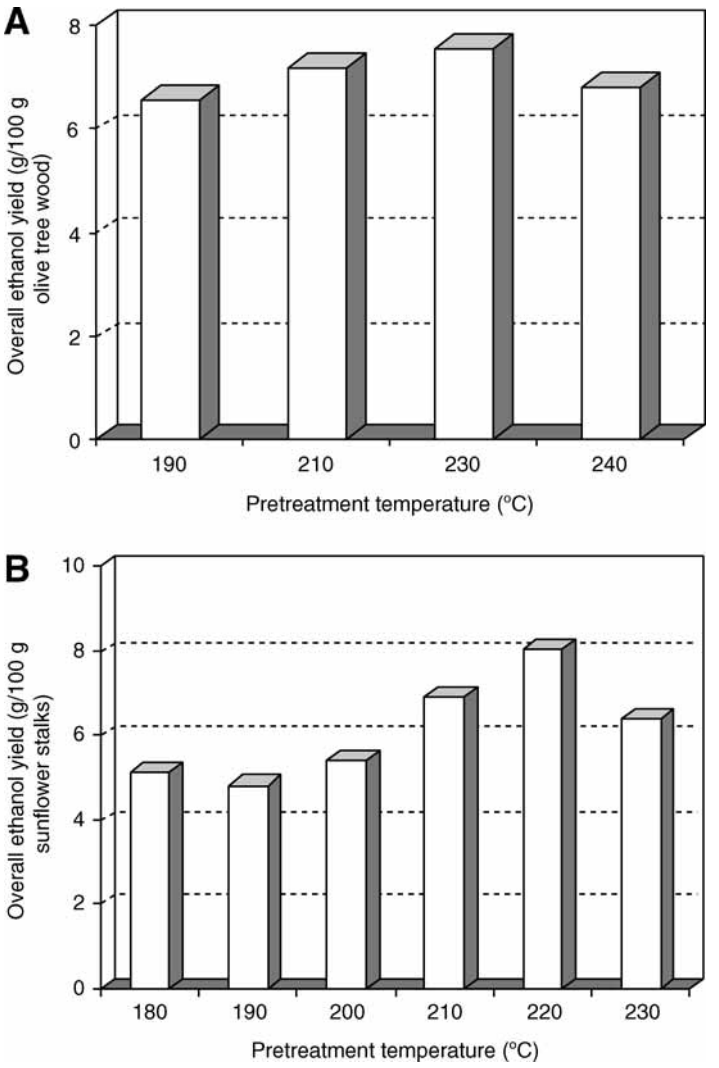

Fig. 3. Overall ethanol yield referred to raw material at different steam-explosion temperatures. **(A)** Olive tree wood (maximum yield attainable: 17.6 g ethanol/100 g raw material). **(B)** Sunflower stalks (maximum yield attainable: 17.3 g ethanol/100 g raw material).

from 180°C to 220°C resulted in an increase in overall ethanol yield because of improvement in the susceptibilty of pretreated material to enzymatic action. Beyond 220°C, the decline in overall ethanol yield is attributed to lower recovery of cellulose.

From 100 g of olive tree wood, steam-explosion pretreatment at 230°C, further alkaline-peroxide delignification on the solid-pretreated residue, and submission to SSF process, 7.5 g of ethanol is obtained, which corresponds to 43% of theoretical if all glucose in the raw material was available for fermentation. Slight differences in the overall ethanol yield are found for other assayed steam temperatures.

Comparing the global process for both residues, process yields are a little better for sunflower stalks and the global scheme implies only two steps (steam explosion and SSF) instead of three for olive tree wood. On the contrary, more concentrated ethanol solutions are obtained for the latter as a consequence of a higher cellulose content in the pretreated solid after delignification. Thus, 1 L of ethanol may be obtained from 2.7 kg of steamed, delignified olive tree residue, comparing to 3.8 kg of steam-exploded sunflower stalks. This must be taken into account for an eventual scale-up of the process. Referred to a raw material basis, 95.1 L ethanol/t olive tree wood and 101.4 L ethanol/t sunflower stalks may be obtained.

As a conclusion, SSF process is an interesting option for producing ethanol from both agricultural residues. Nevertheless, it is assumed that yields obtained are all relatively low for industrial ethanol production processes and that further improvements in terms of increased ethanol yieds are necessary to achieve an economical process. Research on advanced reactor configuration or on the utilization of the whole slurry generated in the pretreatment step appear to be a promising means to increase final ethanol yieds in SSF process.

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