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Increase in Fermentable Sugars of Olive Tree Pruning Biomass for Bioethanol Production: Application of an Experimental Design for Optimization of Alkaline Pretreatment

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Abstract

Olive Tree Pruning (OTP) biomass can be considered a suitable source of fermentable sugars for the production of second-generation bioethanol. The present study proposes a remarkable alternative for the valorization of olive tree pruning residues. OTP biomass was processed using a sequential calcium hydroxide pretreatment/enzymatic hydrolysis. A 2^{4-1} half fractional factorial design was adopted for the screening of process variables and a central composite design was used for the optimization stage. Temperature and lime loading resulted statistically significant. The following optimal conditions were obtained: 0.01 g of $Ca(OH)_2$/g of dry material, 20 g of H_2O/g of dry material at 160 °C for 2 h. The mathematical model that governs this alkaline pretreatment was obtained with a 76% adjusted determination coefficient, which means that it is a good representation of the process. Under optimal operating conditions, 13% of the cellulose and 88% of the hemicellulose was solubilized. Moreover, the fermentable sugar content increased 1800% compared with the initial conditions, obtaining 240 g of glucose per kg of OTP residue. The fermentable sugars obtained after the calcium hydroxide pretreatment and enzymatic hydrolysis of OTP biomass yielded 2.8 g of ethanol/100 g of raw material.

Keywords

Olive Tree Pruning (OTP), calcium hydroxide pretreatment, optimum conditions, fermentable sugars, bioethanol

1 Introduction

Energy policies that have been implemented in recent years are oriented towards the exploitation of renewable energy sources, greatly encouraged by economic and tax incentives [1]. These policies are leading to significant changes through the combination of different types of energy, progressively replacing conventional fuels by renewable energy sources. Worldwide, countries intend to reach objectives on renewable energies with a relevant paradigm change in the exploitation of renewable sources [2]. These sources do not generate hazardous waste and help reduce the greenhouse effect. Lignocellulosic materials have been proposed within the concept of precursors for a wide range of value-added products [3]. This concept also stimulates the participation of agriculture and involves energy recovery from waste [4]. The residues generated during olive cultivation, e.g. during tree pruning, are lignocellulose-rich raw materials and abundant. Currently, about 11 million hectares in the world are cultured with olive trees, with an estimated annual Olive Tree Pruning (OTP) biomass production of 3000 kg/ha [5]. Removal of olive tree pruning biomass is necessary to keep clean fields and to avoid propagation of plant diseases or burning practices, which cause air pollution. Moreover, its great abundance, low cost and lignocellulosic composition, make this residue appropriate as a renewable energy source, with many environmental advantages [3, 6].

Second generation bioethanol is obtained from raw materials (lignocellulosic material) that have no negative impact on the food chain [7]. Conversion into ethanol generally includes four steps: pretreatment, hydrolysis, fermentation and product recovery [8]. Pretreatment is the most complicated and expensive step. Cellulose is usually sheathed by hemicelluloses forming a cellulose-hemicellulose complex that works as a chemical barrier and prevents

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access of enzymes to the complex under natural conditions [9]. In addition, the cellulose-hemicellulose complex is encapsulated with lignin and this structure limits enzymatic hydrolysis of biomass to produce fermentable sugars [10]. Therefore, a pretreatment is required to remove the lignin-cellulose-hemicellulose complexes through changes in the macroscopic, submicroscopic and microscopic structures of the biomass, making it accessible to the hydrolytic enzymes that convert cellulose and hemicellulose into fermentable sugars [11]. Pretreatments can be roughly classified into four groups: physical, chemical, physicochemical and biological techniques [8]. Numerous different pretreatments have been carried out with a wide range of lignocellulosic biomasses to determine the most appropriate method and special requirements of each biomass [12]. Within chemical pretreatments, different substances and conditions have been assayed [13]. For example, alkaline pretreatments do not require extreme conditions such as high temperature and high pressure [14]. Hydroxide has been used as an alkaline pretreatment with significant advantages over others, as it is cheap and safe to handle [15]. Pretreatment with $Ca(OH)_2$ generally avoids significant loss of carbohydrates. A disadvantage is its poor solubility, but this can be solved by varying the operating conditions [16, 17]. Pretreatments with $Ca(OH)_2$ have been studied using rice straw, switch grass, sugarcane bagasse, corn stover and wheat straw [18]. Presently, there are no reports on $Ca(OH)_2$ pretreatment of olive tree pruning biomass assessing a set of procedures that support the statistical analysis of different experiments. Factorial designs, based on a combination of factors, are classical experimental designs that have been widely used in scientific experiments to simultaneously examine multiple factors and their interactions while reducing the degree of bias in the experiments [19].

The main goal of the present study was valorization of Olive Tree Pruning residues by its use for bioethanol production. The study is focused on the use of an alkaline treatment with lime, a low cost reagent, under mild conditions, to remove lignin without degrading carbohydrates, increasing the porosity of the raw material and enhancing enzymatic hydrolysis of Olive Tree Pruning residues. The operating conditions were optimized using a half fraction factorial experimental design and a composite central design. It proposes a simple operation scheme with a less aggressive chemical reagent, leading to environmental and economic advantages, due to a lower energetic demand and equipment investment. Besides, it presents an evaluation of the use of native yeasts, isolated from the wine process, in the fermentation stage, an innovation thought under the circular economy concept, for a region where agribusiness is one of the main economic activities.

2 Materials and methods

2.1 Material collection and preparation

Branches and leaves were collected during March and April 2019 after olive tree pruning (*Olea europaea*) in San Juan province, Argentina (31°41'00" S; 68°35'00" W). Samples were washed with tap water to remove contaminants and placed in direct sunlight at ambient temperature for 7 days. The dried material was ground and sieved to the desired particle size for each analysis.

2.2 Material characterization

Moisture content (%) was determined by drying at 105±3 °C for 3 h and calculated as the percentage of mass loss (ASTM D4442-92 [20]). Moisture, Total Solids (TS) and ash content were calculated as weight percentage. To determine the ash content, samples were placed in crucibles which were located in a muffle furnace at 575 °C until carbon was completely eliminated (ASTM D1102-84 [21]). Volatile Matter (VM) was estimated using a muffle furnace at 950 °C for 7 min (ASTM E872-82 [22]). Fixed Carbon (FC) was calculated through the difference in moisture percentage between ash and volatiles. Total sugars were determined using the Dubois method [23], a spectrophotometric technique that involves the reaction of sugars with phenol and sulfuric acid to form a colored compound. The percentage of cellulose, hemicellulose, and lignin was calculated from Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), and Acid Detergent Lignin (ADL) using Eqs. (1)–(3).

$$\%\text{Lignin} = \%\text{ADL} \tag{1}$$

$$\%\text{Cellulose} = \%\text{ADF} - \%\text{ADL} \tag{2}$$

$$\%\text{Hemicellulose} = \%\text{NDF} - \%\text{ADF} \tag{3}$$

2.3 Calcium hydroxide pretreatment

OTP biomass was ground and sieved to a particle size below 1.4 mm, and then contacted with different volumes of distilled water and high purity calcium hydroxide in a 1 liter Parr reactor. Different reaction times were assayed under constant stirring and automatic temperature control. Pretreatment stages were performed according to two

experimental designs; one for screening variables and one for optimization of these variables. Commercial software (Design-Expert 11.0.3) was used to analyze the results and optimize the conditions.

2.3.1 Screening design

The following variables were assayed: temperature (40–120 °C), lime loading (0.05–0.5 g $Ca(OH)_2$/g of dry mass), water loading (10–20 g H_2O/g of dry mass), and pretreatment time (120–480 min), according to a 2^{4-1} half fractional factorial design. A total of 18 experiments, including four central point experiments, were performed. The reducing sugar content, determined by the DNS method, was chosen as response variable to evaluate the effect of the alkaline pretreatment.

2.3.2 Optimization design

RMS analysis was employed to determine the effects of different operating factors on the reducing sugar content and reveal the optimum conditions to increase this value, this technique was also used to build models. The effects of the screened variables, obtained with the screening experimental design, on the increase in reducing sugars were assayed to optimize the pretreatment process. The objective was to maximize the content of reducing sugars. The following two variables were optimized: temperature (100–220 °C) and lime loading (0.005–0.015 g $Ca(OH)_2$/g of dry mass) according to a rotatable central composite design with a total of 11 experiments. Four star points, distributed at a distance of 1.4 from the central point, and two central points were included to gather important information on the reproducibility of the experiments and on the suitability of the proposed model [24]. The response variable was the reducing sugar content.

2.4 Enzymatic hydrolysis

Pretreated biomass was subjected to enzymatic hydrolysis to examine the effect of different pretreatment conditions on the enzymatic digestibility. Hydrolysis of all pretreated samples and raw material was performed using a mixture of two enzymes: cellulase from *Trichoderma reesei* ATCC 26921 (≥ 700 units/g) (Sigma Aldrich, Denmark) and hemicellulase from *Aspergillus niger* (0.3–3 units/mg) (Sigma Aldrich, USA). Enzymes were suspended in 0.05M sodium citrate buffer (pH 4.9). Enzymatic hydrolysis was carried out in a rotary shaker at 45 °C for 24 h, with a rotational speed of 100 rpm and at a substrate concentration of 4% (w/v). All experiments were performed in duplicate. A small aliquot of hydrolysate was taken from each sample to determine reducing sugars according to the method aforementioned.

2.5 Validation of the statistical model

Statistical analysis is useful for optimum operating conditions to obtain maximum fermentable sugar content and to build models. Validation of the statistical model is a fundamental step to ensure reliable results. The screening and optimization designs provided models that show the mathematic relationship between the response variable (reducing sugars) and the variables assayed (temperature, lime loading, water loading and pretreatment time concerning the screening experimental design and lime loading and temperature to optimize the experimental design). These models can be described by Eqs. (4), (5):

$$Y = \alpha_0 + \alpha_1 \times A + \alpha_2 \times B + \alpha_3 \times C + \alpha_4 \times D + \alpha_{12} \times A \times B + \alpha_{13} \times A \times C + \alpha_{14} \times A \times D \quad (4)$$

$$Y = \beta_0 + \beta_1 \times A + \beta_2 \times B + \beta_{11} \times A^2 + \beta_{22} \times B^2, \quad (5)$$

where Y is the predicted response, A, B, C and D are the factors studied, and α_i and β_i are coefficients estimated from regression analysis, which represent the linear, quadratic and cross-products of A, B, C, D on the responses. The validity of the equations was analyzed with ANOVA, and the goodness of fit of the equations was judged with the determination coefficients of the P-value.

The validation step consisted in contrasting the experimental results of the response variable against the values predicted by the model. Besides, experimental assays were performed in triplicate under optimum conditions predicted with the model and this biomass is hereafter referred to as OPH.

2.6 Determination of pretreatment effects

SEM was used to compare the surface of untreated and pretreated biomass. Samples were dried, coated with gold and examined with a JEOL JSM-6610LV (MEB) electron microscope. Analyses were performed at 100 μm (250× magnification) and 5 μm (3000× magnification). The specific surface area of untreated OTP biomass was determined through N2 adsorption isotherms at −196.1 °C (Micromeritics ASAP-2000). Before adsorption analysis, samples were outgassed at 100 °C for 12 h, and subsequently surface areas were determined using the BET theory.

2.7 Fermentation

Fermentation assays were carried out in 25 mL test tubes, inoculating 10 mL of OPH biomass (pH 4.9) with 2×10^6 cells/ml of *Saccharomyces cerevisiae* BSc114, a native yeast isolated from an enological environment in San Juan province. Tubes were overlaid with Vaseline-paraffin, statically incubated at 28±2 °C and monitored for 3 days every 24 h for CO_2 production. Assays were considered positive when the Vaseline-paraffin overlay was displaced. Positive tests were submitted to sugar [25] and ethanol determination. The production of ethanol was measured by Gas Chromatography (GC). Samples were taken from the supernatant, diluted, stirred, and filtered through a 0.45 µm syringe filter (Microlar 26 mm, nyl). Analysis of each sample was then carried with a SHIMADZU GC-2010 Plus gas chromatograph equipped with a Flame Ionisation Detector (FID) and a CHROMPACK column (30 m × 0.25 mm id, and 0.25 µm film thickness). Nitrogen was used as carrier gas at a flow rate of 1 ml/min. GC operation always started with an injector temperature and detector temperature set at 240 °C and 260 °C, respectively and with a 1:10 split ratio. 0.1 µl of each sample was injected, by triplicate, and the ethanol concentration was measured by maintaining the GC oven temperature at 35 °C, for 9.5 minutes.

3 Results and discussion

3.1 Material characterization

The physicochemical properties of the biomass are affected by handling, storage and transport facilities while the biomass composition determines the efficiency of the conversion from raw material into energy [26]. The amount of water in the raw material is represented by the moisture content, which is determined as the percentage of air-dried biomass weight. The OTP biomass assayed contained 7.64% water, suggesting an appropriate drying process and suitable storage conditions. Other parameters like TS, VM, FC and ash reported 92.36%, 74.70%, 12.36%, and 5.30%, respectively. NDF, ADF and ADL determined in air-dried biomass were 58.40%, 41.14% and 9.26%, respectively. During NDF analysis it was possible to dissolve digestible cell contents such as sugar, starch, protein and pectin from the biomass and leave the fibrous residues [27]. The high NDF value obtained in our study showed that only 41% of OTP biomass consisted of soluble substances. Therefore, 58.40% of air-dried OTP biomass was solid, mainly composed of cellulose (31.88%), hemicellulose (17.26%) and lignin (9.26%), the principal cell wall components. Consequently, alkaline pretreatment of OTP biomass under different conditions might be a suitable method to deal with the lignin barrier and dissolve carbohydrates.

3.2 Calcium hydroxide pretreatment

3.2.1 Statistical analysis of the screening design

Application of a 2^{4-1} half fractional factorial design to the pretreatment generated the following polynomial equation for reducing sugar content Eq. (6).

$$\text{Red. Sugars}_{(\text{mg/g})} = 15 + 0.47 \times A - 33.84 \times B + 0.32 \times C + 0.02 \times D - 0.57 \times A \times B, \quad R^2 = 0.9442 \tag{6}$$

The significance and adequacy of the regression model was tested with the *p*-value and the determination coefficient, and the corresponding results of the Analysis of Variance, ANOVA, are presented in Table 1. This test evidences the statistical significance of each effect comparing their mean square against an estimate of the experimental error. In our case three effects, temperature, lime loading and the interaction between both, showed *p*-value below the level of significance $\alpha = 0.05$, indicating that they significantly affected the variation of the content of reducing sugars with a 95.0% confidence level. The very low *p*-value (less than 0.0001) obtained for the model suggests that it was highly significant for the pretreatment process. The fact that the value obtained for the lack of fit was insignificant means that the quadratic model was perfectly acceptable. Another evaluation of the model was performed through the multiple determination coefficient (R^2), which was 0.9442 (adjusted $R^2 = 0.9052$). The given R^2-value for the reducing sugar content implies that 94.45%

Table 1 ANOVA for the 2^{4-1} screening design

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value
Model	6968.88	7	995.55	24.18	0.0001*
A-Temperature	1388.31	1	1388.31	33.72	0.0002*
B-Lime loading	5100.10	1	5100.10	123.88	0.0001*
C-Water loading	19.36	1	19.36	0.4703	0.5085
D-Time	0.6006	1	0.6006	0.0146	0.9063
AB	419.43	1	419.43	10.19	0.0096*
AC	1.01	1	1.01	0.0245	0.8787
AD	40.07	1	40.07	0.9733	0.3471
Residual	411.69	10	41.17		
Lack of Fit	2.20	1	2.20	0.0483	0.8310
Pure Error	409.49	9	45.50		
Total Correlation	7380.56	17			

Note: * indicate statistical significance in the design (*p*-values < 0.05)

of the variation between the samples can be attributed to the factors and only 5.55% of the total variation is not satisfactorily explained by the model.

The principal effects are shown in a Pareto chart (Fig. 1) in which the negative (blue blocks) and positive effects (ochre blocks) of the pretreatment can be distinguished. The length of each bar is proportional to the standardized effect, which is the estimated effect divided by its standard error. This is equivalent to the calculation of the *t*-value for each effect. The *t*-value limit and Bonferroni limit can be used to judge which variables resulted statistically significant, and the results were taken into account for the optimization design.

The response graph shown in Fig. 2 illustrates the mutual interactive effects of the combination of independent variables on reducing sugar content as 3-D surface plots. It was obtained as a function of two factors by holding the others constant.

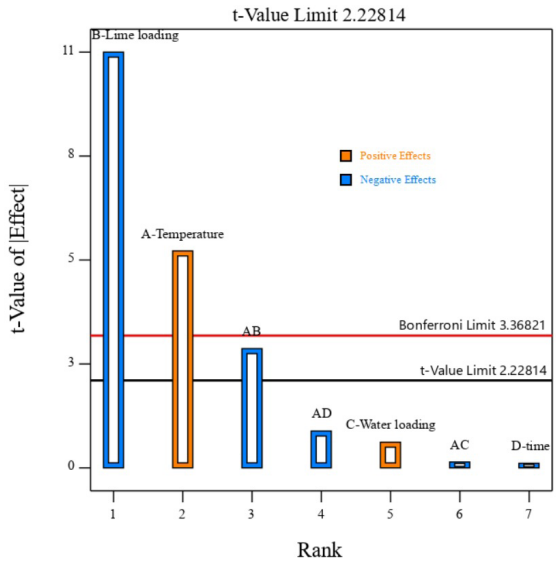


Fig. 1 Pareto chart for screening design

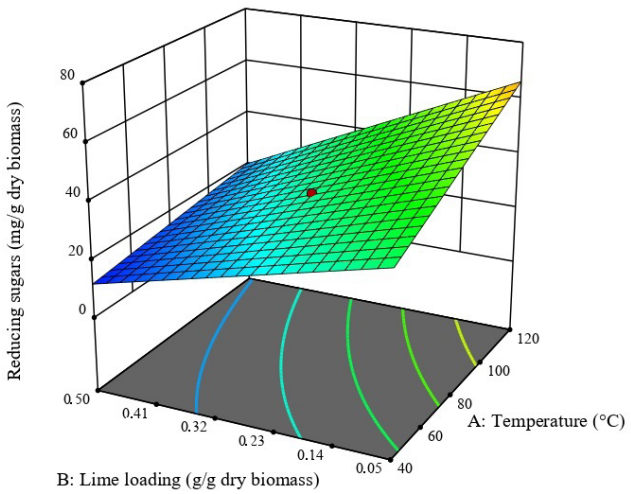


Fig. 2 Surface graph of the screening design for reducing sugars (mg/g)

3.2.2 Statistical analysis of the optimization design

Response surface methodology was also applied to determine optimum pretreatment conditions. The second order polynomial equation corresponding to this response is given by Eq. (7).

$$\text{Red. Sugars}_{(\text{mg/g})} = 18 - 1.32 \times A + 2362 \times B - 21 \times A \times B + 0.02 \times A^2 + 30201 \times B^2, \quad R^2 = 0.979 \tag{7}$$

When the values for A and B were substituted in the Eq. (7), the predicted responses were obtained. Predicted and experimental values were compared and they were in close agreement (Fig. 3).

It can be observed that the model and temperature (A) were significant. In addition, the multiple determination coefficient obtained from the regression (R^2) was 0.979 and the adjusted R^2 was 0.8630. These values ensure a satisfactory adjustment of the theoretical values to the experimental data through this model. The summary of analysis of variance (ANOVA) of the results is presented in Table 2.

Three dimensional response surfaces were plotted to show the interactions between the various parameters in alkaline pretreatment of OTP biomass and to determine the optimum levels of each factor required to obtain maximum response. The effect on reducing sugar content (mg/g of dry biomass) in the optimization design is shown in Fig. 4.

3.3 Validation of the statistical model

Model equations obtained for the response and the response surface were used to determine optimum $Ca(OH)_2$ pretreatment conditions. Optimal values of each factor that optimized the process response were obtained from a

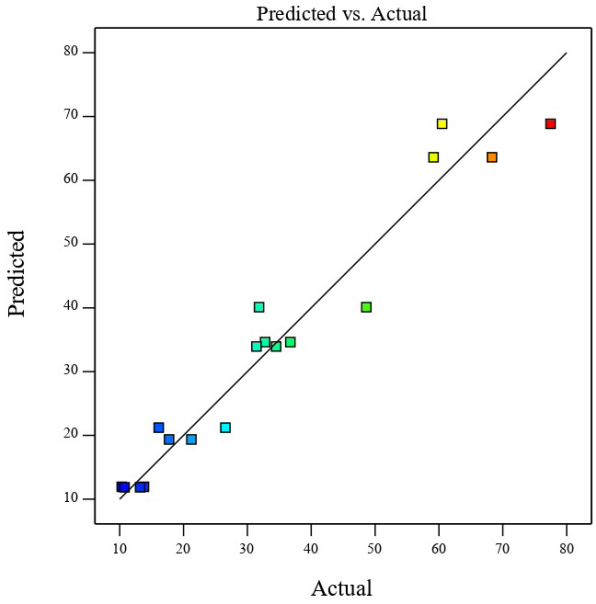


Fig. 3 Predicted vs. actual values optimization design

Table 2 ANOVA optimization design (Response variable: Reducing sugar content (mg/g))

Source	Sum of Squares	df	Mean Square	*F*-value	*p*-value
Block	1349.08	1	1349.08		
Model	31476.76	5	6295.35	11.08	0.0377*
A-Temperature	21128.36	1	21128.36	37.18	0.0089*
B-Lime loading	2681.38	1	2681.38	4.72	0.1182
AB	4603.62	1	4603.62	8.10	0.0653
A^2	3057.83	1	3057.83	5.38	0.1031
B^2	667.16	1	667.16	1.17	0.3579
Residual	1704.78	3	568.26		
Total Correlation	34530.62	9			

Note: * indicate statistical significance in the design (*p*-values < 0.05)

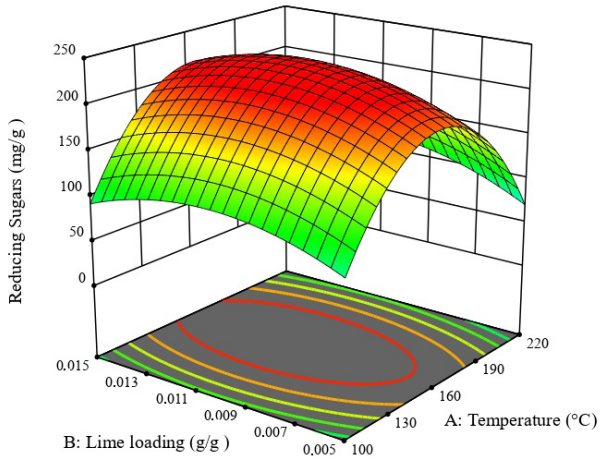


Fig. 4 Response surface graph of the optimization design for reducing sugars (mg/g)

Multi-Objective Numerical Optimization. The optimum conditions obtained from the screening design were 0.05 g $Ca(OH)_2$ and 20 g H_2O per gram of dry biomass at 120 °C for 2 h, and the optimization design rendered 0.01 g $Ca(OH)_2$ per gram of dry mass at 160 °C. Table 3 shows predicted and experimental values of the response variable obtained under optimum operating conditions. The predicted response was 69 and 240 mg reducing sugars per gram of dry biomass for the screening and optimization design, respectively. Validation of the results from the model and regression equation was performed in triplicate and the values obtained were in close agreement, thus confirming the optimization process.

Fig. 5 shows a comparative analysis of total and reducing sugar content in raw material and pretreated and hydrolyzed OTP biomass obtained with screening and optimization experimental designs. It can be seen that the percentage of fermentable sugars significantly increased in both cases after application of the alkaline pretreatment.

Table 3 Maximum content of reducing sugars in the prediction models (screening and optimization design)

	Raw material	**A** Maximum content in the screening design		**B** Maximum content in the optimization design	
		120 °C, 0.05 g $Ca(OH)_2$/g of dry mass		160 °C, 0.01 g $Ca(OH)_2$/g of dry mass	
		Predicted response	Exp. response	Predicted response	Exp. response
Reducing sugars (mg/g)	11.26	68.99	68.32	240	220
Diff		**A**:1%		**B**:8%	

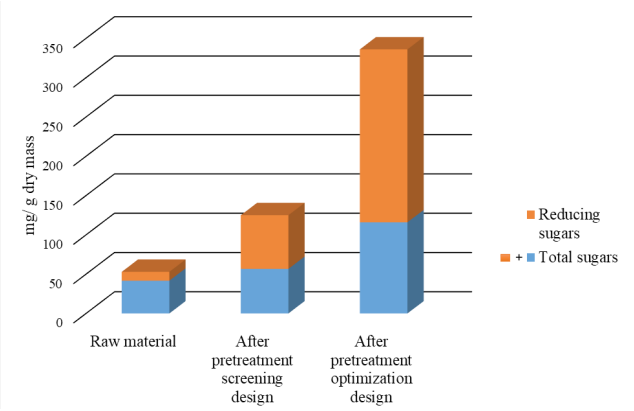
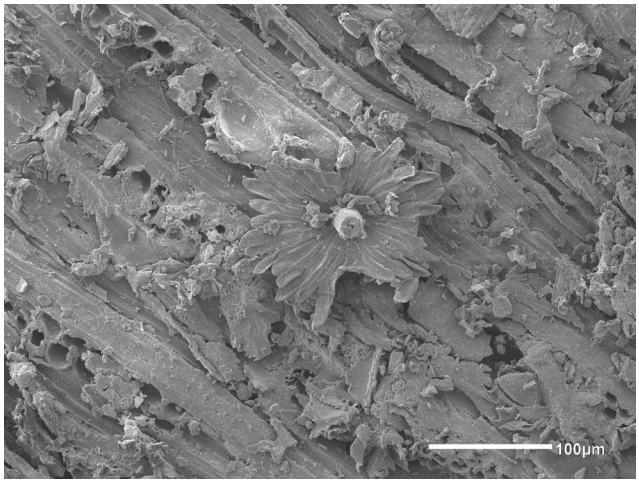


Fig. 5 Comparative graph of maximum sugar content

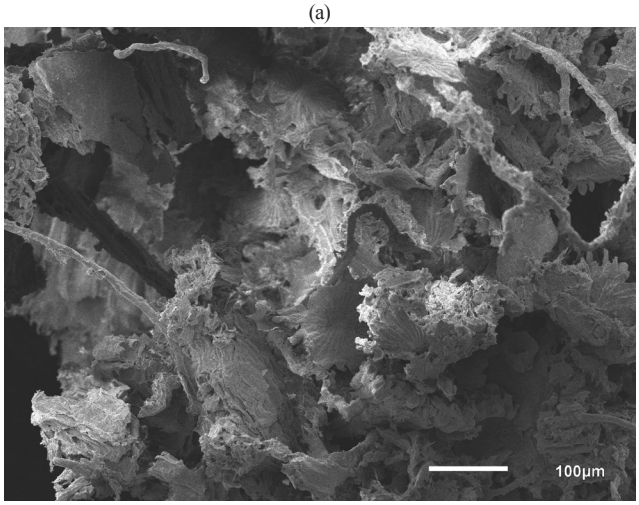
The reducing sugar content increased 500% under optimal conditions of the screening test and 1800% during the optimization stage. Other authors have obtained similar results with the same raw material. Saha and Cotta [28] assayed similar conditions to ours, pretreating rice hulls with $Ca(OH)_2$ (a lime loading of 100 mg/g, 121 °C, 1 h) and three enzymes (cellulase, hemicellulase and *β*-glucosidase) for hydrolysis (45 °C, pH 5, 72 h), obtaining 154 mg sugars per gram of substrate. Mateo et al. [29] carried out pretreatment with 2% H_2SO_4 at 120 °C for 90 min and obtained 12.4 g/l of total sugars from OTP residues, similar to the maximum reached in our study (10.5 g/l).

3.4 Effects of alkaline pretreatment on OTP biomass

SEM was proposed to observe the superficial changes in OTP biomass after alkaline pretreatment. Fig. 6 (a) and (b) shows images of raw and pretreated olive tree pruning residues, respectively. It can be observed that prior to pretreatment the material presented structures that are typical of olive leaves, called trichomes and also known as foliar plates, that constitute part of the epidermis and play a protection role against plagues and diseases [30]. In Fig. 6 (b) it can be seen how the original structures changed, showing holes and interstices produced by the



(a)



(b)

Fig. 6 SEM images of OTP biomass samples before (a) and after (b) treatment with $Ca(OH)_2$

alkaline pretreatment. The peeling effect of alkaline pretreatment is evident, and results in a morphological change in the OTP biomass surface. Delignification leads to the formation of holes in the structure of the cell wall and therefore, its surface seems more fragile when compared with the untreated sample [31].

Fig. 7 shows the general mass balance and lignocellulosic composition of OTP residues and hydrolyzed solid OTP (stream 4) after pretreatment-hydrolysis stages. It can be observed that the lignin content is almost unaffected, whereas cellulose and hemicellulose changed considerably after pretreatment and hydrolysis; both components were hydrolyzed (see streams 3 and 4 of both balances). The greatest change occurs in hemicellulose, which can be expected during pretreatment with calcium hydroxide since it releases the acetyl groups of hemicellulose [32]. The low percentage of solubilized cellulose can be attributed to the fact that alkaline pretreatments do not typically act on this polymer. The minor lignin removal can be ascribed to the low solubility of hardwood lignins in alkaline solutions due to their structural characteristics. Brandt et al. [32] studied the chemical composition of different lignocellulosic biomasses and the chemical effect on delignification, and hence on biomass deconstruction. The lignin composition of softwoods, hardwoods and grasses greatly varies; in softwoods it mostly consists of guaiacyl (G) units, while in hardwoods it also contains a large number of syringyl (S) units. G units are more likely to be cross-linked at the C5 position of the ring during delignification. In contrast, the C5 position in S units is occupied, and therefore it cannot participate in substitution reactions. Consequently, it cannot be hydrolyzed by acids or bases, making delignification of hardwoods more difficult than softwoods [32]. Under the optimum experimental conditions, it was revealed that solubilization of hemicellulose and cellulose prevailed over lignin removal.

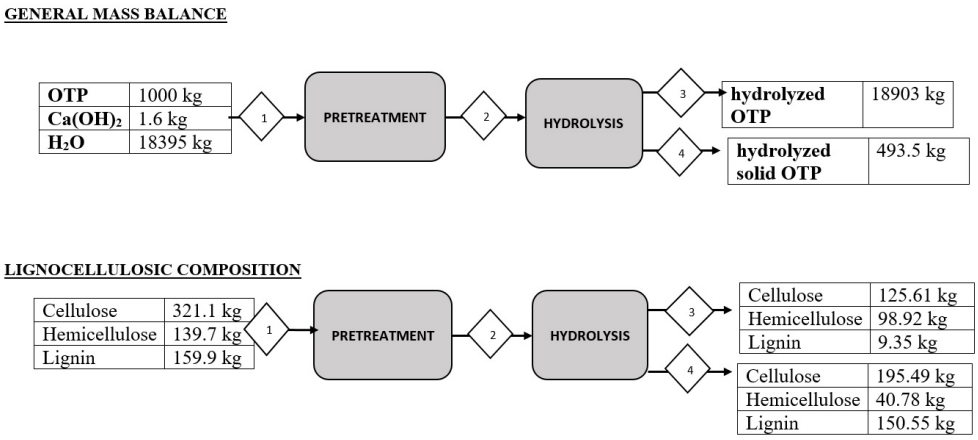


Fig. 7 General mass balance and lignocellulosic components under optimum experimental conditions

3.5 Bioethanol production

The ethanol concentration after 3 days of fermentation was 1.42±0.03 g/l. The notable performance of this native yeast with respect to the bioethanol production is remarkable. Initial sugars concentration was 10.5 g/l and the sugars after fermentation was 6.5 g/l. The yield was 0.35 grams of ethanol per gram of consumed sugar. The performance favorably compares with other studies using the same feedstock. Moya et al. [33] obtained 0.33 g of ethanol per gram of sugar, after acid pretreatment using H_2SO_4, Fonseca et al. [34] reported a yield of 0.20 g of ethanol per gram of sugar from olive tree wood pretreated with sequentially alkaline/acid system ($NaOH/H_2SO_4$) and Carvalho et al. [35] obtained 0.4 g of ethanol per gram of sugar after alkaline pretreatment in eucalypt wood using NaOH. Nevertheless, further research is necessary to optimize this stage of the bioethanol production process. Table 4 shows a comparison of the ethanol values obtained in the present study with those reported by other authors using various lignocellulosic residues and different pretreatments [36–39].

Table 4 Comparative analysis of ethanol production from different lignocellulosic residues

Biomass	Pretreatment conditions	Sugars (mg/g)	Ethanol (g EtOH/g biomass)	Ref.
Olive tree Pruning	1% (v/v) H_2SO_4 15% (w/v) 164 °C 10 min	126	0.13	[37]
Barley straw	NaOH (0.58 M) 10% (w/v) 87 °C	163	0.05	[36]
Sugarcane bagasse pith	1% (v/v) H_2SO_4 10% (w/v) 121 °C 90 min 1.5 bar	537	0.25	[38]
Hardy sugar cane	0.5% NaOH 5% (w/v) 30 min	185	0.03	[39]
Olive Tree Pruning	1% of $Ca(OH)_2$ 5% (w/v) 120 min 160 °C	220	0.03	This study

4 Conclusions

The amount of fermentable sugars obtained from Olive Tree Pruning (OTP) biomass treated with calcium hydroxide makes it a promising raw material for bioethanol production.

Scanning electron microscopy together with the crystallinity index of samples pretreated with $Ca(OH)_2$ showed the peeling effect of the lime, which resulted in morphological changes of the solid surface favoring the subsequent hydrolysis step.

Statistical tools allowed building a mathematical model that governed this alkaline pretreatment and the operating conditions that yielded maximum fermentable sugar content. The sugar concentration increased 1800% compared with the raw material allowing a production of 28.4 g ethanol from 1 kg of untreated solids.

According to these results, the Olive Tree Pruning biomass could yield 85 kg/ha of bioethanol. Our findings are promising for a future bioethanol production from this cheap and abundant material and highlight the importance to recycle and valorize waste.

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