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Date Submitted: 2020-07-02

Keywords: Fermentation, detoxification, acid hydrolysate, lignocellulosic residue, biorefinery

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Record Type: Published Article

Submitted To: LAPSE (Living Archive for Process Systems Engineering)

Citation (overall record, always the latest version):	LAPSE:2020.0801
Citation (this specific file, latest version):	LAPSE:2020.0801-1
Citation (this specific file, this version):	LAPSE:2020.0801-1v1

DOI of Published Version: https://doi.org/10.3390/pr8050533

processes

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Production of Ethanol from Hemicellulosic Sugars of Exhausted Olive Pomace by *Escherichia coli*

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Received: 30 March 2020; Accepted: 29 April 2020; Published: 1 May 2020

Abstract: Exhausted olive pomace (EOP) is the main residue generated in olive oil industries, after the extraction of the residual oil from olive pomace with hexane. This work studies the ethanol production from hemicellulosic sugars of EOP. The fermentability of the sugar solution, resulting from the acid pretreatment of EOP, was evaluated using *Escherichia coli* SL100, although a detoxification step was required before fermentation. Overliming and activated charcoal detoxification were tested to minimize the presence of inhibitory compounds in the hydrolysate and to achieve a fermentable medium. *E. coli* assimilated all sugars in both detoxified hydrolysates and achieved ethanol yields of about 90% of the theoretical one. However, the fermentation time was much shorter when the hydrolysate had been detoxified with activated charcoal (20 h versus 120 h).

Keywords: biorefinery; lignocellulosic residue; acid hydrolysate; detoxification; fermentation

1. Introduction

Currently, the biorefinery concept of lignocellulosic biomass is gaining interest, as it can replace fossil fuels with much greener and renewable alternatives. These biorefineries, based on residual biomass, are considered environmentally friendly facilities because they are able to produce energy, chemicals, and other valuable products without competing with food use [1,2]. In addition, biorefineries can help to mitigate climate change, as the replacement of fossil fuels by biomass resources avoids carbon dioxide emissions and contributes to the circular economy development instead of the current model of a lineal economy since lignocellulosic biomass, used to obtain bio-based products, can be recovered and recycled. Biorefineries could assure energy and chemical supply in many countries around the world, contributing to the development of rural areas and reducing national dependence on imported fossil fuels. On the other hand, a biorefinery, besides producing at least one energy product (biofuel), heat, and electricity, generates platform molecules, which can be used as building blocks for the production of high-value chemicals such as bioplastics, binders, or fibers. Moreover, process energy requirements could be internally supplied by the production of heat and electricity from the combustion of self-residues [3,4].

Exhausted olive pomace (EOP) is the main residue generated in olive oil industries, which are some of the most important agro-industries in Mediterranean countries. EOP is the residual solid generated in the production of pomace olive oil after the residual oil content is extracted with hexane, and it consists of exhausted pulp, skin, seeds, and stones. According to estimates by Manzanares et al. [5], more than 1,182,000 metric tons of EOP are generated in Spain annually in the pomace olive oil

extracting industry. This residual biomass is frequently used as a fuel in home heaters or small industrial boilers. Its utilization for industrial burning systems is very limited because the impurity content leads to pollutant emissions [5,6], including particles and aromatic compounds [7]. However, based on its chemical composition, EOP can be used as a feedstock for a lignocellulosic biorefinery from which a wide range of bioproducts could be produced, including renewable fuels and chemicals. Bioethanol can be obtained from the sugar fraction of EOP, while the presence of phenolics in the extractive fraction of these materials can be used to produce compounds exhibiting antioxidant properties, which present a high interest for the food industry [8].

Pretreatment is an essential step in the conversion process of lignocellulosic biomass into ethanol for breaking the complex structure of EOP and separating its main components. In this way, pretreatment is able to solubilize hemicellulose and/or lignin, modify the lignin structure, enhance the surface area of the lignocellulosic biomass reducing its particle size, and reduce the crystalline structure of cellulose as well as its degree of polymerization. As a consequence of the pretreatment, sugars are generated in monomeric forms that can, in turn, be fermented to bioethanol. A wide variety of pretreatments have been reported in the literature, such as liquid hot water, steam explosion, dilute acid and alkaline solutions, ultrasound, and microwave-assisted treatments or metal salts treatments, among others [9]. Dilute acid pretreatment is considered the most common and economically viable pretreatment on an industrial scale for releasing sugars from lignocellulosic residues. After pretreatment, a liquid fraction containing pentoses (xylose, arabinose) and hexoses (glucose, galactose) is obtained, while a solid fraction rich in cellulose is obtained, presenting easier hydrolytic enzyme access. Valorization of the hemicellulosic sugar stream is essential to making integral use of the biomass. However, acid pretreatment produces inhibitory compounds such as acetic and formic acids, furfural, 5-hydroxymethylfurfural (HMF), and phenolic compounds, which negatively affect microorganism growth in the subsequent fermentation stage [10]. Therefore, the removal of these compounds in the hemicellulosic hydrolysates is crucial to their fermentability. Several methods, such as overliming, liquid–liquid extraction, biological treatment or treatment with ion-exchange resins, organic solvents, or activated charcoal, have been used to detoxify lignocellulosic hydrolysates before fermentation [11]. *Escherichia coli*, an ethanologenic recombinant microorganism, is able to consume both hexose and pentose sugars with high tolerance to inhibitory compounds, mainly acetic acid [12]. This microorganism has been successfully used in the fermentation of hydrolysates (containing a mixture of pentoses and hexoses) from rapeseed straw [13] and rice husk [14]. In addition, the hemicellulose fraction from other olive-derived biomass such as olive tree pruning [15–17] or olive stones [18] has also been valorized by bioconversion into ethanol.

The objective of this work is to evaluate the possibility of ethanol production from hemicellulosic sugars in EOP, released during the acid pretreatment using the ethanologenic *E. coli* SL100 strain. Before the fermentation of pentose and hexose sugars, overliming and activated charcoal treatment were tested to minimize the presence of toxic compounds in the hydrolysate and improve its fermentability. To the best of our knowledge, this is the first study on ethanol fermentation of hemicellulosic hydrolysates from EOP.

2. Materials and Methods

2.1. Raw Material

Exhausted olive pomace was kindly supplied by a pomace oil extracting industry "Spuny SA" in the province of Jaén, Spain. Once in the laboratory, it was kept at 4 °C until use. The chemical composition of EOP is (dry weight): cellulose 9.5%, hemicellulose 11.5% (xylan 9.5%, galactan 1.0%, arabinan 1.0%), lignin 23.7%, ash 9.4%, and extractives 42% [8].

2.2. Preparation of EOP Hemicellulosic Hydrolysate

EOP was extracted with water (100 °C, 30 min) and pretreated at 170 °C, using 2% H_2SO_4 (*w/v*) and a 20% substrate concentration in a 1 L Parr reactor (Parr Instr. Co., Moline, IL, USA) [8]. Once the reactor was cooled, the hydrolysate was separated from the slurry by vacuum filtration. Then, it was used as a fermentation medium for ethanol production. To reduce the effect of the toxic compounds generated in the acid EOP hydrolysate and improve its fermentability, two detoxification methods were tested: overliming and activated charcoal treatment.

In the overliming process, the acid hydrolysate was adjusted at pH 10 using solid $Ca(OH)_2$. The mixture was agitated at 50 °C and 200 rpm for 30 min in an orbital shaker (Certomat-R, B-Braun, Melsungen, Germany). Then, pH was decreased until 2.5 with 96% H_2SO_4 (*w/v*), and the mixture was centrifuged at 3500 rpm for 10 min (Rotina 420, Hettich Zentrifugen, Tuttlingen, Germany).

The treatment with activated charcoal (100 mesh particle size, Sigma-Aldrich, St. Louis, MO, USA) of the acid hydrolysate was performed at 3.5% (*w/v*) of solid–liquid ratio, in an orbital shaker at 45 °C and 200 rpm for 1 h. The detoxified hydrolysate was separated from the residual activated charcoal by vacuum filtration.

The original hydrolysate and both detoxified hydrolysates were analyzed to determine their concentrations of carbohydrates and inhibitory compounds, and then, they were used as fermentation media.

2.3. Inocula and Ethanol Fermentation

Escherichia coli strain SL100, donated by Dr. Ingram (University of Florida, Gainesville, FL, USA), was maintained at −80 °C in 40% glycerol stocks. Cells were incubated in 250 mL Erlenmeyer flasks using 100 mL of AM1 culture medium, which was composed of (mmol/L): $(NH_4)_2HPO_4$, 19.92; $NH_4H_2PO_4$, 7.56; KCl, 2; $MgSO_4{\cdot}7H_2O$, 1.50; Betaine, 1; and of the following salts (µmol/L): $FeCl_3{\cdot}6H_2O$, 8.88; $CoCl_2{\cdot}6H_2O$, 1.26; $CuCl_2{\cdot}2H_2O$, 0.88; $ZnCl_2$, 2.20; $Na_2MoO_4{\cdot}2H_2O$, 1.24; H_3BO_3, 1.21; $MnCl_2{\cdot}4H_2O_2$, 2.50. In addition, glucose and xylose were also added approximately in the same concentration contained in the EOP acid hydrolysate (5 and 24 g/L, respectively). This medium was sterilized by filtration (Millipore GP 0.22 µm, Millipore, Carrigtwohill, Ireland). The inoculum was grown at 37 °C in an orbital incubator at 200 rpm for 20 h, centrifuged (3500 rpm, 10 min), washed, and added to the culture medium [19].

The acid hydrolysate (before and after each detoxification) was supplemented with salts contained in AM1 culture medium (except glucose and xylose) and sterilized by filtration using 0.22 µm membranes. Fermentation tests were performed at 37 °C, 200 rpm (magnetic stirring), pH 6.5 and approximately 0.2 g/L of initial cell concentration (based on the absorbance at 620 nm) for 142 h (overlimed hydrolysate) or 31 h (activated charcoal detoxified hydrolysate), using 300 mL glass flasks equipped with a pH probe, with 150 mL acid hydrolysate. In order to maintain the temperature, a water bath was used. The pH control was carried out automatically by the addition of 2 M KOH solution. Each flask was provided with a rubber cap, including two holes for venting and sampling [13]. Aliquots were taken and centrifuged (11,500 rpm, 10 min) at different fermentation times to measure cell growth, carbohydrate consumption, and ethanol production. Experiments were carried out in duplicate, and the average values and standard deviations are reported. The performance of the fermentation was evaluated according to ethanol yield (g ethanol/g consumed sugar), which was calculated considering that the final ethanol concentration (g/L) is achieved when the subsequent increase is less than 5%, and it was related to the sugar consumed during that period of time (Equation (1)). In addition, ethanol yield was expressed as a percentage of the maximum theoretical ethanol yield (0.51 g ethanol/g sugar).

$$\text{Ethanol yield } (\text{g/g}) = \frac{\text{Final ethanol concentration } (\text{g/L})}{\text{Consumed sugar concentration } (\text{g/L})} \quad (1)$$

2.4. Analytical Methods

High-performance liquid chromatography (HPLC) using a Waters 2695 liquid chromatograph (Mildford, MA, USA) equipped with a refractive index detector (Waters 2414) was employed to analyze the content of sugars (glucose, arabinose, and XGM, which stands for the sum of xylose, galactose, and mannose), acetic acid, compounds from degradation sugars (formic acid, furfural, and HMF), and ethanol. Sugars content was analyzed using a Transgenomic CHO-782 carbohydrate analysis column at 70 °C, with ultrapure water as the mobile phase (0.6 mL/min), while a Bio-Rad HPX-87H column with 5 mM H_2SO_4 as mobile phase (65 °C and 0.6 mL/min) was used to determine the content of acetic acid, compounds from the degradation of sugars and ethanol. A filtration method using 0.2 µm cellulose nitrate filters (Sartorius Stedim Biotech, Göttingen, Germany) was used to estimate the cell content in the fermentation samples [13]. The biomass concentration was determined as the ratio between the mass of dried biomass and the volume of filtered inoculum. The total phenolic compound concentrations were measured by the Folin–Ciocalteu method. Briefly, 0.25 mL of 50% (*v/v*) Folin's reagent, 0.5 mL of 10% (*w/v*) sodium carbonate solution and 3.75 mL water were added to 0.5 mL of a suitably diluted aliquot of the hydrolysate. The sample was maintained at 25 °C in the dark for 60 min, and then absorbance was measured at 765 nm, using gallic acid as standard. The results were expressed as g gallic acid equivalents/L [20]. All analytical determinations were performed in triplicate, and the average results and relative standard deviations were below 1%.

3. Results and Discussion

3.1. Composition and Detoxification of EOP Hydrolysate

Table 1 shows the composition in monomeric sugars and inhibitors of the original and detoxified hydrolysates. The hemicellulosic hydrolysate, resulting from the acid pretreatment of EOP, reached a total sugar concentration of 36.8 g/L, with xylose as the main carbohydrate, accounting for 64.4% of the total carbohydrates in the liquor (Table 1). Sugar solutions highly concentrated (>8% *v/v*) are required to achieve ethanol concentrations higher than 4% (*v/v*) in the subsequent fermentation stage because low ethanol levels increase the cost of the subsequent distillation stage [21].

Table 1. Composition of acid hydrolysate before and after detoxification.

Detoxification Method	Carbohydrates (g/L)				
	Glucose	**Xylose**	**Galactose**	**Arabinose**	**Mannose**
Non-detoxification	4.63 ± 0.24	23.69 ± 0.45	4.15 ± 0.14	3.42 ± 0.14	0.89 ± 0.02
Overliming	4.61 ± 0.01	23.60 ± 0.11	3.78 ± 0.23	2.98 ± 0.18	0.73 ± 0.19
Activated charcoal	4.53 ± 0.06	23.28 ± 0.31	3.39 ± 0.01	2.75 ± 0.02	0.64 ± 0.06
Detoxification Method	**Inhibitory Compounds (g/L)**				
	Formic Acid	**Acetic Acid**	**HMF**	**Furfural**	**Total Phenols ***
Non-detoxification	0.40 ± 0.05	5.66 ± 0.15	0.15 ± 0.01	1.95 ± 0.07	4.49 ± 0.20
Overliming	0.39 ± 0.02	5.66 ± 0.01	n.d.	1.17 ± 0.03	2.47 ± 0.12
Activated charcoal	0.16 ± 0.03	5.38 ± 0.00	n.d.	0.34 ± 0.02	1.25 ± 0.02

n.d.: non-detected, * measured as g gallic acid equivalents/L.

With regards to the presence of toxic compounds in the hemicellulosic hydrolysates, it is worth mentioning that different levels can be achieved depending on the raw material and the pretreatment conditions [9]. In this way, as it is shown in Table 1, under the pretreatment conditions used in this work, the presence of both formic acid, from the dissociation of furfural, and HMF, from the breakdown of hexoses [11], was very scarce, with concentrations lower than 0.4 g/L. However, a higher concentration was determined for furfural (1.9 g/L), which is generated by the degradation of pentoses [22]. Acetic acid and phenolic compounds were much more noticeable in the liquor, with 5.7 and 4.5 g/L detected, respectively. Acetic acid is generated from the acetyl groups of the hemicellulose fraction, while phenolic compounds are produced by lignin degradation [11].

The concentration of some inhibitory compounds contained in the original EOP hydrolysate can affect its fermentability to ethanol by *E. coli*. Therefore, in order to minimize the levels of these compounds, two detoxification methods were applied before fermentation: overliming and activated charcoal treatment. As detoxification will certainly increase the process cost because of the addition of a new step, other alternatives such as the use of strains much more resistant to inhibitory compounds or even the employment of higher inoculum amounts have been reported; nevertheless, this last option, in general, might not be viable at industrial scale [23–25]. In spite of the economic issue, detoxification can be crucial to remove toxic compounds from the hydrolysate and make it a fermentable medium [24]. The reduction of the concentration of specific compounds such as aliphatic acids or phenolics has been proved to enhance the results of fermentation. According to some authors, the main reason for that is that the treatment converts those compounds to less toxic ones, either by making inert some of the reactive compounds or by introducing strongly hydrophilic sulfonate groups [26]. Nevertheless, the way the different detoxification methods act depends on several factors, including the particular substrate.

In this work, the removal of the inhibitory compounds in the EOP hydrolysates achieved by each detoxification method was determined to assess their suitability (Table 1). Detoxification by overliming is regarded as a non-expensive method where inhibitory compounds are removed by precipitation reactions along with the production of calcium sulfate in the case of sulfuric acid pretreatment hydrolysates [23]. Overliming eliminated HMF completely and removed about 40% and 45% of the furfural and phenols, respectively, but the removal of formic and acetic acid was negligible (Table 1). A noticeable reduction of furans has also been reported in the detoxification of hydrolysates of sugarcane bagasse [27], olive tree biomass [15], and red seaweed [28] by overliming.

However, activated charcoal treatment of the EOP hydrolysate, which also fully eliminated HMF, achieved a more effective removal of furfural, 82.6%, and total phenolic compounds, 72.2%. Although the concentration of formic acid was also considerably reduced (60%), the decrease in acetic acid concentration was non-significant ($p < 0.05$; Table 1). Activated charcoal treatment is a commonly used detoxification method. The excellent capacity of activated charcoal treatment for the removal of furans and phenols was reported in the detoxification of olive tree biomass [19], sweet sorghum bagasse [29], palm press fiber [30], *Agave lechuguilla* [31], and brewers' spent grain [32].

Further, the scarce carbohydrate losses (referred to the sugar concentration in the original hydrolysate), lower than 6%, with the two detoxification methods tested is worth highlighting. Therefore, taking into account the removal of fermentation inhibitors achieved in each case, activated charcoal detoxification was more efficient than overliming for detoxifying EOP hydrolysate.

On the other hand, in addition to the hemicellulosic hydrolysate, a cellulose enriched solid was obtained at the pretreatment conditions assayed in this study, which could be enzymatically hydrolyzed and bioconverted into ethanol, which is potentially interesting in the context of a biorefinery based on EOP and other residues from olive oil industries.

3.2. Ethanol Fermentation by *E. coli*

E. coli could not ferment the original EOP hydrolysate, likely because the concentrations of toxic compounds, as well as the synergistic effect of them, inhibited its growth. For this reason, the EOP hydrolysate was detoxified with overliming (Figure 1a) and activated charcoal (Figure 1b), and then, the fermentability of the resulting hydrolysates was evaluated with *E. coli*.

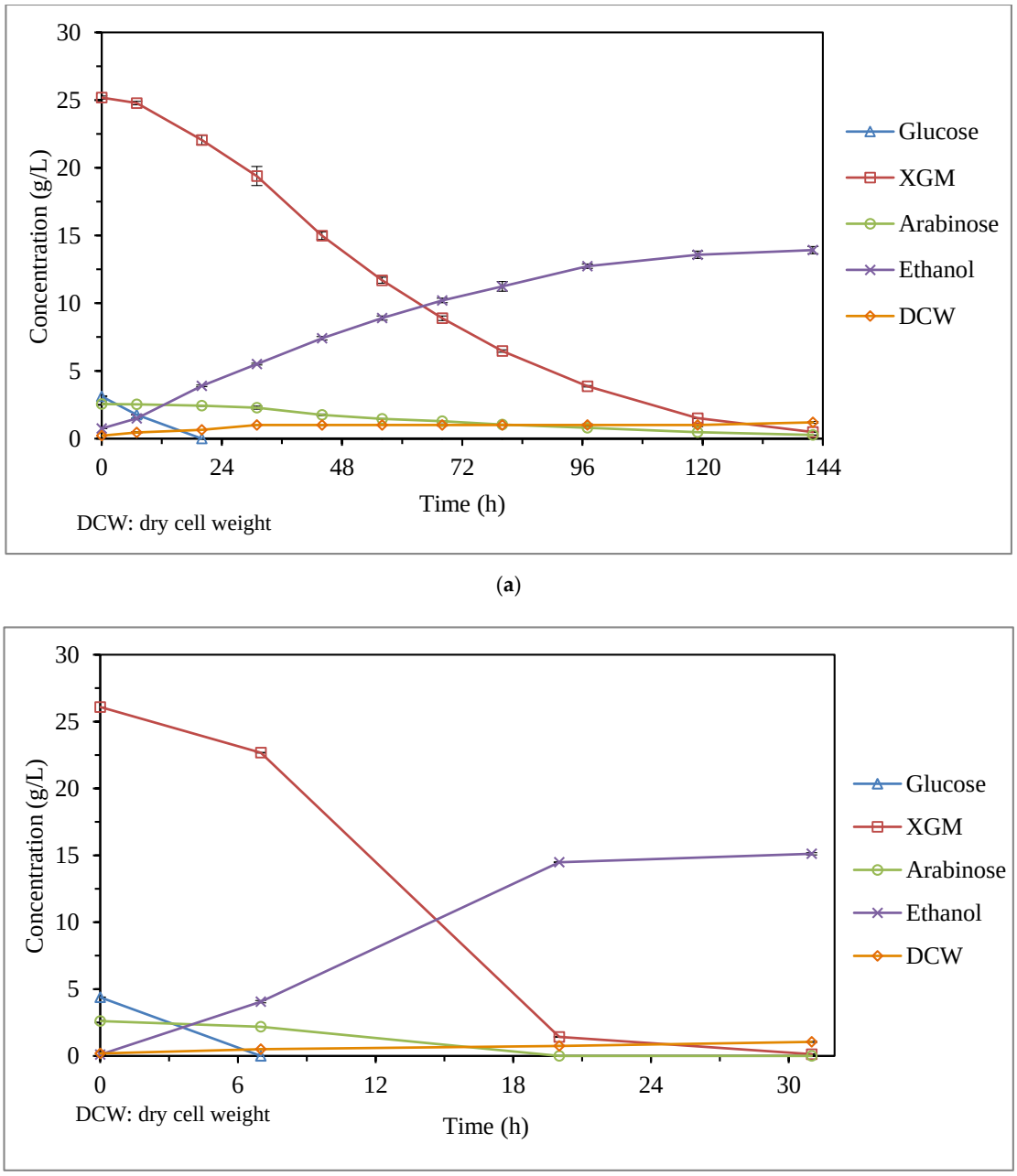

Figure 1. Fermentation of exhausted olive pomace (EOP) hydrolysate by *Escherichia coli* after (**a**) overliming and (**b**) activated charcoal detoxification.

Figure 1a,b shows the fermentation time course of the EOP hydrolysates after detoxification by overliming and activated charcoal treatment, respectively. Glucose was the first carbohydrate exhausted in both cases, with initial concentrations lower than 5 g/L. Nevertheless, *E. coli* required 20 h to assimilate this sugar in the fermentation of the overlimed hydrolysate (Figure 1a), whereas only 7 h was necessary to fully consume glucose in the hydrolysate detoxified with activated charcoal (Figure 1b).

As observed, the simultaneous consumption of glucose and XGM was determined in the fermentation of both detoxified hydrolysates, although the consumption rate of XGM sugars by *E. coli* was also higher for the hydrolysate detoxified with activated charcoal. Simultaneous consumption of glucose and XGM by *E. coli* has also been reported in the fermentation of hemicellulosic hydrolysates

from *Agave lechuguilla* [31], corncobs [33], and rapeseed straw [13]. It is worth mentioning that levels of arabinose identified in the EOP hydrolysates were not relevant (<9% of total sugars).

Fermentation of the overlimed hydrolysate yielded a final ethanol concentration of 13.6 g/L at 120 h (Figure 1a), and only 0.5 g/L of arabinose and 1.5 g/L of XGM remained in the medium without consumption at that time. In this way, *E. coli* consumed 94% of the total carbohydrates contained in this hydrolysate within 120 h of fermentation and achieved an ethanol yield of 0.47 g ethanol/g consumed sugar (Table 2), which corresponds to 92.2% of the theoretical ethanol yield (0.51 g ethanol/g sugar). The ethanol volumetric productivity determined at that time was 0.11 g/L/h. Thus, after 120 h fermentation, *E. coli* assimilated the residual sugar content in the broth, but the increase in ethanol concentration was negligible (Figure 1a).

Table 2. Parameters of the EOP hydrolysate fermentation with *Escherichia coli*.

Fermentation Parameters	Hydrolysate	
	Overlimed Hydrolysate	Activated Charcoal Detoxified Hydrolysate
Ethanol concentration (g/L)	13.58 ± 0.16	14.48 ± 0.47
Fermentation time (h)	120	20
Ethanol yield (g ethanol/g consumed sugar)	0.47	0.46
* Ethanol yield (%)	92.2	89.7
Ethanol productivity (g/L/h)	0.11	0.73

Fermentation time: time at which the final ethanol concentration is obtained. * Based on the maximum theoretical ethanol yield (0.51 g ethanol/g sugar).

When the EOP hydrolysate was detoxified with activated charcoal, *E. coli* assimilated 96% of carbohydrates in a fermentation time of 20 h with an ethanol production of 14.5 g/L, corresponding to 0.46 g ethanol/g consumed sugar (89.7% of the theoretical yield) (Table 2). Nevertheless, residual sugars in the fermentation broth (about 4% of the initial sugar concentration) were consumed after 31 h without additional ethanol production (Figure 1b).

Therefore, activated charcoal was a more effective detoxification method than overliming because it yielded a hydrolysate with lower inhibitory compound concentrations, and therefore, the medium was easier to ferment.

Even though the fermentation of both hydrolysates yielded similar ethanol production, when the hydrolysate was treated with activated charcoal, the highest ethanol concentration was reached at 20 h (14.5 g/L) with a productivity of 0.73 g/L/h. However, the fermentation of the overlimed hydrolysate reached a final ethanol concentration of 13.6 g/L after 120 h, corresponding to the productivity of only 0.11 g/L/h. It can be concluded that there is no statistical difference between both values of ethanol concentration ($p < 0.05$), although the time required by *E. coli* to assimilate all sugars in the overlimed hydrolysate was much longer. This fact can be attributed to the presence of lower levels of toxic compounds (mainly, furfural and phenolic compounds) in the hydrolysate detoxified with activated charcoal. Phenol concentration in overlimed hydrolysate was twice that in the hydrolysate detoxified with activated charcoal and, in the case of furfural, its concentration was three times higher for overlimed hydrolysate (Table 1). Almeida et al. [34] reported that furfural concentrations higher than 1 g/L show a clear negative effect on ethanol-producing microorganisms, affecting their vitality, specific growth rates, phase lag, and ethanol yield and productivity. In addition, phenolic compounds are one of the most common inhibitory compounds in hemicellulosic hydrolysates with the greatest negative effect, even at low levels [35]. According to Klinke et al. [36], the synergistic effect of inhibitory compounds contained in the overlimed hydrolysates could considerably increase the microorganism inhibition. However, although a high acetic acid concentration was also found for the hydrolysate detoxified by activated charcoal (>5 g/L, Table 1), high resistance of *E. coli* to this organic acid has been previously reported [12].

Table 3 summarizes some examples of fermentation of hemicellulosic hydrolysates (containing hexoses and pentoses) from different biomasses. As can be seen, there is a great variety of experimental

process conditions. All of these raw materials were acid pretreated, mainly with H_2SO_4 but also with H_3PO_4 and HCl. As in the case of the EOP, most of them require a detoxification step before fermentation, using physical treatments such as evaporation or chemical methods as those used in this work. The bioconversion of these hydrolysates with xylose-fermenting microorganisms resulted in ethanol concentrations (ranging from 6 g/L to 38 g/L) that are highly dependent on the biomass loading used in the pretreatment. The results of ethanol production obtained in this work for the EOP hydrolysates detoxified with activated charcoal and overliming are comparable to those reported with other biomasses, even with the same ethanologenic microorganism.

Table 3. Ethanol concentrations and yields achieved in the fermentation of hemicellulosic hydrolysates of lignocellulosic residues pretreated at different conditions.

Raw Material	Pretreatment	Detoxification Method	Microorganism	EC (g/L)	EY (%)	Reference
Palm press fiber	121 °C, 60 min, 5% H_2SO_4, 30% DM	Overliming	*Scheffersomyces stipitis* NRRLY 7124	6.1	64.7	[30]
Sweet sorghum bagasse	121 °C, 40 min, 1.75% H_2SO_4, 5% DM	Activated charcoal	*Scheffersomyces stipitis*	22.0	78.4	[29]
Sugarcane bagasse	145 °C, 12 min, 0.5% H_2SO_4, 10% DM	Evaporation	*Spathaspora passalidarum* Y-207907	17.3	84.5	[37]
Corn stover	190 °C, 1 min, 30% DM,0.048 g H_2SO_4/g dry biomass	Ammonium hydroxide	*Zymomonas mobilis* 8b	38.0	80.0	[38]
Rapeseed straw	130 °C, 60 min, 2% H_2SO_4, 10% DM	Ion-exchange resin	*Escherichia coli* MS04	25.0	86.0	[39]
Olive tree biomass	164 °C, 0 min, 0.89% H_2SO_4, 15% DM	Overliming	*Escherichia coli* MM160	14.9	82.4	[19]
	155 °C, 0 min, 2% H_3PO_4, 12.5% DM		*Escherichia coli* SL100	16.0	78.0	[40]
Brewers' spent grain	130 °C, 26 min, 1% H_2SO_4, 12.5% DM	Non detoxification	*Scheffersomyces stipitis* CBS605	11.4	53.0	[41]
			Escherichia coli SL100	17.0	76.0	
	121 °C, 30 min, 1% HCl, 25% DM		*Saccharomyces cerevisiae* 479	13.0	67.0	[42]
Exhausted olive pomace	170 °C, 0 min, 2% H_2SO_4, 20% DM	Overliming	*Escherichia coli* SL100	13.6	92.2	This work
		Activated charcoal		14.5	89.7	

DM: dry matter; EC: ethanol concentration (g/L); EY: ethanol yield, referred to the theoretical ethanol yield (0.51 g ethanol/g sugar).

4. Conclusions

Detoxification prior to fermentation made the bioconversion of sugar content in the acid hydrolysate of EOP with *E. coli* possible. Detoxification with activated charcoal removed more inhibitors than overliming and yielded a more fermentable broth. Thus, although the fermentation of both detoxified hydrolysates resulted in similar ethanol production, about 14 g/L, activated charcoal detoxification achieved this ethanol concentration in a shorter time, 20 h versus 120 h. The bioconversion of the hemicellulosic sugars from EOP proposed in this work contributes to the complete valorization of this residual biomass as a raw material in a biorefinery context.

Author Contributions: Conceptualization, E.R.; investigation, J.C.L.-L. and I.G.-C.; resources, E.C.; writing—original draft preparation, J.C.L.-L.; writing—review and editing, I.R.; supervision, E.C.; project administration, I.R. and E.R.; funding acquisition, I.R. and E.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agencia Estatal de Investigación (MICINN, Spain) and Fondo Europeo de Desarrollo Regional, reference project ENE2017-85819-C2-1-R.

Acknowledgments: I.G.-C. was supported by Universidad de Jaén (research grant R5/04/2017).

Conflicts of Interest: The authors declare no conflict of interest.

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