Xylosaccharides purification from eucalyptus residues for L-lactic acid production by *Weizmannia coagulans*

María-Noel Cabrera[1]*, Eugenia Vila[2], Alberto Liguori[2], Constanza D´Andrada[1], Santiago Moure[1,2], Mairan Guigou[2], Florencia Cebreiros[2], Juan Martín Rodao[1], Laura Camesasca[2], Mario Daniel Ferrari[2], Claudia Lareo[2]

[1] Forest Processes Engineering Group, Chemical Engineering Institute, Faculty of Engineering, Universidad de la República, Julio Herrera y Reissig 565, 11300, Montevideo, Uruguay

[2] Bioengineering Department, Chemical Engineering Institute, Faculty of Engineering, Universidad de la República, Julio Herrera y Reissig 565, 11300, Montevideo, Uruguay

* Correspondence: ncabrera@fing.edu.uy; Tel.: +598-27142714

Abstract: In this work, phosphoric acid pretreatment (0.6% H_3PO_4, 160°C, 40 min) of eucalyptus residues was performed to recover the hemicellulosic fraction for further conversion to L-lactic acid by fermentation with *Weizmannia coagulans* DSM 2314 (formerly *Bacillus coagulans*). The hemicellulosic hydrolysate was composed of xylosaccharides 24.8 g/L (mainly xylose, 20.3 g/L), acetic acid 7.8 g/L, furfural 0.7 g/L, and acid soluble lignin (ASL) 2.1 g/L. It was subsequently purified by using anion exchange or adsorption resins. Different liquor-to-resin ratios were evaluated to obtain a high concentration of xylosaccharides in the eluate and high removal of components that inhibit lactic fermentation. The best performance was observed when using Amberlite®-XAD-4 resin at a liquor-to-resin ratio of 3:1. An eluted hydrolysate was obtained, preserving 80% of the xylosaccharides and effectively removing almost all furfural, 90% of ASL, and 32% of acetic acid. Subsequently, L-lactic acid production by *W. coagulans* DSM 2314 was evaluated using concentrated non-detoxified and detoxified hydrolysate

obtained through vacuum oven. For the non-detoxified hydrolysate, 12 g/L of L-lactic acid was obtained after 48 h showing a yield of 0.56 g $_{lactic\ acid}$/g $_{sugar}$ and a xylose consumption of 62%. The detoxification of the liquor significantly improved the fermentation performance of *W. coagulans*, resulting in a concentration of 16 g/L of lactic acid after 24 h, with a yield of 0.73 $g_{lactic\ acid}/g_{sugar}$, and almost complete xylose consumption.

Keywords: Xylosaccharides purification, adsorptive and anionics resin, L-lactic acid, *W. coagulans*, lignocellulosic residue

Practitioner Points

• Xylan solubilization of 76% was achieved by phosphoric acid pretreatment.

• Resin detoxification of hemicellulosic liquor improved L-lactic acid fermentation.

• W. coagulans achieved complete xylose consumption and L-lactic yield of 0.73 g/g.

1. Introduction

Bio-based products market is expected to grow strongly globally over the next years. Lignocellulosic biorefineries are foreseen to play a key role in this bio-based economy through the efficient conversion of feedstocks from agriculture, forestry, and agro-industrial residues to value-added compounds such as fuels, chemicals, and biomaterials.[1,2] Forestry lignocellulose biomass can be used as a potential substrate in an integrated biorefinery, given the wide variety of products that can arise from the chemical structure of its components (cellulose, hemicellulose, lignin, and extractives). Eucalyptus wood residues are a common forestry byproduct in Latin America, mainly generated in

cellulose pulp mills and through the mechanical processing of wood for timber, plywood, MDF, etc.[3]

The use of forestry residues, particularly wood residues, within a biorefinery enables a more efficient use of the lignocellulosic material through the fractionation of all its main components in an industrial plant, maximizing its value with the commercialization of a broad products portfolio.[4] In the context of a wood residues biorefinery, dilute acid and hydrothermal pretreatments are widely recognized methods for solubilizing the hemicellulosic fraction into monomeric or oligomeric sugars, while most of the lignin and cellulose remains on the solid matrix.[5–7] Thus, the hemicellulosic fraction can be recovered in a separate stream for valorisation.[8–10] However, utilizing this hemicellulosic fraction is more challenging compared to the cellulosic fraction due to the formation of decomposition compounds (such as furans, carboxylic acids, phenols) during pretreatment.

Sulphuric acid (SA) is the most common acid employed for dilute acid pretreatment of lignocellulosic materials.[11] However phosphoric acid (PA) pretreatment has some advantages over SA pretreatment. PA is less corrosive than SA, which implies a lower industrial plant investment; it also generates smaller amount of decomposition compounds as furfural or hydroxymethylfurfural (HMF), which could cause some inhibition during downstream operations such as enzymatic hydrolysis and/or fermentation. In addition, PA pretreatment incorporates a source of phosphorus into the medium, which is a necessary nutrient for the development of microorganisms in the fermentation. This could eliminate the need of salts addition and lower operational costs during downstream operations. [6,12,13]

Lactic acid is one of the main industrials bioproducts from a commercial perspective. It is a highly versatile chemical that can be used in the pharmaceutical, food, and textile

industries. It is also considered a relevant platform product since it can be converted into other demanded chemicals. Its demand has significantly increased due to its use as a monomer to produce polylactic acid (PLA) polymers.[14] The production of lactic acid through microbial fermentation has the advantage of producing only one of the two lactic acid isomers (D or L), depending on the microorganism used for the fermentation process. The production of lactic acid through chemical methods are less environmentally friendly, resulting in racemic mixtures of both D and L isomers. The optical purity of lactic acid results important for its application. For instance, in PLA synthesis, the proportion of the lactic acid isomers affects the physical properties of PLA.[14] In addition, L-lactic acid is desirable for food and pharmaceutical industry since it is better metabolized by the human body. [15]

The production of lactic acid has been studied using different lignocellulosic biomass types such as sugarcane bagasse, wheat straw, elephant grass, among others.[15–18] Studies demonstrate that an efficient bioconversion of lignocellulosic biomass into lactic acid can be achieved through the selection of an optimal combination of pretreatment and fermentation process configuration. Lactic acid bacteria (LAB) are the classical microorganisms used for lactic acid production.[19] However, they present complex nutritional requirements as they are not capable of producing some aminoacids and B-vitamins.[20] Nowadays, *Weizmannia coagulans* DSM 2314 (formerly *Bacillus coagulans*), has gain popularity for its use for microbial lactic acid production from different lignocellulosic substrates. One of the main advantages of this bacteria over other microorganism is its capability of metabolizing pentose (C5) sugars via the pentoses phosphate (PP) pathway and producing optically pure L-lactic acid at high yields. [21] These features make these bacteria a proper candidate for industrial production of L-lactic acid by the valorization of the hemicellulosic sugars under a biorefinery concept.

The hemicellulosic liquor obtained after acid treatment of hardwood biomass, mainly composed of xylose as the main product, contains other components as part of the liquor, like aliphatic acids such as formic and acetic acids, furfural, HMF, lignin fragments, extractives, and other saccharides such as cellulose, glucose and arabinose.[11,13,22] Considering that some of these compounds could result inhibitory to bacterial growth or lactic acid production, it could be interesting to completely or partially remove some of them prior to lactic acid fermentation. Liquid–liquid extraction (LLE), organic solvents precipitation, ion exchange and adsorption resins, and membrane filtration, among others, are some useful methods employed for the purification and concentration of value-added compounds from the hemicellulosic liquor.[9,22–27] While both adsorption and ion exchange involve the retention of compounds from a solution by a solid matrix, the underlying mechanisms are different. Adsorption encompasses diverse interactions, while ion exchange, a specific electrostatic adsorption, relies on counterion exchange governed by Coulombic forces for selectivity. This exchange occurs due to differences in the strength and selectivity of these electrostatic interactions between the exchanging ions and the functional groups.[28] Particularly, anion exchange resins are widely used for the detoxification of glucose or xylose streams prior to the production of ethanol, butanol or xylitol by fermentation. Commonly, anion exchange resins are used in series configuration with other resins (also anionic or cationic resins) or with other detoxification method (such as liming, membrane filtration, solvent precipitation or LLE).[22,29–31] In the same way, non-polar adsorption resins are used to separate lignin derivatives, furfural and HMF from lignocellulosic hydrolysates, with the aim of removing inhibitors prior to the fermentation process[32,33] or to recover valuable compounds. [27,34,35]

Figure 1 shows the biorefinery scheme proposed for the valorisation of wood residues, specifically eucalyptus sawdust, to coproduce a biofuel (bioethanol) and value-added products (L-lactic acid and lignin-derived products). In this work, the conditioning of the hemicellulosic fraction extracted through PA pretreatment from eucalyptus sawdust for L-lactic acid production by microbial fermentation was assessed (left side of the scheme). For this, adsorptive and anionics resins with different resin-to-liquor ratios were first evaluated for the detoxification of the hemicellulosic liquor. Once the operating conditions were selected, a vacuum concentration step was incorporated into the processing scheme to enhance product concentration. The novelty of this work relies on the implementation of adsorptive and anionics resins as a detoxification method prior to L-lactic acid fermentation from eucalyptus hemicellulosic liquor. To the best of the authors' knowledge, there are no reports on lactic acid production from eucalyptus residues within a biorefinery context where lignin-derived products and bioethanol are also co-produced. Furthermore, studies on L-lactic acid production from eucalyptus wood hemicellulosic liquors are scarce, particularly liquors obtained after PA pretreatment. While sulfuric acid is the most widely used acid, the utilization of PA in the pretreatment as an alternative, offers advantages such as reduced formation of toxic compounds and lower corrosiveness.

2. Materials and Methods

2.1. Raw material

Eucalyptus sawdust was obtained from a local pulp mill (UPM, Fray Bentos, Uruguay), corresponding to the discard fine fraction of the chips screening process. Eucalyptus sawdust was composed of a mixture of three *Eucalyptus* species: *Eucalyptus grandis* (~55%), *Eucalyptus globulus* (~30%) and *Eucalyptus dunnii* (~15%). Particle

size of the sawdust was as follows: 87% between 1198 and 3360 µm, 7% between 500 and 1198 µm, and 6% higher than 3360 µm. The material was dried at 40°C until moisture content of 8% and stored in closed bags at room temperature in a dry place.

2.2. Phosphoric acid (PA) pretreatment

The eucalyptus sawdust was subjected to phosphoric acid (PA) treatment, which was performed in a Parr reactor model 4522M, with a nominal capacity of 2 L and a solid stirrer to ensure proper mixing of the material. The reactor was provided with automatic sensors for pressure, temperature, and reaction time control. The sawdust was mixed with distilled water in a liquid to solid ratio (L/S) of 7 g/g. The pretreatment conditions were determined previously based on the maximal recovery of xylosaccharides and the maximal xylose to xylosaccharides ratio content in the liquid fraction with the minimum PA consumption.[36] Accordingly, the pretreatments were carried out with a PA concentration of 0.6% (g of H_3PO_4 per 100 g of dry sawdust), and a reaction time of 40 min at the maximum temperature of 160°C.

After PA pretreatment, the slurry obtained was separated in two fractions (solid and liquid) by centrifugation at 1000 rpm, followed by filtration through a nylon tissue in a Büchner funnel. The solid (pretreated sawdust) was washed by immersion in distilled water for 30 min at room temperature and then filtered using a Büchner funnel. This procedure was repeated four times until pH 5 in the washing waters. Part of the liquid fraction (PA hemicellulosic liquor) was analyzed and purified by resins and the other part was concentrated in a vacuum oven at 60°C until volume was reduced to one half. The concentrated liquor was then separated into two batches: one of them was subjected to lactic acid fermentation and the other one was subjected to purification by resins prior to fermentation. Both batches were stored below 4°C.

2.3. Resin treatment of hemicellulosic liquors

The PA hemicellulosic liquor was subjected to anionic and adsorption resins treatment to evaluate the removal of possible fermentation inhibitors that were produced during sawdust pretreatment. The performance of a weak anion exchange resin (Dowex® 66 Free Base), a strong base anion (type I) resin (Dowex® Marathon 11) and an adsorption resin (Amberlite® XAD-4) were compared. All resins were purchased to Sigma-Aldrich and washed with distilled water before the first used. Dowex® 66 Free Base was regenerated with NaOH 4%, and Dowex® Marathon 11 with a mixture of 10% NaCl / 1% NaOH according to producers' recommendation; Amberlite® XAD-4 was regenerated with 75% acetone according to Scwartz and Lawoko.[37] Experiments were firstly performed at a small scale to determine the best resin and the conditions to be employed for the liquor detoxification. For this, the three different PA hemicellulosic liquor-to-resin ratios of 1:1, 3:1 and 5:1 (v:v) were studied, and the experiments were performed at room temperature. The procedure was as follows: a fixed volume of the PA hemicellulosic liquor (10, 30, or 50 mL) was passed through the column filled with one of the resins at a time. It was stirred with a rod for 1 min to ensure contact with the liquor and then left undisturbed for 10 min. This time was previously set as the minimum time that assures complete exchange in the resin system. Subsequently, the detoxified liquor was eluted from the column at a 1 mL/min rate, and then the resin was washed to recover eventually occluded xylosaccharides. The resin was rinsed twice with 30 mL of distilled water, eluting from the column at 1 mL/min. The washing waters were also collected and analyzed along with the detoxified hemicellulosic liquors.

Resin treatment was also evaluated for the concentrated hemicellulosic liquor. For this, Dowex® 66 Free Base and Amberlite® XAD-4 resins were used at the former liquor-to-resin ratios tested, following the procedure previously described. Finally, once the best

resin and operation conditions were selected, a bigger assay using 450 mL of concentrated liquor was performed in order to detoxify the concentrated hemicellulosic liquor used for lactic acid fermentation.

2.4. Microorganism and inoculum preparation

Weizmannia coagulans DSM 2314 was obtained as a freezed dried stock from the Germany Collection of Microorganisms and Cell Cultures (DSMZ, Leibniz Institute, Germany). Stock cultures were maintained in Tryptic Soy Broth and 20% glycerol, and stored at −80°C. For inoculum development, stocks were cultured on Tryptic Soy Agar (Merck Millipore) at 55°C for 27 h. Biomass used for inoculation was grown in 500 mL-Erlenmeyer flasks with 200 mL of Man, Rogosa and Sharpe (MRS) broth (Merck Millipore) at 55°C in an orbital shaker at 150 rpm for 13 h.

2.5. L-lactic acid fermentation

L-lactic acid fermentation by *W. coagulans* was studied in a 1-L Biostat A Plus (Sartorius) bioreactor with 350 mL of working volume at 55°C, pH 7, 150 rpm and under anaerobiosis condition. The pH was controlled by automatic inyection of 2 M NaOH. Two media cultures were prepared from the concentrated PA hemicellulosic liquor as a source of xylose. The composition of the media was equal to the MRS broth except for the replacement of glucose as carbon source by the concentrated hemicellulosic liquor which was detoxificated by resin XAD-4. Also, a control test was performed with XMRS media culture, using xylose instead of glucose as carbon source. Initial xylose concentration was 25-29 g/L for all the media cultures. Samples were taken periodically to monitor cell growth, L-lactic and acetic acid production, and xylose consumption.

2.6. Analytical methods

The chemical composition of the raw material, pretreated raw material, PA hemicellulosic liquor, concentrated PA hemicellulosic liquor, eluted liquor and resin washing waters were determined by the NREL laboratory analytical procedures.[38,39] Xylo-oligosaccharides (XOS) and gluco-oligosaccharides (GOS) contents in the liquors and washing waters were determined as the difference between the xylose or glucose content before and after hydrolysis by dilute sulfuric acid. Xylosaccharides (XS) was also reported as xylose measured after acid hydrolysis. HPLC was used for the quantification of sugars (glucose, xylose, arabinose), organic acids (acetic, formic, lactic), furfural and 5-hydroxymethylfurfural (HMF) concentrations as previously reported.[40] Acid soluble lignin (ASL) in the liquors and washing waters were determined with NREL procedure and using 210 nm wavelength and an absorptivity coefficient of 110 L/g.cm (TAPPI UM250-91). Analytical conditions used were the same as previously reported.[9] Cell growth during fermentation was measured by optical density at 600 nm with a Genesys 10S UV–vis (Thermo Scientific) spectrophotometer. Samples were diluted with distilled water to obtain absorbance values lower than 0.9 units. The relationship between optical density and dry cell weight was determined, and biomass concentrations were reported as grams of dry cells per liter of culture medium (g/L).

3. Results

3.1. Raw material and composition after PA pretreatment

The composition of the eucalyptus sawdust and the PA pretreated solid are presented in Table 1. After PA pretreatment, the solid recovery was 70.0 ± 0.3 %, which was mainly due to hemicelluloses and extractives solubilization. Particularly, xylan solubilization accounts for 76.4 ± 3.5 % of the xylan initially present in the raw material. Table 2 presents the PA hemicellulosic liquor composition. In the hemicellulosic fraction, as

expected, XS were the major components. Due to the extent of the treatment, XS not only separated from the lignocellulosic matrix but also hydrolysed to their monomeric form. In addition, there was an undesired, slight decomposition to furfural.[11,41] Xylose-to-XOS ratio in the PA hemicellulosic liquor was 81.9 ± 1.0. The second compound present in the liquor was acetic acid, produced from the excision of the acetyl groups of the eucalyptus hemicelluloses.[41] Consequently, since cellulose and lignin were degraded to a much lower extent, the spent solid was richer in these compounds (62.9% glucan and 36.4% lignin). Therefore, it is interesting to consider further valorisation of these compounds, as shown in Figure 1.

3.2. Resin treatment of hemicellulosic liquor

Figure 2 shows the fraction of XS, acetyl groups, AIL and ASL, and furfural retained after the diferent resin treatments. Each fraction was calculated considering the concentrations determined in the eluate liquor (detoxified liquor) and the washing waters for each component. The aim of the resin treatment was to minimize the retention of XS while maximizing the retention of the other compounds which could result inhibitory to the downstream lactic acid fermentation. All three resins evaluated were capable of retaining a large amount of AIL, with complete retention observed under all conditions except for the strong base anion resin (Marathon® 11), used in the most exhaustive condition applied (hemicellulosic liquor-to-resin ratio of 1:1) (Figure 2). XAD-4 showed the best results for the retention of ASL. For acetyl groups retention, the best results were obtained using the Dowex® 66 resin with a hemicellulosic liquor-to-resin ratio of 5:1, reaching a maximum retention of 55.6 ± 0.3 %. Due to the strong anion base character of Marathon® 11 resin, it was not possible to remove acetyl groups with this resin. XS retention in the resins ranged from 4.8 ± 0.1 % in the Dowex® 66 resin with a hemicellulosic liquor-to-resin ratio of 5:1 to 31.2 ± 0.3 % in the XAD-4 resin with a

hemicellulosic liquor-to-resin ratio of 1:1. It is important to mention that the XS that were not bound to the resin, but occluded in the bed, were recovered in the washing waters. In the best case, only 4% of them were recovered after the second washing. Also, both acetyl groups and ASL compounds eluted with the washing water, but in a much lower percentage than XS. This behaviour was previously reported by Schwartz and Lawoko.[37]

A vacuum concentration step was incorporated in order to increase the xylose concentration in the hemicellulosic liquor with the aim to maximize the lactic acid production. In this step, some of the volatile compounds present in the hemicellulosic liquor were lost by evaporation (in particular, acetyl and formic groups, and HMF). This represent an advantage of this method over other concentration methods, since the removal of these components would result beneficial for the downstream processing as they may be inhibitors of the lactic acid fermentation. This behaviour explained why some components increased their concentration in a lower factor. On the other hand, xylose concentration was expected to duplicate in the concentrated hemicellulosic liquor, but this was not observed. A clear explanation for this phenomenon was not found, assuming that the mild conditions used are not sufficient to dehydrate xylose to furfural. Additionally, the concentration of furfural did not increase to an extent that could justify the losses of XS.

The results obtained after resin treatment of the concentrated hemicellulosic liquor is shown in Figure 3. In this case, AIL retention was also complete in all the resin treatments evaluated, even though the liquor concentration was higher and the resin exchangeable sites were less available. The XAD-4 resin had better performance in removing ASL than Dowex® 66, reaching retention yields over 80% with all three ratios tested. Also, XAD-4 resin retained more furfural than Dowex® 66. However, as it was expected, due to resins intrinsic characteristics, Dowex® 66 had a better performance regarding acetyl

groups retention. Within each resin, the expected behaviour was an increase in the retention of components as the liquor-to-resin ratio decreased. However, the retention of different components in various assays did not follow this trend. The selectivity of the resin for the different compounds present in the hemicellulosic liquor could explain this phenomenon. Regarding XS retention, Dowex® 66 resin generally presented the lowest values. However, it was observed that as the concentration of components in the hemicellulosic liquor decreased in relation to the resin exchange sites, the retention of XS, which have lower affinity to the resin, increased. Figure 4 shows the appearance of the hemicellulosic liquors before and after resin treatment, along with the washing waters. It is clear that compounds contributing to the colour of the liquor were retained in the resin, as the eluted fraction had lower absorbance and a lighter colour. Also, it should be mentioned that the resin changed its colour from a faint to brownish yellow (not shown).

3.3. L-lactic acid production from eucalyptus hemicellulosic liquor by *W. coagulans* DSM 2314.

Hemicellulosic liquor obtained from the PA treatment and vacuum-concentrated by oven (non-detoxified and detoxified by resin) was fermented to evaluate its potential as substrate for L-lactic acid production by *W. coagulans* DSM 2314. Additionally, fermentation with XMRS medium was performed as a control to evaluate the possible inhibitory effect of certain compounds (e.g., furfural, HMF) present in the hemicellulosic liquor on lactic acid production by *W. coagulans*. Figure 5 shows the biomass, substrate and products concentration profiles obtained, and Table 3 summarizes the fermentation parameters. In non-detoxified liquor-based media, the maximum L-lactic acid concentration (12 g/L) was reached at 48 h (Figure 5b). However, xylose consumption was only 52% of the initial xylose concentration. Instead, the L-lactic acid concentration in detoxified liquor-based media reached a higher concentration (16 g/L) compared to

non-detoxified based media. As shown in Figure 5c, in detoxified liquor-based media, maximum L-lactic acid concentration was reached at 24 h, reaching a complete xylose consumption at approximately 30 h. Consequently, L-lactic acid volumetric productivity resulted in 0.7 g/Lh and 0.3 g/Lh, and L-lactic acid yield based on xylose consumption was 0.73 g/g and 0.92 g/g in the detoxified and non-detoxified liquor-based media, respectively. According to the chemical characterization (Table 2), the non-detoxified hemicellulosic liquor presented, before the resin treatment, 0.3 g/L of 5-HMF, 0.3 g/L of furfural and 2.4 g/L of phenols. After resin treatment, concentrations decreased to 0.1 g/L for 5-HMF and 0.6 g/L for phenols, with complete removal of furfural. Therefore, the lower L-lactic acid concentration and volumetric productivity reached in the non-detoxified media could be attributed to the presence of these inhibitory compounds. Acetic acid was the only by-product detected during fermentation of this strain, but a formation below 0.5 g/L was observed for all the media evaluated. A maximum biomass concentration of around 3.5 g/L was achieved in both the detoxified liquor-based and the XMRS medium, which resulted higher than that obtained in the non-detoxified liquor (2.5 g/L).

The results achieved in the detoxified liquor-based media resulted similar to those obtained in the control fermentation, except for lactic acid yield (Table 3, Figure 5a). These results showed that *W. coagulans* was able to produce L-lactic acid from both non-detoxified and detoxified hemicellulosic liquor fractions. However, the application of the resin treatment as a detoxification method improved the lactic acid production in approximately 33%.

4. Discussion

Composition of the raw material was similar to previous work of the group using eucalyptus sawdust provided by the same company. However, in this case the biomass

has a slightly higher amount of glucan and acetyl groups and a lower amount of lignin.[8,42] The biomass composition is also comparable with eucalyptus residues reported by other authors, despite being different eucalyptus species.[43,44]

XS production directly from the PA treatment of eucalyptus sawdust was effective in a large extent. As it was mentioned, most of XS were in the liquid fraction as xylose, with minimum degradation to furfural. Rojas-Chamorro *et al.* studied the best PA pretreatment conditions to produce bioethanol with brewer´s spent grain, working at a maximum temperature range of 140-180°C with 2-6 % (w/w) of PA.[45] They reported hemicellulose (mostly xylose and arabinose) solubilization ratios above 70% for the conditions evaluated, achieving a maximum solubilization of 89.4 % at 140°C and 6% of PA. The results obtained by these authors regarding hemicelluloses solubilization resulted in the range or higher than the results reported in this work. However, they worked with a higher PA charge (even 10 times in the best condition achieved) and a biomass that had a higher hemicellulosic content (25%) and a lignin content of 12.5%, which implies lower carbohydrate-lignin complexes interactions, making the lignocellulosic biomass more susceptible to acid hydrolysis.[45]

In the same way, Nair *et al.* used wheat straw sawdust and optimize the extraction conditions for the further production of bioethanol. They worked in the range of 0.5–3.0% (w/v) PA concentrations, 150–210°C of temperature, and 5-20 min of reaction time at maximum temperature. They reported a xylan hydrolysis yield of 76%, similar to the results reached in this work.[46]

Comparing the results of this study with those reported by other authors using the same type of biomass, the results were higher than those reached by Castro *et al.*[13] They reported xylose yields ranging from 23.5% to 58.3% working with dilute PA steam explosion pretreatment of *Eucalyptus benthamii* chips at 180-200°C and 5-15 min, with

a PA concentration of 0.5-1.0 % (w/w). In their study, chips were utilized, and a liquid-to-solid ratio of 14 was used during pretreatment. Probably, the smaller size of the raw material (eucalyptus sawdust) used in this study and the more concentrated system used during pretreatment (liquid-to-solid ratio of 5) could explain the better results in terms of xylose yields obtained in this work.

Regarding resin treatment, as it was expected, weak anionic resin resulted effective to remove acetic acid at a great extent, while adsorption resin resulted better in terms of phenolic retention. While the exchange capacity of anionic resins is primarily attributed to the presence of hydrogen bonds, the factors responsible for the adsorption effectiveness of adsorption (non-polar) resins are believed to be hydrophobicity, large specific surface area and highly interconnected macroreticular structure. Particularly, the adsorption capacity of phenolic compounds on adsorption resins is attributed to the hydrophobic Π- Π interaction between the phenolic rings and the resin exchangeable groups.[32] Also, strong anion resins were selected because they are cheaper, and are normally available in the industries, for power boiler water treatment. Despite previous authors reported very good results in the removal of acetyl acids with this type of resin, its performance in this study was insufficient. Although acetate and formate, which are conjugate bases of weak acids with relatively high dissociation constants (Ka $1.8x10^{-5}$ for acetic acid and Ka $1.8x10^{-4}$ for formic acid), could theoretically be adsorbed by the strong anion resin, the presence of phosphate groups in the medium hindered their removal. This competition for binding sites arose from the resin's higher affinity for phosphate, a consequence of its strong negative charge and greater electron density compared to the carboxylic anions.

The capacity of XAD-4 resin in the removal of fermentation inhibitory compounds from autohydrolysis pretreatment liquors is widely reported.[32,33,37] For instance, Weil *et*

al.[33] studied the effect of XAD-4 treatment on corn fiber rich hydrolysate to produce ethanol, with particular focus on furfural removal. They worked with an 8% (w/v) XAD-4 resin in batch mode for a contact time of 1.5 h at room temperature and found that the resin did not remove a significant quantity of sugars, but furfural removal was of 0.5 % to 0.02% (w/w). Schwartz and Lawoko[37] analyzed the performance of XAD-4 resin on the removal of ASL from autohydrolysis liquors from (w/w) a mixed of southern U.S. hardwood chips, and the fermentation of the detoxified liquors with *E. coli* K011 to produce ethanol. They worked in a relation of 1.2 g resin/mL of hydrolysate in column and with a contact time of 10 min. Their results showed that 88% of ASL and 20% of acetyl groups could be removed with a loss of sugars lower than 5% (after the second water wash of the resin). Yu and Christopher[32] worked on the detoxification of hemicellulose-rich poplar hydrolysate in batch placing the hydrolysate and resin (in a ratio of 1.6 - 3.6 $g_{hydrolysate}/g_{resin}$) in a flask overnight at 150 rpm. They found that the adsorption capacities of XAD-4 for xylose and XOS were negatively correlated with the liquor-to-resin ratio, indicating that the XAD-4 resin had a higher affinity for phenolic compounds than for XS. They reported a removal of 97% for ASL using XAD-4 resin, as well as with a strong anion resin (Amberlite® IRA-400 (OH^-)), both working at a ratio of 1.6 $g_{hydrolysate}/g_{resin}$, with XS higher losses than 90% and 40%, respectively. When they worked with a 3.6 g $_{hydrolysate}/g_{resin}$ ratio, the ASL removal was about 75% with XAD-4 resin, and with a XS loss nearly 20%; and about 90% with the anion strong resin with a XS loss of 60%. They did not report acetic acid removal. The results obtained by these authors were similar to the ones obtained in this work when XAD-4 resin was used in a 3:1 ratio. In this work, XS losses were lower than those reported by the authors when a lower resin-to-hydrolysate ratio was used. The results obtained with the strong

anion resin were not comparable but, the performance of the resin in terms of XS retention was detrimental in both cases.

Regarding Dowex® 66 resin performance, it could be mentioned that this type of resin was developed for the purification of carbohydrate-rich solutions in the sugar industry. Therefore, effective aliphatic acids removal and high recovery of carbohydrate was expected. However, in this work, the removal of phenolic compounds was also important. Previous reports have indicated the use of weak anionic resins for removal of acetic acid from XS solutions. Vallejos *et al.* evaluated different serial detoxification strategies for xylitol production.[22] They used liquors from the hydrothermal treatment of sugar cane bagasse. For acetic acid removal, they used a weak anionic resin, Amberlite® IRA-67 in 6.6 and 10 $g_{hydrolysate}/g_{resin}$. This procedure was carried out after stages of liming, calcium removal with cationic resins, and the use of activated carbon. They reported 80 - 85% of acetic acid removal, higher than those obtained in this work, but XS retention was not reported by the authors. As the other inhibitors were eliminated in previous steps, their extraction in the anion exchange were also not reported. Maciel de Mancilha and Karim[47] worked with sugarcane bagasse acidic hydrolysate, using a weak anionic resin developed for the sugar industry (Purolite® A 103S) in continuous mode. They reported the complete removal of acetic acid, HMF, and 95% of the furfural in a corn stover hydrolysate with a xylose recovery of 94%. Phenolic compounds removal was not reported.

Han *et al.*[48] used the weak anionic resin 335 for the detoxification of acidic corncob hydrolysate. The adsorption of acetic acid, furfural, lignin, and xylose in 335 resin was evaluated in different resin-to-hydrolysate ratios (1:5 to 1:40 w/v), placing the mixture in a baker for 2 h at 150 rpm. They achieved the best inhibitors removal when the resin had a higher ratio of exchangeable sites (1:5), reaching removal yields higher than 80%

for acetic acid, furfural, and lignin, with XS losses of about 20%. These results show more promising outcomes than those obtained in this work. However, as the resin-to-hydrolysate ratios decreased, the removal rates decreased, reaching 40% for acetic acid and lower than 15 % for furfural and lignin (with a XS losses lower than 10%). The authors also analyzed the effects of initial XS concentration on the inhibitor removal (20 to 55 g XS/L). As the concentration of XS increased, the removal of the inhibitors decreased, and the XS retention decreased accordingly. Consequently, the best resin-to-hydrolysate depends on the concentration and distribution of inhibitors and XS in the solution, and the retention of inhibitors and XS is strongly influenced by the availability of the active sites in the resin. Previous works from our group, using the same type of resins but with eucalyptus hydrolysate from steam-exploded treatment, confirmed this behavior.[9,49]

The XAD-4 resin, with a liquor-to resin ratio of 3:1, was chosen for detoxifying the concentrated liquor to be fermented. This selection was based on the recognition that lignin compounds are considered more toxic for fermentation than acetyl groups,[32] and the XS losses in this selected case were acceptable to achieve good fermentation yields.

Studies on L-lactic acid production from eucalyptus wood hemicellulosic liquors are scarce, particularly liquors obtained after PA pretreatment. Considering that no previous work carried out by other authors with adsorption or weak anionic resins as detoxification methods has been found, the results obtained in this work were compared to reported works which addressed the fermentation of hemicellulosic hydrolysates from different lignocellulosic residues using *W. coagulans* strains and different detoxified methods (Table 4). The results achieved in this study were similar to the lactic acid yields and productivities obtained in previous studies using different *W. coagulans* strains. For instance, Yamakawa *et al.*[50] studied lactic acid production from

hemicellulosic hydrolysate of *Pinus tadea* by *W. coagulans* DSM 2314. The detoxification process used by the authors involved various steps as acid post-hydrolysis, solid-phase extraction, and liquid-liquid extraction. The highest lactic acid production (5.32 g/L) was achieved by diluting the raw hydrolysate to 28% to reduce the effect of inhibitors and supplementing it with a high concentration of yeast extract (24 g/L). The experiments with non-diluted partially detoxified hydrolysate did not succeed, indicating that the removal of the majority of phenolic compounds was insufficient to promote an efficient microbial growth. Moreover, Cubas-Cano *et al.*[51] studied hemicellulosic hydrolysates from gardening residues for lactic acid production by *Bacillus coagulans* DSM 2314. The results reached by these authors were similar to those obtained in this work, being lactic acid concentration (~22.5 g/L) and volumetric productivity (1.6 g/Lh) slightly higher. However, the *Bacillus* strains used in their work were reported as resistant to inhibitory compounds.

On the other hand, higher initial sugar concentrations were used by other authors. Although higher lactic acid concentrations and volumetric productivities were achieved by using a strain of *B. coagulans* DSM ID 14-300 isolated from hemp leaves, the similar lactic acid yields indicated similar utilization of the sugars for lactic acid production. Further research could be carried out using high sugar concentrations in eucalyptus hemicellulosic hydrolysates.

5. Conclusions

The present study highlights an efficient alternative for the valorization of hemicellulose recovered from eucalyptus sawdust through acid pretreatment within the framework of a biorefinery concept. The application of resin treatment proved to be an effective detoxification method for removing compounds that adversely affect the fermentation performance of the LAB strain. Particularly, adsorption resin was chosen

as a detoxification method due to its better efficiency in the removal of acid-soluble lignin, which is considered more toxic for fermentation than acetyl groups. Consequently, the fermentation performance of *W. coagulans* improved, achieving complete xylose consumption and a higher L-lactic acid yield.

W. coagulans demonstrated its ability to produce L-lactic acid from eucalyptus hemicellulosic liquor, both in the presence and absence of well-known inhibitory compounds. The novelty of this work relies on the implementation of adsorptive and anionics resins as the sole detoxification method prior to L-lactic acid fermentation from eucalyptus hemicellulosic liquor.

6. Acknowledgments

The authors would like to thank UPM Fray Bentos (Uruguay) for providing the raw material, and to Agencia Nacional de Investigación e Innovación (ANII_ 541 FSE_1_2019_1_159574) and Comisión Sectorial de Investigación Científica (CSIC, MIA2023-423) for financial support.

7. References

1. Karlsson H, Börjesson P, Hansson PA, Ahlgren S. Ethanol production in biorefineries using lignocellulosic feedstock - GHG performance, energy balance and implications of life cycle calculation methodology. J Clean Prod. 2014 Nov 15;83:420–7.
2. Rajesh Banu J, Preethi, Kavitha S, Tyagi VK, Gunasekaran M, Karthikeyan OP, et al. Lignocellulosic biomass based biorefinery: A successful platform towards circular bioeconomy. Fuel. 2021 Oct 15;302.
3. Dieste A, Cabrera MN, Clavijo L, Cassella N. Analysis of wood products from an added value perspective: The Uruguayan forestry case. Maderas: Ciencia y Tecnologia. 2019;21(3):305–16.
4. IEA Bioenergy Executive Committee. IEA Bioenergy Annual Report 2022 [Internet]. 2023 [cited 2023 Oct 19]. Available from: https://www.ieabioenergy.com/blog/publications/iea-bioenergy-annual-report-2022/
5. Galbe M, Wallberg O. Pretreatment for biorefineries: A review of common methods for efficient utilisation of lignocellulosic materials. Biotechnol Biofuels [Internet]. 2019;12(1):1–26. Available from: https://doi.org/10.1186/s13068-019-1634-1
6. Solarte-Toro JC, Romero-García JM, Martínez-Patiño JC, Ruiz-Ramos E, Castro-Galiano E, Cardona-Alzate CA. Acid pretreatment of lignocellulosic biomass for energy vectors production: A review focused on operational conditions and techno-economic assessment

for bioethanol production. Renewable and Sustainable Energy Reviews. 2019 Jun 1;107:587–601.
7. Gullón P, Romaní A, Vila C, Garrote G, Parajó JC. Potential of hydrothermal treatments in lignocellulose biorefineries. Vol. 6, Biofuels, Bioproducts and Biorefining. 2012. p. 219–32.
8. Bariani M, Cebreiros. Florencia, Guigou M, Cabrera MN. Integrated production of furfural and second-generation bioethanol from Eucalyptus wood residues: experimental results and process simulation. Wood Science and Technology . 2022 Jul;56:1149–73.
9. Cebreiros F, Risso F, Cagno M, Cabrera MN, Rochón E, Jauregui G, et al. Enhanced production of butanol and xylosaccharides from Eucalyptus grandis wood using steam explosion in a semi-continuous pre-pilot reactor. Fuel. 2021;290:119818.
10. Kumar V, Bahuguna A, Ramalingam S, Kim M. Developing a sustainable bioprocess for the cleaner production of xylooligosaccharides: An approach towards lignocellulosic waste management. Vol. 316, Journal of Cleaner Production. Elsevier Ltd; 2021.
11. Trajano HL, Wyman CE. Fundamentals of Biomass Pretreatment at Low pH. In: Wyman CE, editor. Aqueous Pretreatment of Plant Biomass for Biological and Chemical Conversion to Fuels and Chemicals. 1st Ed. United Kingdom: John Wiley & Sons, Ltd.; 2013. p. 103–23.
12. de Vasconcelos SM, Santos AMP, Rocha GJM, Souto-Maior AM. Diluted phosphoric acid pretreatment for production of fermentable sugars in a sugarcane-based biorefinery. Bioresour Technol. 2013;135:46–52.
13. Castro E, Nieves IU, Mullinnix MT, Sagues WJ, Hoffman RW, Fernández-Sandoval MT, et al. Optimization of dilute-phosphoric-acid steam pretreatment of Eucalyptus benthamii for biofuel production. Appl Energy. 2014 Jul 15;125:76–83.
14. Albuquerque TL De, Da Silva IJ, De MacEdo GR, Rocha MVP. Biotechnological production of xylitol from lignocellulosic wastes: A review. Process Biochemistry. 2014;49(11):1779–89.
15. Alves WR, da Silva TA, Zandoná Filho A, Pereira Ramos L. Lactic Acid Production from Steam-Exploded Sugarcane Bagasse Using Bacillus coagulans DSM2314. Fermentation. 2023 Sep 1;9(9).
16. Yankov D. Fermentative Lactic Acid Production From Lignocellulosic Feedstocks: From Source to Purified Product. Front Chem. 2022 Mar 4;10.
17. Ren Y, Wang X, Li Y, Li YY, Wang Q. Lactic Acid Production by Fermentation of Biomass: Recent Achievements and Perspectives. Sustainability (Switzerland). 2022 Nov 1;14(21).
18. Zou L, Ouyang S, Hu Y, Zheng Z, Ouyang J. Efficient lactic acid production from dilute acid-pretreated lignocellulosic biomass by a synthetic consortium of engineered Pseudomonas putida and Bacillus coagulans. Biotechnol Biofuels. 2021 Dec 1;14(1).
19. Cubas-Cano E, González-Fernández C, Ballesteros M, Tomás-Pejó E. Biotechnological advances in lactic acid production by lactic acid bacteria: lignocellulose as novel substrate. Vol. 12, Biofuels, Bioproducts and Biorefining. John Wiley and Sons Ltd; 2018. p. 290–303.
20. Chopin A. Organization and regulation of genes for amino acid biosynthesis in lactic acid bacteria. FEMS Microbiol Rev. 1993 Sep;12(1–3):21–37.
21. Cubas-Cano E, López-Gómez JP, González-Fernández C, Ballesteros I, Tomás-Pejó E. Towards sequential bioethanol and L-lactic acid co-generation: Improving xylose conversion to L-lactic acid in presence of lignocellulosic ethanol with an evolved Bacillus coagulans. Renew Energy. 2020 Jun 1;153:759–65.
22. Vallejos ME, Chade M, Mereles EB, Bengoechea DI, Brizuela JG, Felissia FE, et al. Strategies of detoxification and fermentation for biotechnological production of xylitol from sugarcane bagasse. Ind Crops Prod [Internet]. 2016;91:161–9. Available from: http://dx.doi.org/10.1016/j.indcrop.2016.07.007

23. Cebreiros F, Guigou MD, Cabrera MN. Integrated forest biorefineries: Recovery of acetic acid as a by-product from eucalyptus wood hemicellulosic hydrolysates by solvent extraction. Ind Crops Prod. 2017;109(August):101–8.
24. Gullón P, González-Muñoz MaríaJ, Domínguez H, Parajó JC. Membrane processing of liquors from Eucalyptus globulus autohydrolysis. J Food Eng. 2008;87(2):257–65.
25. Dávila I, Gullón B, Alonso JL, Labidi J, Gullón P. Vine shoots as new source for the manufacture of prebiotic oligosaccharides. Carbohydr Polym. 2019 Mar 1;207:34–43.
26. Peng F, Peng P, Xu F, Sun R cang. Fractional purification and bioconversion of hemicelluloses. Biotechnol Adv. 2012;30:879–903.
27. Oriez V, Peydecastaing J, Pontalier PY. Lignocellulosic biomass fractionation by mineral acids and resulting extract purification processes: Conditions, yields, and purities. Molecules. 2019 Nov 23;24(23).
28. Berrios M, Siles JA, Martín MA, Martín A. Ion Exchange. In: Ramaswamy S, Huang HJ, Ramarao BV V., editors. Separation and Purification Technologies in Biorefineries. 1st Ed. John Wiley & Sons, Inc; 2013. p. 149–65.
29. Carvalheiro F, Duarte LuisC, Lopes S, Parajó JC, Pereira H, Gírio FranciscoM. Evaluation of the detoxification of brewery's spent grain hydrolysate for xylitol production by Debaryomyces hansenii CCMI 941. Process Biochemistry. 2005;40(3–4):1215–23.
30. Nilvebrant NO, Reimann A, Larsson S, Jönsson LJ. Detoxification of lignocellulose hydrolysates with ion-exchange resins. Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology. 2001;91–93:35–49.
31. Li J, Shi S, Tu M, Via B, Sun FF, Adhikari S. Detoxification of Organosolv-Pretreated Pine Prehydrolysates with Anion Resin and Cysteine for Butanol Fermentation. Appl Biochem Biotechnol. 2018 Nov 1;186(3):662–80.
32. Yu Y, Christopher LP. Detoxification of hemicellulose-rich poplar hydrolysate by polymeric resins for improved ethanol fermentability. Fuel [Internet]. 2017;203:187–96. Available from: http://dx.doi.org/10.1016/j.fuel.2017.04.118
33. Weil JR, Dien B, Bothast R, Hendrickson R, Mosier NS, Ladisch MR. Removal of Fermentation Inhibitors Formed during Pretreatment of Biomass by Polymeric Adsorbents. Ind Eng Chem Res. 2002;41:6132–8.
34. Hu L, Zheng J, Li Q, Tao S, Zheng X, Zhang X, et al. Adsorption of 5-Hydroxymethylfurfural, Levulinic Acid, Formic Acid, and Glucose Using Polymeric Resins Modified with Different Functional Groups. ACS Omega. 2021 Jul 6;6(26):16955–68.
35. Nitzsche R, Gröngröft A, Kraume M. Separation of lignin from beech wood hydrolysate using polymeric resins and zeolites – Determination and application of adsorption isotherms. Sep Purif Technol. 2019;209(February 2018):491–502.
36. Moure S, Liguori A, Guigou M, Cebreiros F, Cabrera MN, Vila E, et al. Optimization of phosphoric acid pretreatment conditions to produce lactic acid from eucalyptus residues. In: 11th World Congress of Chemical Engineering. Buenos Aires, Argentina; 2023.
37. Schwartz TJ, Lawoko M. Removal of acid-soluble lignin from biomass extracts using Amberlite XAD-4 resin. Bioresources. 2010;5(4):2337–47.
38. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples: Laboratory Analytical Procedure (LAP) NREL/TP-510-42623 [Internet]. 2008 [cited 2018 Mar 11]. Available from: http://www.nrel.gov/docs/gen/fy08/42623.pdf
39. Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, et al. Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP) NREL/TP-510-42618 [Internet]. Laboratory Analytical Procedure (LAP) - NREL/TP-510-42618. 2012. Available from: http://www.nrel.gov/docs/gen/fy13/42618.pdf

40. Guigou M, Cabrera MN, Vique M, Bariani M, Guarino J, Ferrari MD, et al. Combined pretreatments of eucalyptus sawdust for ethanol production within a biorefinery approach. Biomass Convers Biorefin. 2019;9:293–304.
41. Garrote G, Kabel MA, Schols HA, Falqué E, Domínguez H, Parajó JC. Effects of Eucalyptus globulus wood autohydrolysis conditions on the reaction products. J Agric Food Chem. 2007;55(22):9006–13.
42. Guigou M, Guarino J, Chiarello LM, Cabrera MN, Vique M, Lareo C, et al. Steam Explosion of Eucalyptus grandis Sawdust for Ethanol Production within a Biorefinery Approach. Processes. 2023 Aug 1;11(8).
43. Penín L, López M, Santos V, Alonso JL, Parajó JC. Technologies for Eucalyptus wood processing in the scope of biorefineries: A comprehensive review. Bioresour Technol. 2020 Sep 1;311.
44. Chiarello LM, Ramos CEA, Neves P V., Ramos LP. Production of cellulosic ethanol from steam-exploded Eucalyptus urograndis and sugarcane bagasse at high total solids and low enzyme loadings. Sustainable Chemical Processes. 2016;4(1):15.
45. Rojas-Chamorro JA, Cara C, Romero I, Ruiz E, Romero-García JM, Mussatto SI, et al. Ethanol Production from Brewers' Spent Grain Pretreated by Dilute Phosphoric Acid. Energy and Fuels. 2018 Apr 19;32(4):5226–33.
46. Nair RB, Lundin M, Lennartsson PR, Taherzadeh MJ. Optimizing dilute phosphoric acid pretreatment of wheat straw in the laboratory and in a demonstration plant for ethanol and edible fungal biomass production using Neurospora intermedia. Journal of Chemical Technology and Biotechnology. 2017 Jun 1;92(6):1256–65.
47. Maciel de Mancilha I, Karim MN. Evaluation of Ion Exchange Resins for Removal of Inhibitory Compounds from Corn Stover Hydrolyzate for Xylitol Fermentation. Biotechnol Prog. 2003;19(6):1837–41.
48. Han J, Xu B, Wang H, Huang G, Zhang X, Xu Y. Purification of acidic lignocellulose hydrolysate using anion-exchange resin: Multicomponent adsorption, kinetic and thermodynamic study. Bioresour Technol. 2022 May 1;351.
49. Rochón E, Cabrera MN, Scutari V, Cagno M, Guibaud A, Martínez S, et al. Co-production of bioethanol and xylosaccharides from steam-exploded eucalyptus sawdust using high solid loads in enzymatic hydrolysis: Effect of alkaline impregnation. Ind Crops Prod. 2022 Jan 1;175.
50. Yamakawa CK, D'Imperio I, Bonfiglio F, Mussatto SI. Valorization of Pinus taeda hemicellulosic hydrolysate for the production of value-added compounds in an ethanol biorefinery. Fuel. 2022 Jun 15;318.
51. Cubas-Cano E, Venus J, González-Fernández C, Tomás-Pejó E. Assessment of different Bacillus coagulans strains for L-lactic acid production from defined media and gardening hydrolysates: Effect of lignocellulosic inhibitors. J Biotechnol. 2020 Nov 10;323:9–16.
52. Alves de Oliveira R, Schneider R, Vaz Rossell CE, Maciel Filho R, Venus J. Polymer grade L-lactic acid production from sugarcane bagasse hemicellulosic hydrolysate using Bacillus coagulans. Bioresour Technol Rep. 2019 Jun 1;6:26–31.
53. Jiang S, Xu P, Tao F. L-Lactic acid production by Bacillus coagulans through simultaneous saccharification and fermentation of lignocellulosic corncob residue. Bioresour Technol Rep. 2019 Jun 1;6:131–7.

Figure captions:

Figure 1. Process scheme for the coproduction of L-lactic acid and bioethanol from eucalyptus sawdust under a biorefinery approach. The processes studied in this work are depicted with thick lines.

Figure 2. Percentage of retention for the different components (inhibitors and xylosaccharides) after the different resin assays performed using the PA hemicellulosic liquor.

Figure 3. Percentage of retention for the different components (inhibitors and xylosaccharides) after the different resin assays performed using the concentrated PA hemicellulosic liquor.

Figure 4. From left to right: concentrated PA hemicellulosic liquor; eluted liquor (detoxified liquor) from XAD-4 resin (3:1); washing water from the first resin wash; washing water from the second resin wash.

Figure 5. Biomass, xylose, L-lactic acid, and acetic acid concentrations profile during fermentation of *W. coagulans* DSM 2314 in bioreactor at 55°C, pH 7, 150 rpm under anaerobiosis in (a) XMRS media, (b) concentrated non-detoxified liquor-based media, and (c) concentrated detoxified liquor-based media.

Table 1. Chemical composition of untreated and pretreated raw material (% dry weight of each solid).

Component	Raw material	Pretreated raw material
Glucan	44.5 ± 1.6	62.9 ± 1.9
Xylan	13.7 ± 0.8	3.3 ± 0.3
Arabinan	< 0.3	Not detectable
Acid-insoluble lignin	25.8 ± 0.5	34.5 ± 0.1 (*)
Acid-soluble lignin	2.8 ± 0.2	1.9 ± 0.2
Acetyl groups	4.4 ± 0.5	1.4 ± 0.2
Ash	0.17 ± 0.03	Not detectable
Extractives in water + ethanol	4.6 ± 0.7	(*) see AIL

(*) Includes acid insoluble lignin and the fraction of the extractives that are insoluble in acid media.

Table 2. Characterization of hemicellulosic liquor from PA pretreatment of eucalyptus sawdust.

	PA hemicellulosic liquor	**PA hemicellulosic liquor after concentration at vacuum oven**
pH	2.13 ± 0.04	2.09 ± 0.04
Xylose (g/L)	20.3 ± 0.2	29.4 ± 0.4
XOS (g/L)	4.5 ± 0.2	9.7 ± 0.1
Glucose + GOS (g/L)	2.1 ± 0.1	3.9 ± 0.1
Arabinose (g/L)	0.12 ± 0.02	0.25 ± 0.02
Acetyl groups (g/L)	7.8 ± 0.1	6.7 ± 0.1
Formic groups (g/L)	0.62 ± 0.02	0.86 ± 0.02
Acid-soluble lignin (g/L)	2.1 ± 0.1	3.8 ± 0.1
Acid-insoluble lignin (g/L)	0.8 ± 0.2	2.1 ± 0.2
Furfural (g/L)	0.72 ± 0.05	0.75 ± 0.03
HMF (g/L)	0.25 ± 0.08	0.17 ± 0.05

Table 3. Results obtained for the L-lactic acid production by *W. coagulans* in XMRS (control) and concentrated non-detoxified and detoxified hemicellulosic liquor-based media.

Parameter	Control	Concentrated non-detoxified liquor-based media	Concentrated detoxified liquor-based media
Biomass (g/L)	3.6	2.2	3.4
Fermentation time (h)	24	48	24
Xylose consumption (%)	100	54	92
L-lactic acid (g/L)	19	12	16
$Y_{P/S}$ ($g_{lactic\ acid}/g_{sugar}$)	0.95	0.92	0.73
Q_P ($g_{lactic\ acid}$/Lh)	0.8	0.3	0.7

Table 4. Comparison of L-lactic acid production by microbial fermentation using *B. coagulans spp*. from lignocellulosic biomass with other reported data.

Substrate	**Pretreatment**	**Strain**	**Initial sugars (g/L)**	**Lactic acid (g/L)**	$Y_{P/S}$ ($g_{lactic\ acid}/g_{sugar}$)	Q_P ($g_{lactic\ acid}$/Lh)	**Reference**
Eucalyptus sawdust hemicellulosic hydrolysate	Phosphoric acid (160°C, 40 min, 0.6% H_3PO_4) + Resin detoxification	*B. coagulans* DSM 2314	~ 25	16	0.73	0.7	This study
Steam-exploded sugarcane bagasse	Steam explosion (195°C, 7.5 min) + Activated carbon detoxification	*B. coagulans* DSM 2314	13.9	13.4	0.96	0.54	[15]
Diluted *Pinus taeda* hemicellulosic hydrolysate (28%)	Steam explosion (200°C, 10 min)	*B. coagulans* DSM 2314	7.6	5.32	0.7	0.22[1]	[53]

Gardening residues hemicellulosic hydrolysate	Acid-catalyzed steam explosion (180°C, 10 min, 0.33 M H_2SO_4)	*B. coagulans* DSM 2314	~ 28	22.5	0.80[1]	1.63	[51]
Concentrated sugarcane bagasse hemicellulosic hydrolysate	Hydrochloric acid (140°C, 15 min, 0.5% (v/v) HCl)	*B. coagulans* DSM ID 14-300	62	56	0.87	1.70	[52]

[1] Parameter calculated from reported data.

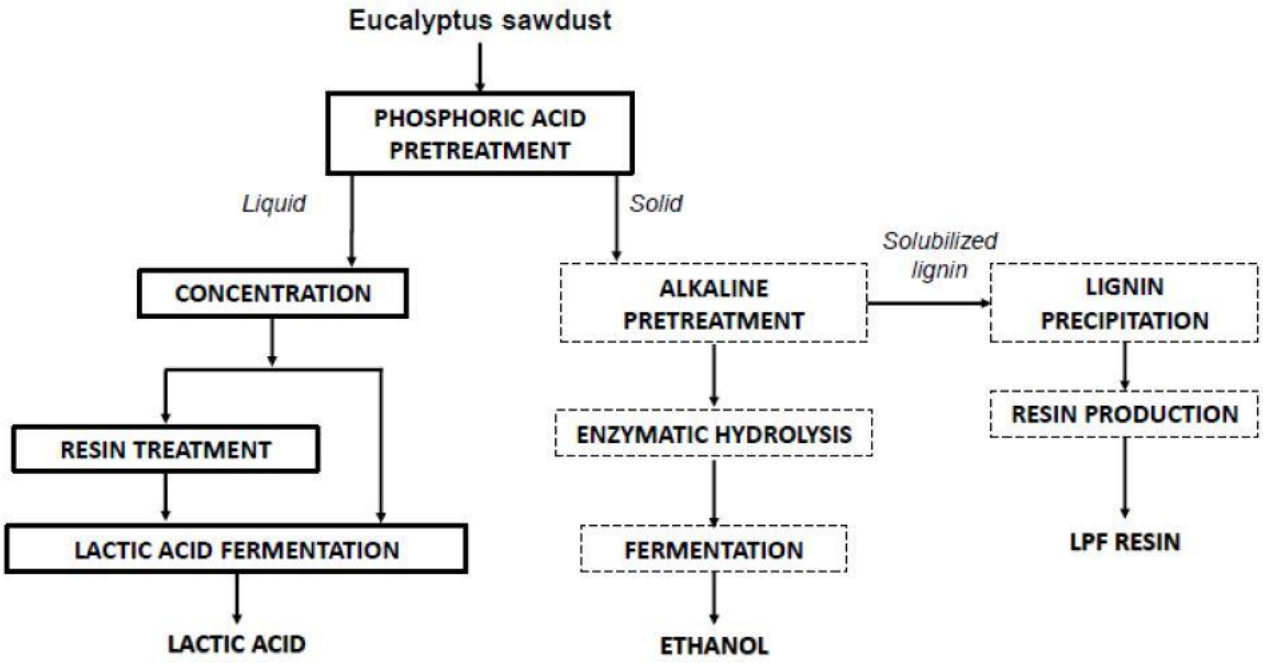
Eucalyptus sawdust
PHOSPHORIC ACID PRETREATMENT
Liquid
Solid
CONCENTRATION
RESIN TREATMENT
LACTIC ACID FERMENTATION
LACTIC ACID
ALKALINE PRETREATMENT
Solubilized lignin
LIGNIN PRECIPITATION
RESIN PRODUCTION
LPF RESIN
ENZYMATIC HYDROLYSIS
FERMENTATION
ETHANOL

Fig. 1

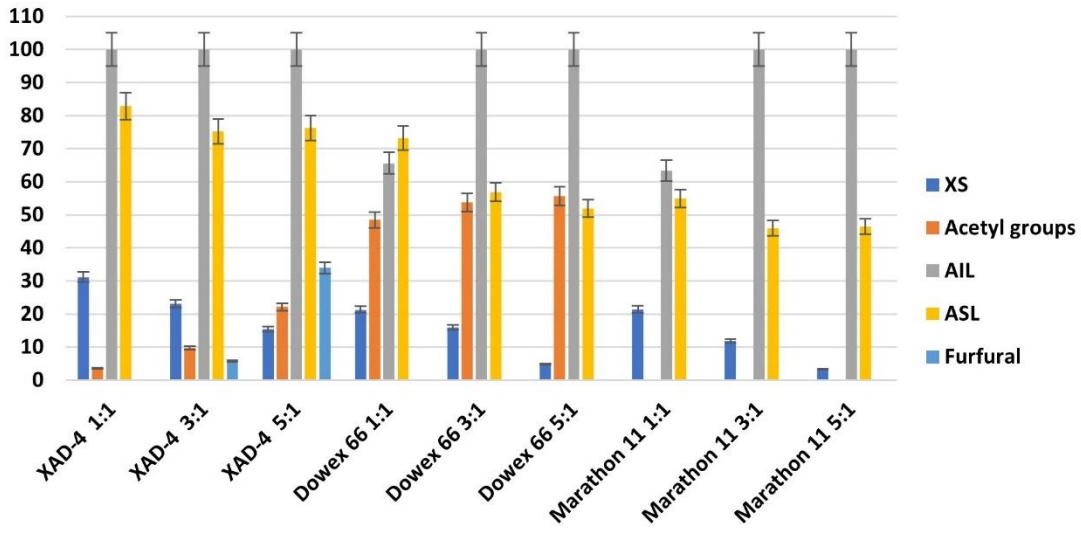
110
100
90
80
70
60
50
40
30
20
10
0
XAD-4 1:1
XAD-4 3:1
XAD-4 5:1
Dowex 66 1:1
Dowex 66 3:1
Dowex 66 5:1
Marathon 11 1:1
Marathon 11 3:1
Marathon 11 5:1
XS
Acetyl groups
AIL
ASL
Furfural

Fig. 2

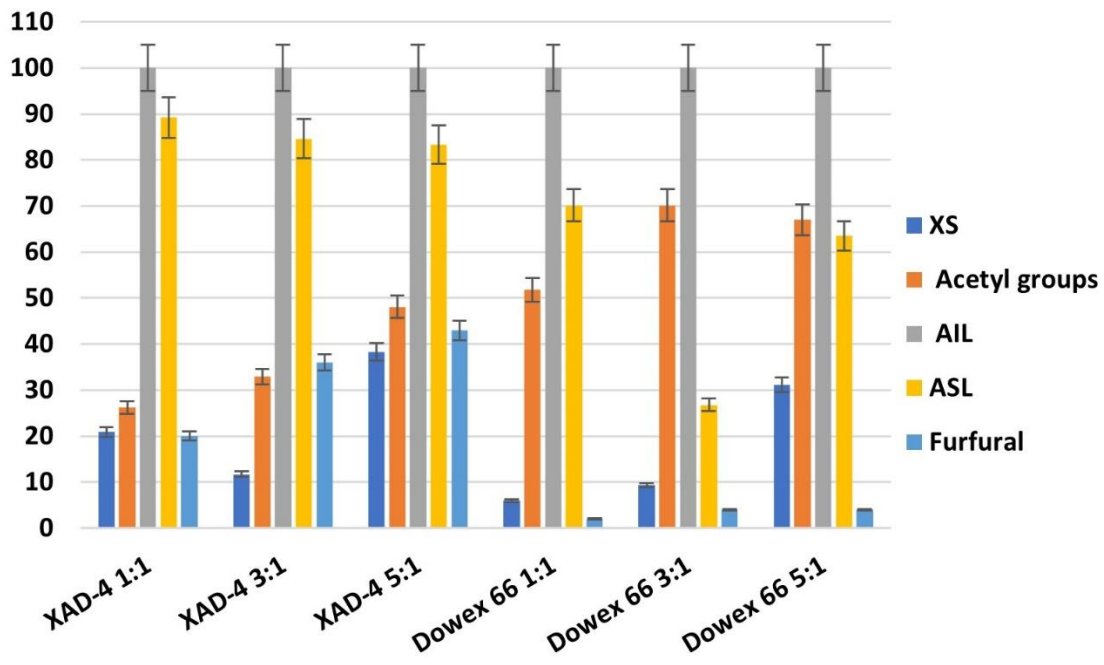
110
100
90
80
70
60
50
40
30
20
10
0
XAD-4 1:1
XAD-4 3:1
XAD-4 5:1
Dowex 66 1:1
Dowex 66 3:1
Dowex 66 5:1
XS
Acetyl groups
AIL
ASL
Furfural

Fig. 3

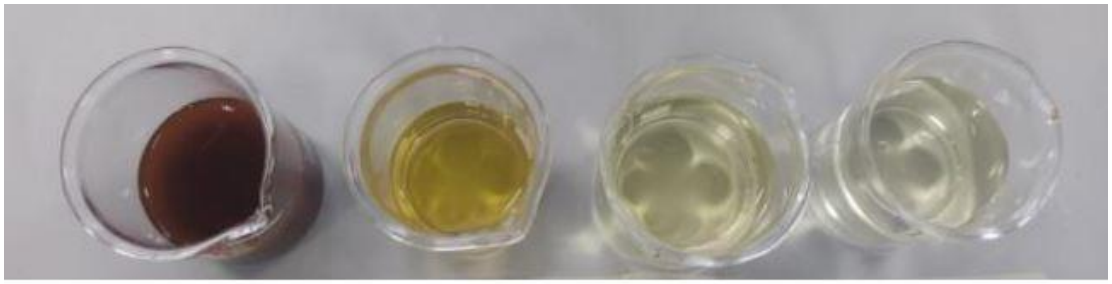

Fig. 4

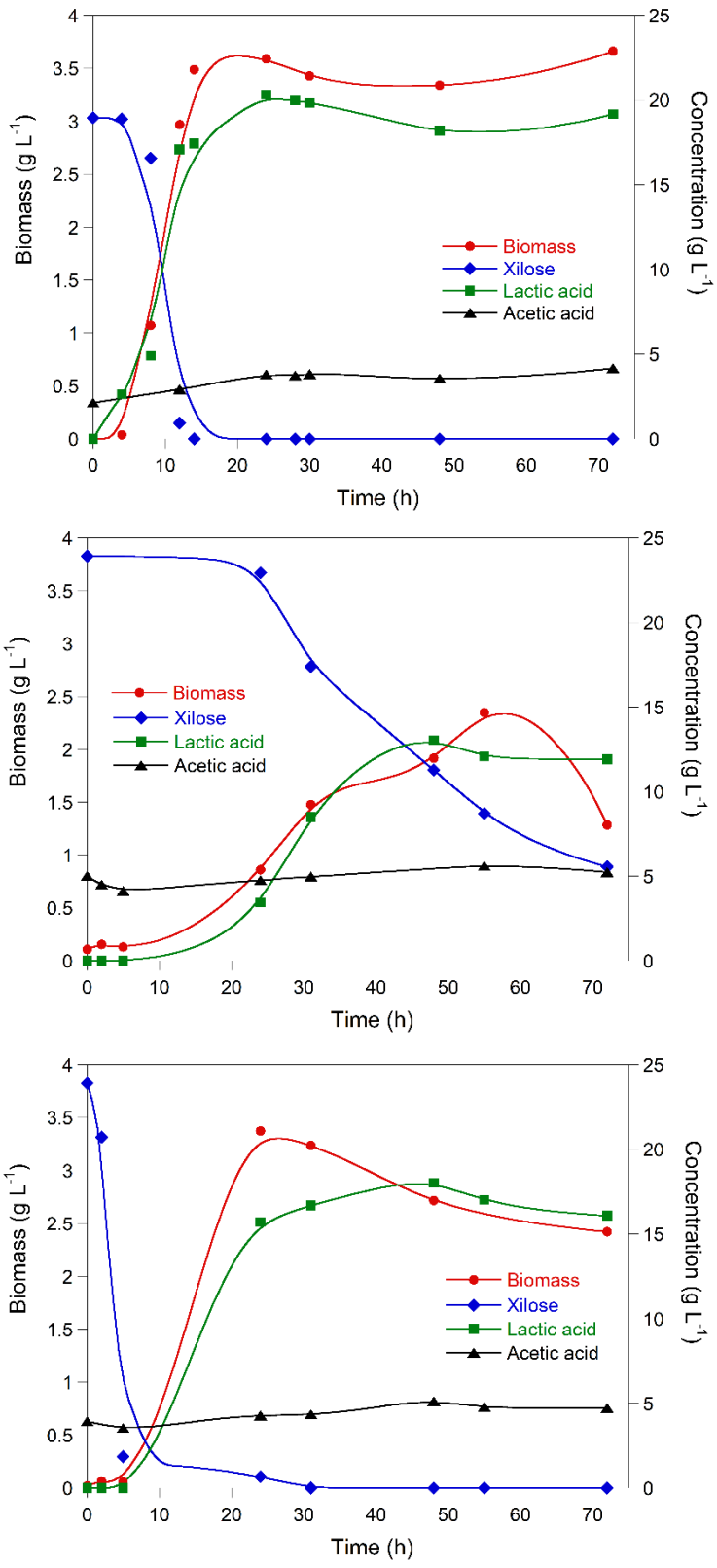
Biomass (g L-1)
Concentration (g L-1)
Time (h)
Biomass
Xilose
Lactic acid
Acetic acid

Fig. 5