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# Detoxification of a Lignocellulosic Waste from a Pulp Mill to Enhance Its Fermentation Prospects

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**Abstract:** Detoxification is required for sugar bioconversion and hydrolyzate valorization within the biorefining concept for biofuel or bio-product production. In this work, the spent sulfite liquor, which is the main residue provided from a pulp mill, has been detoxified. Evaporation, overliming, ionic exchange resins, and adsorption with activated carbon or black carbon were considered to separate the sugars from the inhibitors in the lignocellulosic residue. Effectiveness in terms of total and individual inhibitor removals, sugar losses and sugar-to-inhibitor removal ratio was determined. The best results were found using the cation exchange Dowex 50WX2 resin in series with the anion exchange Amberlite IRA-96 resin, which resulted in sugar losses of 24.2% with inhibitor removal of 71.3% of lignosulfonates, 84.8% of phenolics, 82.2% acetic acid, and 100% of furfurals. Apart from exchange resins, the results of evaporation, overliming, adsorption with activated carbon and adsorption with black carbon led to total inhibitor removals of 8.6%, 44.9%, 33.6% and 47.6%, respectively. Finally, some fermentation scenarios were proposed in order to evaluate the most suitable technique or combination of techniques that should be implemented in every case.

**Keywords:** biorefinery; detoxification; fermentation; inhibitors; spent sulfite liquor; sugars

## 1. Introduction

Pretreatment and delignification followed by hydrolysis and detoxification (fractionation) prior to fermentation is usually required to assure an efficient bioconversion of lignocellulosic biomass into bio-based chemicals, biopolymers, biofuels and energy. Optimization of these stages permits the integral use of the feedstock, increasing the lignocellulosic biorefinery economic margins and the global demand for the use of biomass renewable resources. Therefore, a proper deconstruction of the lignin polymer from the plant cell wall (pretreatment and delignification stages); followed by an efficient depolymerisation of carbohydrate chains (hydrolysis); and separation of the main undesirable inhibitor compounds forming part of the lignocellulosic hydrolyzates should be carried out before fermentation. The main objectives to be taken into account in detoxification processes are [1]: (i) low cost of chemicals, solvents and other reagents employed; (ii) minimization of waste generation; (iii) preservation of hemicellulose sugars making them more accessible for fermentation; (iv) minimal conditioning costs; and (v) little sugar losses. The projected use of biofuels is raising day by day because of the oil dependency and greenhouse gas emissions. Some examples found in the literature in Europe [2], Brazil [3], China [4] or Canada [5] for second-generation bioethanol production from non-food raw materials.

Nowadays, most challenges in lignocellulosic fractionation consist of overcoming technical and economic barriers. Detoxification treatments have the purpose of reducing the toxic effects of inhibitory degradation compounds formed during the hydrolysis of biomass. In addition, due to the

use of these compounds in the production of value-added products, the term fractionation instead of detoxification has been used. Such treatments are based on separation and purification technologies carried out by physical, chemical or biological methods [6]. Detoxification prior to fermentation becomes significant when working with lignocellulosic residual streams obtained from textile, agricultural or food industries. Such residues are typically partially or totally hydrolyzed [7,8]. Many examples can be found in the literature for inhibitor removal in diverse situations. Overliming [9–11], ultrafiltration [12–14], chromatographic separation [15], treatment with reducing agents [16,17], ion exchange resins [18–20], adsorption with activated carbon [20–22], organic solvent extraction [23–25], or microbial treatment [24,25] are the most common processes. The separation mechanisms, advantages and disadvantages were already analyzed and published by our research group [26].

This study contemplates the detoxification or fractionation of a Sulfite Spent Liquor (SSL) from an industrial facility located in the north of Spain, which is a sugar-rich residue generated in a pulp mill. The factory produces dissolving pulp for hygienic, sanitary or textile purposes. The demand for dissolving pulps has substantially increased during the last decade in the entire world (not only in China or other Asian countries). Within this framework, the sulfite pulping process becomes significant and the spent liquors generated constitute an important renewable resource for biorefining purposes. A drawback of these liquors is their low pH and high levels of lignosulfonates which make it very difficult to separate and recover the different inhibitory compounds from the sugar substrate [26,27]. Slow advances were recorded in recent years for the sulfite pulping process [28]. Previous studies have reported the presence of 87.2% and 98.5% of hemicellulose and lignin in the hardwood feedstock into this SSL [29] and showed that this residue is already hydrolyzed, therefore, external hydrolysis before fermentation is not worthwhile [30]. However, during the extraction of cellulose from *Eucalyptus globulus* in the sulfite mill, different inhibitors are also formed. Such inhibitors constitute a complex mixture of microbially-derived toxins, including acetic acid, furan dehydration products, aliphatic acids, assorted phenolic compounds and inorganic ions [25,26]. Lignosulfonates, which are the major component of the SSL, are not toxins themselves, but they hinder the acetic acid removal ability, for instance, by strong base resins [19]. Consequently they must to be removed as well. A previous work of our research group [26] studied the main inhibitors forming part of lignocellulosic matrices and specifically of the SSL. Coz et al. [26] and also other consulted authors [31,32] suggested similar inhibitor classifications for lignocellulosic hydrolyzates: (i) furan derivatives such as furfural and HMF; (ii) phenolic compounds; (iii) weak organic acids (levulinic, formic and acetic acids); (iv) raw material extractives (acidic resin, tannic acids and terpene acids) and (v) heavy metal ions (like iron, nickel, aluminum, chromium, etc.), concluding that weak acids, furan derivatives and phenolics are the main fermentation inhibitors.

One of the main conclusions of Coz et al. [26] is that all of the compounds can be inhibitors in the post-fermentation process, and, depending on the fermenting scenario, the maximum concentration of the inhibitors can vary. Parajó et al. [33] also reported that the maximum allowable concentration for each inhibitor cannot be established as general rule, since it is strongly dependent on factors such as: (i) the microorganism utilized and its degree of adaptation; (ii) the fermentation process; and/or (iii) the synergistic effects caused by the simultaneous presence of several other inhibitors.

As the SSL has already been subjected to pretreatment, delignification and hydrolysis, all the efforts should be focused on detoxification and fermentation. In addition, the recovery of the inhibitors present in the SSL should be a priority together with the sugar bioconversion. With this in mind, the main goal of this study was to achieve cost-effective detoxification processes able to transform the existing pulp mill into a modern lignocellulosic biorefinery by producing not only cellulosic pulp, but also value-added products from the sugar platform. Among the detoxification alternatives previously reviewed by the authors [26], evaporation, overliming, exchange resins, and adsorption were selected for this kind of lignocellulosic material as they can be used in these kinds of inhibitors and because of their selectivity, simplicity of design, operation, scale-up and low cost, among other advantageous features. Apart from the selected techniques, in previous papers, the authors applied membranes [34]

and organic solvents [24] to separate SSL sugars from the rest of inhibitors. Membranes were adequate for lignin-derivatives such as lignosulfonates but fouling problems might lead to inefficient process operations and increased costs; on the other hand, organic solvents were suitable only in the case of valorizing phenolic compounds.

In this research, the following tasks have been developed: in a first step, evaporation, overliming, anionic resins, cationic resins, adsorption with activated carbon and black carbon were applied to separate the sugars from the inhibitors in the industrial SSL; in a second step, technical effectiveness of these fractionation processes was evaluated in terms of total sugar losses (TSL), individual inhibitors removal (IIR), total inhibitors removal (TIR) and the sugar-to-inhibitors removal ratio (S/I); finally, a discussion about the best separation processes coupled to several fermentation purposes within different scenarios. Most common fermentation scenarios, microorganisms, conditions and constraints for fermenting lignocellulosic hydrolyzates from the literature were reviewed. As a result, it was possible to devise an approach for determining the best detoxification technique or combination of techniques suitable in every single case from a technical point of view. In recent years, many publications have studied a single detoxification technique coupled with a specific fermentation scenario [2,9,16,19,23,35]. Other papers have studied the combination of detoxification techniques experimentally [10,17,18,20,21,36,37] or from a theoretical viewpoint [26,31,38,39]. Nonetheless, the present research is the first paper working with spent sulfite liquor which compares different detoxification techniques showing which one of them would be adequate to produce seven different fermentation and value-added products (ethanol, bacterial cellulose, xylitol, polyhydroxyalkanoate (PHA), acetone-butanol-ethanol (ABE), succinic acid and polyhydroxybutyrate (PHB).

## 2. Materials and Methods

### *2.1. Resource Material: The Spent Sulfite Liquor*

Industrial liquors from calcium-magnesium sulfite pulping of *Eucalyptus globulus* were supplied by Sniace S.A. Company (Torrelavega, Spain). The liquor is transferred to a multiple effect evaporation plant (MEEP). In this work liquors collected at the beginning and at the end of the MEEP called respectively diluted-SSL and concentrated-SSL were used: (i) diluted-SSL for the laboratory evaporation experiments; (ii) concentrated-SSL for overliming, ion exchange resins and adsorption experiments.

### *2.2. Fractionation Procedures*

Evaporation, overliming, adsorption and ion exchange resins have been carried out in duplicate.

#### 2.2.1. Evaporation

Evaporation of the SSL was done in a rotary evaporator LR-4000 (Heidolph, Schwabach, Germany) at laboratory scale, coupled with a vacuum pump MZ2C NT (Vacuubrand, Wertheim, Germany) and was controlled and monitored by a CVC 3000 device (Vacuubrand, Wertheim, Germany). With the equipment described above, the effects of the industrial evaporation plant were reproduced. Ten samples corresponding to five concentrates (bottom phase) and five condensates (light phase) of each effect were collected. By means of a rotary evaporator it was possible to reproduce the industrial MEEP at laboratory scale. MEEP consists of five evaporators connected in series where the output vapor from one unit acts as a heat source for the next effect. Pressure and temperature conditions were taken from the plant. However, the data is not displayed in this paper because it is confidential information from the pulp mill. In our case, we show the quantity of dry matter of every effect to be the same values as at industrial scale, being 10.0%, 14.4%, 18.5%, 27.6% and 55.3% $w/w$ respectively. Every sample was analyzed in triplicate.

#### 2.2.2. Overliming

The experiments were conducted in 250 mL Erlenmeyer flasks. The SSL was placed in a 1-L beaker and neutralized up to pH 10. After adjusting the pH, the SSL/alkali was distributed in Erlenmeyer flasks and maintained at a fixed temperature in an orbital incubator at 120 rpm until equilibrium was reached. Afterwards, samples were placed in Falcon tubes and centrifuged. The supernatant was adjusted with commercial $H_2SO_4$ to pH 5.5. Three different sets of experiments were carried out according to the following information:

(1) The effect of the kind of alkali was studied. Two different neutralizing agents, $Ca(OH)_2$ 2.5 M and $NH_4OH$ 2.5 M, were used to raise the pH up to 10. Temperature at 25 °C was chosen.
(2) The effect of temperature was assayed. Tests were performed at 25, 30, 35 and 40 °C, at pH 10, using $Ca(OH)_2$ 2.5 M as neutralizing agent.
(3) The effect of pH was analyzed. Temperature was set at an intermediate value of 30 °C and using $Ca(OH)_2$ 2.5 M as neutralizing agent, experiments at pH of 5, 6, 7, 8 and 9 were done.

#### 2.2.3. Adsorption with Activated Carbon and Black Carbon

Granular activated carbon (AC) and powdered black carbon (BC) were used in this work as adsorbents. AC supplied by Panreac (Barcelona, Spain) with 1.25–3.15 mm particle size, density of 920 g/cm$^3$ and a 878 m$^2$/g of surface area was used. The BC N220 supplied by Columbian Carbon S.L. (Cantabria, Spain) presents a particle size in the range of 0.044 and 0.5 mm and surface area in the range of 1150 and 2000 m$^2$/g. SSL was neutralized at pH 7 with $Ca(OH)_2$ and adjusted to pH 5.5 with $H_2SO_4$ [36,37]. After neutralization, batch experiments were conducted in 250 mL Erlenmeyer flasks. Kinetic isotherms at 30 and 50 °C were constructed at adsorbent-to-SSL ratios of 1:10 and 1:5 $w/v$ (grams of dry adsorbent per mL of neutralized-SSL). Equilibrium trials were also done varying the ratio at 1:5, 1:10, 1:20 and 1:50 weight/volume ($w/v$, in grams of AC or BC per mL of SSL). Then 1:5 and 1:10 $w/v$ were selected as the best conditions in terms of phenolics and LS separation degree. In order to recover the adsorbent, spent carbon was washed and equilibrated sequentially with 0.4 N HCl or 0.05 M $H_2SO_4$, then washed with distilled water and dried at room temperature.

#### 2.2.4. Ion Exchange Resins

Dowex 50WX2 cation exchange resin (mesh 100–200) in H+ form and Amberlite IRA-96 anion exchange resin (mesh 20–50) in $OH^-$ form were used according to Fernandes et al. [18]. Experiments were carried out at batch mode in a rotary shaker at 60 rpm, room temperature at different settling times. Trials were extended until equilibrium was reached under settling ratios. Times between 5 and 120 min were considered (5, 15, 20, 40, 60 and 120 min). In this case, there is no pH adjustment considering results of Fernandes et al. [18] working also with acid hydrolyzates. The effect of resin-to-SSL ratio was also contemplated, varying from 1.2 to 60 mL SSL/g wet resin. A total of 7 ratios were studied: 1.2, 1.5, 2, 3, 6, 30, 60 mL/g. Resin activation was performed with 5% $w/w$ HCl for cation exchange resin, or 4% $w/w$ NaOH for anion exchange resin. After activation and regeneration, resins were washed using ultrapure water until neutral pH.

### *2.3. Analytical Methods*

Diluted-SSL, concentrated-SSL and detoxified-SSL samples were analyzed for sugars, acids and furfurals by HPLC/RI; $Mg^{+2}$ and $Ca^{+2}$ by flame atomic absorption spectrometry; and low molecular phenolics and lignosulfonates by UV-Vis spectrophotometry. All samples were analyzed in triplicate.

#### 2.3.1. Determination of Lignosulfonates and Phenolic Hydroxyl Groups

Lignosulfonates (LS) and hydroxyl groups (OH) were determined following the standard UNE EN 16109 [40]. The UV-Vis spectrophotometer (Lambda 25-Perkin Elmer, Waltham, MA, USA) was

used for LS and OH quantification. The LS and phenolic hydroxyl groups were calculated as follows using Equations (1) and (2):

$$LS\ (\%) = \frac{Abs\ (232.5\ \text{nm}) \cdot D}{f \cdot P(\text{g}) \cdot 10} = \frac{Abs\ (232.5\ \text{nm}) \cdot 100 \cdot 250}{f \cdot P(\text{g}) \cdot 30} \quad (1)$$

where $f$ is a factor which is 35.2 for calcium lignosulfonate; $P$ is the sample weight in grams; $D$ is the dilution factor in mL; *Abs* (232.5 nm) is the absorbance at 232.5 nm.

$$OH_{phenolics}\ (\%) = 0.192 \cdot \frac{Abs\ (255\ \text{nm}) - Abs_{\text{min}}\ (340 - 220\ \text{nm})}{Conc\ (\text{g/L})} \quad (2)$$

where *Conc* (g/L) is the value of dry lignosulfonate concentration in 50 mL flask solutions, *Abs* (255 nm) is the absorbance peak value of about 255 nm and $Abs_{\text{min}}$ (220−340 nm) the minimum valley to either side.

#### 2.3.2. Analysis of Sugars and Degradation Products

Samples were passed through the HPLC-RI for sugars, aliphatic acids (acetic, levulinic and formic acids), methanol, furfural and 5-hydroxymethylfurfural (HMF) quantification. The HPLC system used was a Prominence LGE-UV Shimadzu (IZASA, Madrid, Spain). Sugars were separated using a CARBOSep CHO-782 Pb column operating at 0.3 mL/min of ultrapure water at 68 °C with 20 µL of injection volume. Acids, methanol and furfurals were determined by using a Shodex SH-1011 column working with 0.5 mL/min of 0.005 M $H_2SO_4$ M at 60 °C and sample injection of 20 µL.

#### 2.3.3. Analysis of Metals and Sulfur Dioxide

The SSL contains high amounts of calcium and magnesium arising from the dolomite used in the cooking process. Metal compositions were determined using flame atomic absorption spectroscopy AA-7000, Shimadzu (IZASA, Madrid, Spain). Calcium lamp operated at 10 mA and spectral bandwidth of 0.1 nm, setting the analytical wavelength at 422.7 nm. Magnesium lamp operated at 4 mA with 0.1 nm of bandwidth at 285.2 nm. The fuel in both cases was a mixture of synthetic air and acetylene. Calcium standards at 0.5, 1, 2, 5, 10 and 50 mg/L and magnesium standards at 0.1, 0.2, 0.4 and 1 mg/L were prepared. Standards consisted of aqueous solutions acidified with 4 mL of commercial HCl (Panreac). Four mL of a releasing agent were also added to every standard, being 210 g/L of $LaCl_3$ in case of calcium and 99.77 g/L of $SrCl_2$ for magnesium. $LaCL_3$, $SrCl_2$, Ca and Mg standards were supplied by Panreac. Samples were prepared using 25 µL of releasing agent per 25 mL of sample and ultrapure water. As the pH is acid, the addition of hydrochloric acid is not required.

Furthermore, free, combined and total $SO_2$ in the SSL and evaporated samples were determined by volumetric titration, following the methodology described in the TAPPI T 604 om-00 standard [41].

## 3. Results and Discussion

Before the evaporation stage, the so-called diluted spent liquor is composed of LS (42% *w*/*w* dry basis), sugars (24%), acetic acid (7.4%), Ca (3.6%), $SO_2$ (2.3%), phenolics (2.01%), cellobiose (1.9%), Mg (0.74%), methanol (0.49%), furfural (0.31%), formic acid (0.26%), Na (0.28%), HMF (0.15%), K (0.13%), levulinic acid (0.11%), and Fe (0.049%). Ashes at 900 °C constitute 9.9% dry matter, giving a 4.8% of non-identified compounds.

After evaporation, the SSL used in overliming, adsorption and ionic resins experiments is composed of 422 ± 31.0 g/L of LS and 214 ± 20.1 g/L of sugars which constitute about 82% *w*/*w* of dry matter. Minor compounds were, in descending order: Ca (1.9%), phenolics (1.6%), Mg (1.3%), acetic acid (1.02%), cellobiose (0.68%), $SO_2$ (0.29%), Na (0.27%), K (0.14%), Fe (0.045%), formic acid (0.031%), methanol (0.022%), furfural (0.019%), HMF (0.016%), and levulinic acid (0.014%). Ashes at 900 °C constitute 8.7% dry matter, giving a 1.3% of non-identified compounds. Considering high amounts

of LS after evaporation stage, which is around 56% of the total dry matter, the effect of LS cannot be ignored. LS have adhesive and dispersant properties which make them feasible for fabrication of composite boards. As pointed out by Coz et al. [26], it is more difficult to separate the sugar substrate from the rest of the inhibitors, mainly because the lignosulfonates act as glue in the mixture.

### *3.1. Pre-Treatment of the SSL by Evaporation*

Concentration by evaporation is considered as a previous detoxification stage, removing the most volatile compounds. In the factory, there are five effects working in series in order to concentrate the spent liquor. Two kinds of streams are generated at the end of every effect: condensates and concentrates. Condensates are the fraction rich in volatile compounds. Such condensates are currently being reintroduced in the accumulators for fresh liquor $SO_2$ enrichment in the factory. At the end of the process, the last concentrate stream (bottom phase) is directly sent to another enterprise where the lignosulfonates are reused and the sugars are burned.

In order to study the evaporation step, some laboratory trials were carried out. Results of the evaporation at laboratory scale were similar to the industrial plant. Results were expressed as concentrations and removals in Figure 1. Concentrations were reflected as percentages, where FormC, LevC, FuC, SuC and LSC, are formic acid, levulinic acid, furfurals, sugars and lignosulfonates concentrations respectively. On the other hand, removals were also expressed as percentages, where HAcR, MetOHR, co$SO_2$R and TIR, represent acetic acid, methanol, combined $SO_2$ and total inhibitors removals respectively.

As combined $SO_2$, water, methanol, acetic and formic acids are partially removed, the pH increases from 1.8 to 2.3. Acetic removals reached 53% in the last effect whereas 95.3% MetOHR and 28.6% co$SO_2$R were the maximum removals of methanol and combined $SO_2$ respectively. For these reasons, the fifth effect constitutes the most suitable fraction for SSL detoxification and valorization.

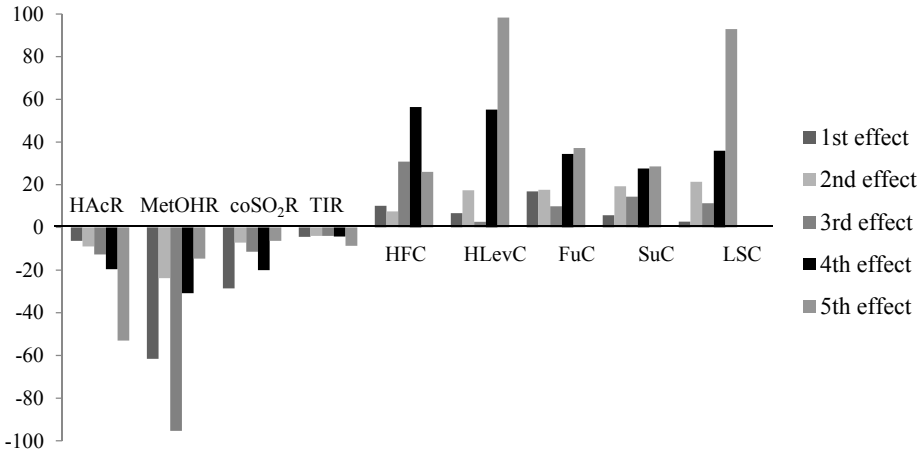


**Figure 1.** Results of inhibitors and sugars of SSL within the five evaporation effects.

Based on Figure 1 results, it can be concluded that evaporation accomplished a double objective. Evaporation can be considered as a preliminary detoxification stage with total inhibitor removals of 8.61% at the last effect. Thus, evaporation is a pre-treatment where not only LS and sugars increased, but also several volatile toxic inhibitors i.e., $SO_2$, methanol or acetic acid totally or partially disappeared.

### 3.2. Overliming

Once the evaporation has been carried out, other separation techniques were studied. Precipitation of lignosulfonates from SSL can be achieved by addition of lime or other alkali, forming the insoluble calcium lignosulfonate compounds according to the following reaction:

$$(\text{Lignin-SO}_3)_2\text{Ca} + \text{Ca(OH)}_2 \rightarrow (\text{Lignin-SO}_3)_2\text{Ca}\cdot\text{Ca(OH)}_2\downarrow \quad (3)$$

Thus, the insoluble lignin is also contained on the precipitate. Alkali treatment has been reported to be one of the best and most common detoxification methods because of its low cost and high efficiency [9,10]. Unlike neutralization, which consists of working within a pH range of 5 and 7, overliming can raise the pH from 10 to 12. In addition to the removal of lignosulfonates, this effect promotes precipitation of low molecular weight phenolics and furfurals. Besides, some carboxylic acids with pKa 3–4 are converted to salts, which decrease their ability to form hydrogen bonds with the lignin network [6]. Overliming has a drawback in terms of sugar losses. There are variables that can drastically contribute to sugar losses like high pH values, temperatures or residence times. Based on the literature, moderate temperatures below 60 °C and pH under 12 should be used [9].

In this work, the effect of the kind of alkali at 25 °C, pH of 10 for 30 min was studied firstly. Two different alkalis were used, $Ca(OH)_2$ and $NH_4OH$, because they are the most frequently used in literature [9,10,35]. Experiments were carried out until equilibrium and the results showed higher LS removals by using $Ca(OH)_2$ in comparison with $NH_4OH$. Lignosulfonate removals were respectively 36.5% and 13.3%. Sugar losses were slightly higher in case of $Ca(OH)_2$ with losses of 11.5% whereas using $NH_4OH$ there were registered sugar losses of 5.5% in the SSL.

The effect of time and temperature is plotted in Figure 2a,b using 0.5 M $Ca(OH)_2$. Error bars are presented in the figures. These experiments were performed holding the pH at 10 which is a moderate pH [9]. Due to the fact that overliming mainly affects LS removal, the concentration of these compounds has been represented. Furthermore, phenolics decreased from 12.60 g/L to an average value of 7.57 g/L regardless of the temperature checked, and only a small decrease in the concentration of acetic acid, from 7.85 to 7.80 g/L, and furfurals, from 0.27 to 0.20 g/L, was obtained. With the purpose of determining optimal experimental conditions, only LS removals in Figure 1a and sugar losses in Figure 2b will be considered. As was expected, both LS and sugars decrease by increasing temperature reaching a minimum at 45 °C with 218.7 g/L of LS and 174.2 g/L of sugars.

The effect of pH over LS, furfurals and sugar losses is plotted in Figure 2c,d. It can be observed how an increase of pH resulted in depletion of inhibitors. Consequently, an increase of pH enhances detoxification and therefore increases the fermentability of the hydrolyzates. Maximum LS precipitation occurs at pH 10 where the LS decrease from 421.9 to 260.4 g/L, phenolics from 12.60 to 8.0 g/L and furfurals from 0.27 to 0.076 g/L. In addition, acetic acid decreased from 7.85 g/L up to 5.6 g/L at 25 °C and pH 10.

The effect of pH is not as drastic as the effect of temperature. The treatment at 25 °C and pH 10 resulted in a better fermentability regarding sugar concentration and LS precipitation. Higher pH or temperatures were not contemplated due to the drawback of sugars precipitation according the studies of Millati et al. [9] and Persson et al. [10].

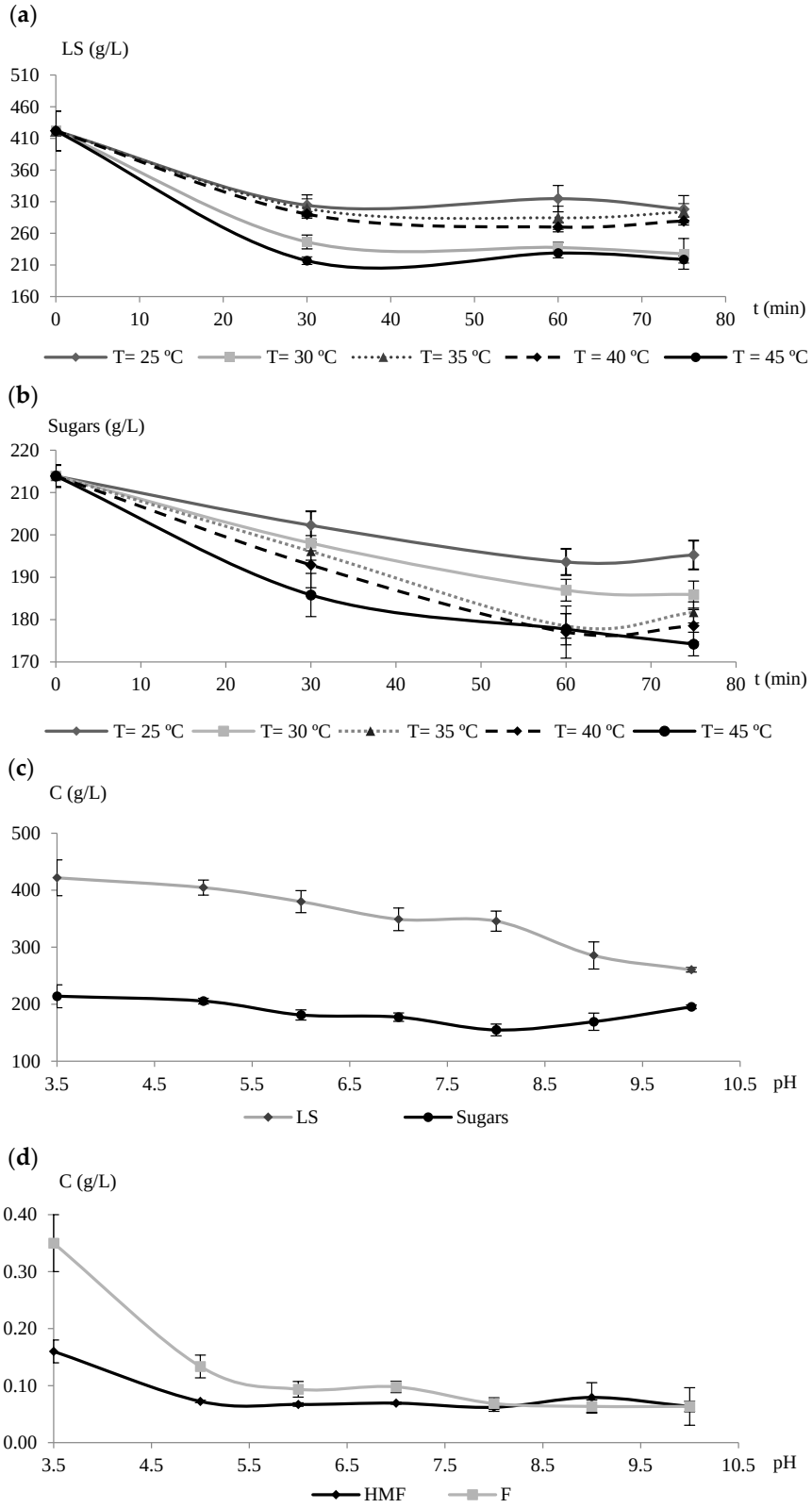


**Figure 2.** (**a**) Variation in time and temperature over lignosulfonate detoxification; (**b**) and the sugar losses in the hydrolyzates; (**c**) Effect of pH over lignosulfonates and sugar; (**d**) and furfural concentration.

### 3.3. Adsorption with Activated Carbon and Black Carbon

Adsorption enables the separation of selected organic compounds from aqueous streams. Among the advantages, its design simplicity, operation and scale up, high capacity and favourable rate, insensitivity to toxic substances, easy regeneration and low cost can all be highlighted. Additionally, adsorption avoids the use of toxic solvents and minimizes degradation [38]. Firstly, the effect of the time was studied in both AC and BC. Because the shape of curves was quite similar in all studied cases, Figure 3 shows only the experiments using BC at 50 °C, 1:5 $w/v$. Error bars are also presented in both figures. Furfurals were not plotted because separation took place almost immediately. Selectivity of AC and BC towards the analyzed components is, in descending order: furfurals, acetic acid, phenolics and LS. In all cases, saturation times were reached fast (before 20 min) regardless of the adsorbent, temperature or ratio employed.

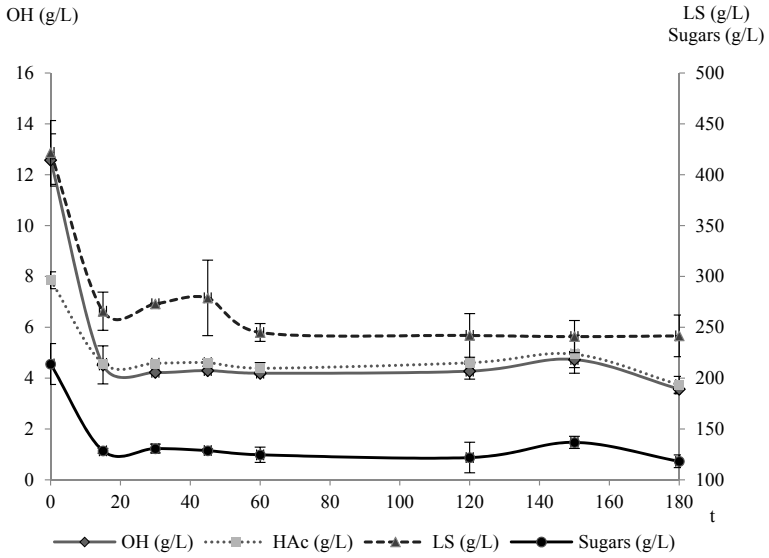


**Figure 3.** Concentration of sugars and inhibitors over time, using BC at 50 °C and 1:10 $w/v$ ratio.

Once the equilibrium was reached, final concentrations and consequently maximum removals are summarized in Table 1 at different temperatures, adsorbents and ratios. The two adsorbents, AC and BC, at 30 and 50 °C and solid-to-liquid ratios of 1:10 and 1:5 g per mL of SSL were considered based on literature [21,22].

**Table 1.** Detoxification by means of adsorption: influence of temperature, adsorbent and ratio.

| (g/L) | LS | OH | HAc | Furfurals | Sugars |
|---|---|---|---|---|---|
| **SSL** | 422 ± 31 | 12.6 ± 1.0 | 7.85 ± 0.33 | 0.27 ± 0.19 | 214 ± 20 |
| **BC 30 °C 1:10 *w/v*** | 231 ± 5 | 5.24 ± 0.96 | 3.57 ± 1.32 | <DL [a] | 119 ± 23 |
| **BC 50 °C 1:10 *w/v*** | 218 ± 55 | 3.69 ± 0.12 | 2.93 ± 0.08 | <DL | 77.5 ± 0.6 |
| **BC 30 °C 1:5 *w/v*** | 112 ± 0.6 | 3.63 ± 0.13 | 4.14 ± 0.1 | <DL | 99.7 ± 1.4 |
| **BC 50 °C 1:5 *w/v*** | 100 ± 10 | 4.19 ± 0.37 | 4.49 ± 0.37 | <DL | 125 ± 9 |
| **AC 30 °C 1:10 *w/v*** | 271 ± 52 | 7.70 ± 1.25 | 5.03 ± 1.35 | <DL | 117 ± 25 |
| **AC 50 °C 1:10 *w/v*** | 228 ± 25 | 6.31 ± 0.07 | 3.27 ± 0.50 | <DL | 104 ± 28 |
| **AC 30 °C 1:5 *w/v*** | 193 ± 16 | 4.90 ± 0.06 | 1.96 ± 0.09 | <DL | 94.5 ± 15.1 |
| **AC 50 °C 1:5 *w/v*** | 193 ± 15 | 5.21 ± 0.20 | 1.76 ± 0.11 | <DL | 86.3 ± 5.1 |

[a] Under Detection Limit.

Among the two adsorbents, BC seems to be the most suitable in terms of low molecular phenolic and LS removal. Another advantage of BC with respect to AC is related to the sugar losses. Despite the fact that sugar losses are inherent to any detoxification technique as they are chemically bonded to

the LS structure, sugar losses were lower using BC in comparison with AC under the same conditions. The only toxic substance, which is better removed by AC is acetic acid when working at 1:5 $w/v$ either at 30 or 50 °C. The affinity of BC towards toxic substances under consideration in this research is higher than using AC, and therefore the adsorption efficiency is better using BC. In this work, maximum LS, phenolic, acetic and furfural removals of 76.2%, 71.4%, 77.1% and 100% respectively were achieved under conditions checked. As will be seen further in Section 3.6, detoxification with BC or AC seems to be adequate working with yeasts or bacteria because of the high removals of LS, phenolics and acetic acid. This research gave better results in case of furfurals and weak acids in comparison with Guo et al. [17] and Lee et al. [22] who removed 94%–96% of furfurals, 14%–28% of acetic acid or 88% of total phenolics.

### *3.4. Ion Exchange*

Two kinds of resins, Dowex 50WX2 cationic resin (CR) and Amberlite IRA-96 anionic resin (AR), were used. CR separates mainly $Ca^{+2}$ and $Mg^{+2}$ while AR removes mainly LS, phenolics and acetic acid. SSL was treated in 18 experiments, working with only one of the resins and working with CR and AR in series. The first seven experiments were developed with AR, using 1.2 mL/g to 60 mL/g of SSL/AR ratio. In the case of CR, seven experiments from 0.67 mL/g to 60 mL/g were used. Finally, four sets of experiments were done working in series: Set I consists of CR at 3 mL SSL/g wet resin followed by AR treatment at 2 mL SSL/g wet resin; set II was run with CR at 3 mL/g and AR at 6 mL/g; Set III using ratios of 5 mL/g CR and 6 mL/g AR; and finally, Set IV at 5 mL/g CR and 8 mL/g AR. Table 2 presents the main results under equilibrium at tested conditions.

**Table 2.** Ion exchange resin experiments.

| | Ca | Mg | LS | OH | HAc | Furfurals | Sugars |
|---|---|---|---|---|---|---|---|
| | (g/L) | (g/L) | (g/L) | (g/L) | (g/L) | (g/L) | (g/L) |
| **SSL** | 14.6 ± 0.7 | 10.0 ± 0.8 | 422 ± 31 | 12.6 ± 1.0 | 7.85 ± 0.33 | 0.27 ± 0.19 | 214 ± 20 |
| **AR 1.2 mL/g** | 6.18 ± 0.08 | 4.77 ± 0.70 | 13.7 ± 1.12 | 0.16 ± 0.03 | 2.72 ± 0.13 | <DL | 71.9 ± 1.2 |
| **AR 1.5 mL/g** | 4.51 ± 1.28 | 4.10 ± 0.10 | 15.9 ± 3.3 | 0.26 ± 0.05 | 3.04 ± 0.47 | <DL | 78.4 ± 13.3 |
| **AR 2 mL/g** | 1.59 ± 0.22 | 7.51 ± 0.96 | 57.1 ± 15.4 | 1.29 ± 0.33 | 4.60 ± 0.27 | <DL | 128 ± 8 |
| **AR 3 mL/g** | 2.22 ± 0.31 | 8.27 ± 1.42 | 95.0 ± 5.3 | 2.84 ± 0.37 | 4.60 ± 0.33 | <DL | 128 ± 11 |
| **AR 6 mL/g** | 3.96 ± 0.55 | 9.74 ± 0.10 | 159.9 ± 5.4 | 5.69 ± 0.06 | 5.87 ± 0.34 | <DL | 180 ± 4 |
| **AR 30 mL/g** | 12.55 ± 1.30 | 11.90 ± 0.36 | 326 ± 18 | 7.99 ± 3.26 | 7.14 ± 1.41 | <DL | 170 ± 41 |
| **AR 60 mL/g** | 14.07 ± 2.15 | 10.90 ± 0.20 | 341 ± 10 | 9.90 ± 0.60 | 7.44 ± 0.48 | <DL | 181 ± 3 |
| **CR 0.67 mL/g** | 0.18 ± 0.01 | 0.028 ± 0.01 | 99.5 ± 9.0 | 5.69 ± 0.50 | 1.11 ± 0.14 | <DL | 31.3 ± 2.3 |
| **CR 1.33 mL/g** | 0.32 ± 0.02 | 0.20 ± 0.01 | 209 ± 20 | 5.84 ± 0.66 | 3.15 ± 0.24 | <DL | 83.7 ± 5.9 |
| **CR 2 mL/g** | 0.55 ± 0.02 | 0.33 ± 0.01 | 233 ± 2 | 6.02 ± 0.02 | 3.49 ± 0.17 | <DL | 91.7 ± 7.2 |
| **CR 3 mL/g** | 1.28 ± 0.10 | 0.41 ± 0.09 | 272 ± 6 | 9.28 ± 0.50 | 5.16 ± 0.07 | <DL | 134 ± 3 |
| **CR 4 mL/g** | 1.32 ± 0.08 | 0.94 ± 0.19 | 281 ± 13 | 11.7 ± 1.3 | 5.34 ± 0.21 | <DL | 143 ± 9 |
| **CR 30 mL/g** | 12.01 ± 0.33 | 7.93 ± 1.05 | 354 ± 1 | 12.5 ± 2.40 | 7.67 ± 0.74 | <DL | 189 ± 24 |
| **CR 60 mL/g** | 11.48 ± 3.10 | 9.60 ± 1.73 | 367 ± 1 | 10.84 ± 1.76 | 7.65 ± 0.72 | <DL | 189 ± 24 |
| **Series-I** | 0.34 ± 0.05 | 0.53 ± 0.02 | 4.3 ± 1.3 | <DL | 0.63 ± 0.05 | <DL | 80.5 ± 8.8 |
| **Series-II** | 1.04 ± 0.03 | 0.97 ± 0.06 | 90.8 ± 4.4 | 1.80 ± 0.06 | 0.81 ± 0.37 | <DL | 131 ± 17 |
| **Series-III** | 0.81 ± 0.01 | 1.69 ± 0.03 | 99.5 ± 3.9 | 2.03 ± 0.03 | 1.31 ± 0.02 | <DL | 157 ± 6 |
| **Series-IV** | 0.85 ± 0.05 | 1.67 ± 0.05 | 121 ± 16 | 1.91 ± 0.59 | 1.40 ± 0.09 | <DL | 162 ± 29 |

CR working at 0.67 mL/g exhibited metal removals of 98.8% for $Ca^{+2}$ and 99.7% for $Mg^{+2}$ in one single fractionation step. Similar results were obtained by Fernandes et al. [18] after two-step fractionation working in series. AR has an amine group that can selectively adsorb anions with the following selective order: $OH^- < CH_3COO^- < Cl^- < HSO_3^- < HSO_4^-$. This fact reveals that major inhibitor losses were produced in the case of LS and phenolics. Major removals were registered using AR at 1.5 mL/g: 96.1% of LS, 98.1% of OH, 61.3% of acetic acid, 65.0% metals and 100% of furfurals were trapped by AR. As will be seen in Section 3.6 ion exchange resins are, from a technical point of view, an excellent detoxification method not only for yeasts but also for bacteria. The ion exchange results were better than the rest of detoxification processes, especially considering the high initial

amounts of LS in the SSL. Takahashi et al. [19] observed that the presence of lignosulfonates hinders the ability of acetic acid removals by strong base resins. 120 g/L of LS decreased the efficiency of acetic removals from 98% to only 30% [19]. Looking at phenolic removals, results decrease from 12.6 ± 1.03 g/L towards 0.26 ± 0.05 g/L. According Nilvebrant et al. [42], 21% phenolic losses were exhibited, i.e., from 3.7 g/L to 0.77 g/L. The connection of individual and total inhibitor removals with sugar losses will be discussed in detail in Section 3.5.

One of the advantages of ion exchange resins is their ability to be regenerated, recovering the LS and phenolics. Because of the results shown in Table 2, the ability of the AR to be reused after cleaning and regenerating was determined. Figure 4a,b show the obtained results. Sixteen consecutive cycles can be used regarding the recovery efficiency of LS, acetic acid and furfurals. However, only the low molecular phenolic efficiency decreases after seven cycles. Furthermore, these compounds can be recovered from the regenerated solution for valorization purposes. For instance, low molecular phenolics are high-value added by-products with strong antioxidant capacities [23,24]. Therefore, six cycles of regeneration are recommended in this paper.

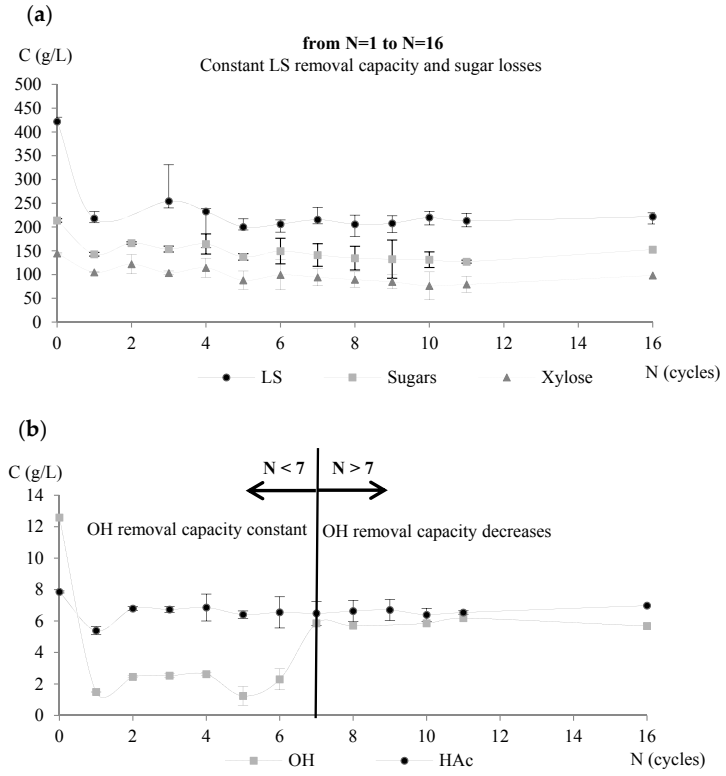


**Figure 4.** Sequential use of anionic resins: (**a**) Evolution of LS and sugars; (**b**) evolution of phenolics and acetic acid.

### *3.5. Comparison of Detoxification Techniques*

The best results of the detoxification techniques studied in this work are presented in Table 3. Technique effectiveness under equilibrium was calculated in terms of Individual Inhibitor Removal (IIR). In addition, Total Inhibitor Removal (TIR) and Total Sugar Losses (TSL) were also obtained. IIR is calculated as the difference between the initial and final concentration of the inhibitor before and after detoxification divided by the initial inhibitor concentration and multiplied by 100. The IIR values are denoted with MetalR, LSR, OHR, HAcR and FuR for metals, lignosulfonates, phenolics, acetic acid and furfurals removals, respectively. TIR is calculated as the difference between 442.6 and the total inhibitors removed divided by 442.6 and multiplied by 100, where 442.6 g/L is the total amount of inhibitors in the initial SSL. Finally, TSL is calculated as the difference between 213.9 and the sum of

total sugars after detoxification, divided by 213.9 and multiplied by 100. The concentration 213.9 g/L corresponds to the total amount of sugars in the initial SSL. In addition, a new ratio was calculated for the detoxification effectiveness, namely sugar-to-inhibitor removal ratio (S/I) calculated as the TSL divided by TIR. Among all the experiments checked, only the optimum ones regarding maximum TIR and IIR within the minimal TSL were displayed in Table 3.

Regarding IIR and TIR, detoxification with resins is better than detoxification by means of adsorption. CR-AR Series I of resins has the best results with a total toxic separation of 98.5%. The coefficient S/I is also low (0.63) demonstrating that this method has a good affinity for inhibitors with respect to sugars. A slightly inferior result was obtained with one-single resin, AR 1.5 mL/g, presenting very good TIR of 91.8%, S/I of 0.69 but a high TSL value of 63.3. Series IV preserves sugars, giving acceptable TIR of 70.8% with TSL of only 24.2%. Regarding the adsorption methods, BC reduces sugar (TSL of 42.6%) and inhibitors content (47.6%) almost in the same proportion and consequently S/I is almost 1. Nevertheless, it is a good solution in those cases working with microorganisms affected in a moderated way by phenolics, lignosulfonates and acetic acid. AC adsorption seems to be the most adequate adsorption option for acetic acid removal, presenting losses from 74.5% to 92%. Regarding all the effectiveness parameters, the adsorption with BC is a better alternative in comparison with AC because it eliminates more inhibitors with low sugar losses. Overliming should not be considered as a unique detoxification alternative, but it might be used in combination with adsorption or ion exchange resins. In any case, a pH adjustment of the lignocellulosic hydrolyzate has to be done in order to create a medium where yeasts can be grown and survive.

**Table 3.** Optimal results of detoxified-SSL applying different detoxification techniques.

| | MetalR (%) | LSR (%) | OHR (%) | HAcR (%) | FuR (%) | TSL (%) | TIR (%) | S/I Coef. |
|---|---|---|---|---|---|---|---|---|
| **AR 1.5 mL/g** | 65.0 | 96.2 | 98.1 | 61.3 | 100 | 63.3 | 91.8 | 0.69 |
| **AR 6 mL/g** | 44.3 | 62.1 | 54.8 | 25.2 | 100 | 15.8 | 55.1 | 0.29 |
| **CR 1.3 mL/g** | 97.9 | 50.5 | 53.7 | 59.9 | 100 | 60.9 | 50.5 | 1.21 |
| **CR-AR Series I** | 96.5 | 99.0 | 100 | 92.0 | 100 | 62.4 | 98.5 | 0.63 |
| **CR-AR Series IV** | 89.8 | 71.3 | 84.8 | 82.2 | 100 | 24.2 | 70.8 | 0.34 |
| **BC 50 °C 1:5** | NM [a] | 76.2 | 66.7 | 42.7 | 100 | 42.6 | 47.6 | 0.89 |
| **AC 30 °C 1:5** | NM | 54.3 | 61.1 | 74.5 | 100 | 56.1 | 33.6 | 1.7 |
| **OV [b] 30 °C pH 10** | NM | 45.9 | 39.6 | 31.3 | 50.1 | 11.2 | 44.9 | 0.25 |

[a] NM not measured; [b] OV overliming.

Biofuel potentials [29,43] would increase significantly after detoxification in comparison with the untreated SSL. This phenomenon is due to the separation of the inhibitors from the SSL whom hinders the ability of microorganisms to produce bioethanol or biogas. However, calculations performed by Thomsen et al. [43] and implemented to the untreated SSL by Llano et al. [29] do not take into account the inhibition caused, for instance, by the presence of acetic acid. Olsson and Hahn-Hägerdal [44] reported that acetic acid concentrations above 4.3 g/L inhibit fermentation by 50% with *S. cerevisiae* at pH of 5.5. In the case of *P. stipitis* concentrations above 8 g/L inhibit 98% or 25% at pH of 5.1 or 6.5 respectively. Considering HAcR of Table 3, OV is not the best option for fermenting SSL towards EtOH using *S. cerevisiae*. Within this framework resin in series (Series I and IV) or AC at 30 °C and 1:5 *w*/*v* would be suitable for sugars biological conversion into ethanol with low inhibition grade using *S. cerevisiae* or *P. stipitis*.

Considering the results of Table 3, treatments with ion exchange resins are the most suitable techniques for SSL detoxification. Prior to sugar fermentation, the best method being the utilization of CR at 3 mL SSL/g wet resin followed by AR treatment at 2 mL. Apart from the technical analysis recent studies of Rueda et al. [45] reported the techno-economic analysis of detoxification techniques. Guthrie's method was applied to get fixed capital investment (FCI) costs and manufacturing costs (COM). Regarding the FCI costs, anion exchange resins were chosen due to their lower costs in comparison with adsorption with BC, adsorption with AC or ultrafiltration.

### *3.6. Best Solutions for the Most Common Fermenting Scenarios*

The results of detoxification processes depend strongly on the application of the following step in biorefinery because the microorganisms are more or less sensitive depending on the type of inhibitor and its concentration. For this reason, the most common fermenting scenarios are proposed in this paper. Different detoxification techniques were contemplated making SSL sugars more accessible for fermentation. Substrate quality, inhibitor tolerance or the product of interest are some of parameters affecting fermentation. Because of this, one of the objectives pursued in this work is to establish the most suitable technique for every single case.

Regarding the effectiveness of total inhibitor removal, ion exchange resins working in series seem to be the most adequate detoxification technology. The maximum separation degree of the toxic substances plays an important role in terms of yield, productivity or rate of fermentation working with sensitive microorganisms under adverse conditions. Nevertheless, a drawback of resins is their cost in comparison with overliming, organic solvent extraction, or adsorption.

Figure 5a highlights the importance of a convenient detoxification technique for an integral use of the SSL. In order to study some biorefinery options of the pulp mill, not only dissolving pulp, but also other products such as weak acids, phenolics, lignosulfonates and sugars can be valorized according to Figure 5a. To get this objective, some detoxification methods must be carried out. The experimental results obtained in this paper together with the fermentation requirements of a specific microorganism permit to establish the detoxification possibilities that might be implemented in different case studies. It should be noted that the study cases represented in Figure 5b belong to hydrolyzates similar to the industrial liquors used in this work such as hardwood hydrolyzates [22,46], softwood hydrolyzates [9,17] and spent sulfite liquors [19,23,35,47].

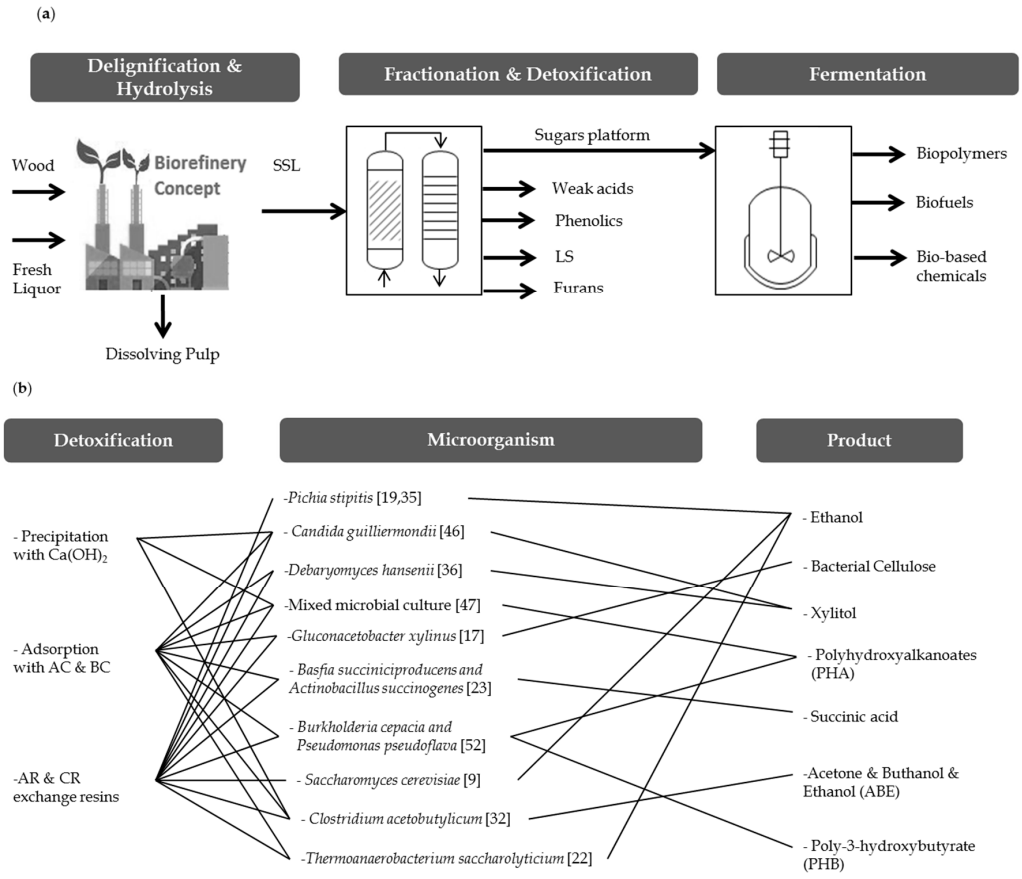


**Figure 5.** (**a**) Main steps involved in transformation of a sulfite pulp mill into a modern biorefinery; (**b**) Detoxification pathways of lignocellulosic hydrolyzates within common fermenting scenarios.

Main SSL inhibitors can be classified into weak acids, furan derivatives and phenolics among others [48,49]. The separation of LS, the major compound of the SSL, is also important since LS hindered the ability to separate other toxic compounds [19].

The first group of inhibitors corresponds to furfurals which inhibit the growth of yeast and decrease ethanol yield and productivity [25]. There are studies demonstrating that furfural concentrations above 2 g/L stopped the cell growth of *Pichia stipitis* almost completely, whereas a concentration of 1.5 g/L reduced the ethanol yield by 90% and the productivity by 85% [50]. Although HMF is not as serious an inhibitor as furfural, it was also reported by Sainio et al. [50] that the concentrations in the range of 1 to 5 g/L reduce ethanol production by 71%–96%. Ylitervo et al. [48] also checked the impact of furfural on ethanol production using *Saccharomyces cerevisiae* and observed a rapid decrease of ethanol production with the presence of 20.6 g/L of furfural. Regarding furfurals concentration of the untreated SSL, this group is not problematic because of the presence of 0.20 g/L of furfural and 0.07 g/L of HMF.

The second group of inhibitors is formed by the weak acids whose toxic effects strongly depend on the pH, due to partial dissociation of acetic formic and levulinic acid. Weak acids inhibit cell growth and undissociated forms of the acid are liposoluble and diffuse across the plasma membrane. The bibliography results suggest that acetic acid concentration should be reduced to less than 1 g/L to effectively produce ethanol from hardwood SSL using *Pichia stipitis* [15]. *Saccharomyces cerevisiae* can only survive at external pH higher than 4.5 and maximum concentration of undissociated acetic acid of less than 5 g/L [51]. Concentrations higher than 1.45 g/L completely inhibited the growth of the yeast *Pachysolen tannophilus*, concentrations above 1.2 g/L inhibit the growth of *Candida utilis* and concentrations higher than 2.7 g/L reduced the pentose utilization by *Pichia stipitis* [33]. The negative effect of acetic acid on yeasts is almost the same producing either xylitol or ethanol. Levulinic acid and formic acid do not have any toxic effect at the concentrations in the SSL, which were 0.11 and 0.23 g/L, respectively. However, the acetic acid can constitute a serious problem because of its high concentration of 7.85 g/L.

The third group of inhibitors is the low molecular weight phenolics which may act on biological membranes, causing loss of integrity, destroying the electrochemical gradient by transporting the protons back across the mitochondrial membranes [49]. In many cases, phenolic compounds are considered the major inhibitors in lignocellulosic hydrolyzates, even more than weak acids and furfurals [32]. This behavior becomes significant when working with bacterial strains for succinic acid production [23], PHA production [52], ABE fermentation [32] or bacterial cellulose [17]. Alexandri et al. [23] observed an increase of 62% on succinic acid production when fermenting with two wild-type bacteria strains after SSL organic solvent detoxification which partially removed phenolic compounds. In the case of the SSL phenolics are problematic for fermentation purposes because of the initial concentrations of 12.6 g/L. Thus, detoxification methods that efficiently remove phenolics benefit bacterial growth. An exception of this is observed if working with mixed cultures consuming acetic acid, a part of phenolics and a low amount of sugars for PHA production [47]. Afterwards, remaining sugars can be used for other bioprocesses like bioethanol production.

The detoxification technique was selected in every case considering the inhibitor tolerance previously analyzed towards furfurals, weak acids and phenolics. The fact that SSL detoxification is completely necessary was already discussed by Coz et al. [26]. Discussion of Figure 5b was possible assuming total and individual inhibitor losses obtained in this work for overliming, exchange resins and adsorption. Based on the results and the information collected it can be concluded: (i) bioprocesses working with bacterial strains require detoxification processes like anionic exchange resins or overliming coupled with adsorption, capable of removing LS and phenolic compounds in large amounts; (ii) bioprocesses working with yeasts mostly require acetic acid and furan removal or a convenient pH adjustment, and therefore only detoxification by adsorption coupled with overliming could be enough.

## 4. Conclusions

Detoxification or fractionation of lignocellulosic residues is an attractive way to transform pulp mills into biorefineries in order to valorize all of the main components and transform sugars into bioethanol or other biofuels or bioproducts. In this work, spent sulfite liquor has been treated by evaporation, overliming, adsorption and resins, giving a maximum total inhibitor removal of 98.5% working with cationic and anionic exchange resins in series. Apart from exchange resins, the results of evaporation, overliming, adsorption with activated carbon and adsorption with black carbon lead to total inhibitor removals of 8.6%, 44.9%, 33.6% and 47.6%, respectively. Evaporation was studied in order to conclude with the main stream for valorization purposes, with the fifth effect of the evaporation plant giving the best results. Since the maximum allowable concentration for each inhibitor cannot be established as a general rule, total sugar losses, sugar-to-inhibitor ratio, and inhibitor removals were evaluated. Separation by ionic resins was proposed as the best solution, using a maximum of six cycles of regeneration to recover lignosulfonates and phenolic compounds.

Lignosulfonates are one of the main inhibitors in the process. In addition, these kinds of compounds can be easily valorized. However, there is a link between the quantity of lignosulfonates and other inhibitors and sugars; therefore, in some cases a compromise solution should be taken into account. Overliming and resins give the best separation results between lignosulfonates and sugars.

Thanks to the literature review analyzing the main fermentation scenarios of residual lignocellulosic hydrolyzates and the inhibitory effects across such scenarios together with the experimental results obtained in this work it can be concluded that: (i) bioprocesses working with bacterial strains require detoxification processes like AR or OV coupled with adsorption that can remove large amounts of LS and phenolics; (ii) bioprocesses working with yeasts mostly require acetic acid and furan removal or a convenient pH adjustment, and therefore only detoxification by adsorption coupled with overliming would be enough.

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

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