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Lactic Acid Production from Steam-Exploded Sugarcane Bagasse Using *Bacillus coagulans* DSM2314

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Abstract: This work aimed at producing lactic acid (LA) from sugarcane bagasse after steam explosion at 195 °C for 7.5 and 15 min. Enzymatic hydrolysis was carried out with Cellic CTec3 and Cellic HTec3 (Novozymes), whereas fermentation was performed with *Bacillus coagulans* DSM2314. Water washing of pretreated solids before enzymatic hydrolysis improved both hydrolysis and fermentation yields. The presence of xylo-oligosaccharides (XOS) in substrate hydrolysates reduced hydrolysis efficiency, but their effect on fermentation was negligible. The presence of fermentation inhibitors in C5 streams was circumvented by adsorption on activated carbon powder with no detectable sugar losses. High carbohydrates-to-LA conversions ($Y_{p/s}$) of 0.88 $g \cdot g^{-1}$ were obtained from enzymatic hydrolysates of water-washed steam-exploded materials that were produced at 195 °C, in 7.5 min, and the use of centrifuged-but-never-washed pretreated solids decreased $Y_{p/s}$ by 16%. However, when the detoxified C5 stream was added at a 10% ratio, $Y_{p/s}$ was raised to 0.93 $g \cdot g^{-1}$ for an LA productivity of 2.55 $g \cdot L^{-1} \cdot h^{-1}$. Doubling the pretreatment time caused a decrease in $Y_{p/s}$ to 0.78 $g \cdot g^{-1}$, but LA productivity was the highest (3.20 $g \cdot L^{-1} \cdot h^{-1}$). For pretreatment at 195 °C for 7.5 min, the elimination of water washing seemed feasible, but the use of longer pretreatment times made it mandatory to eliminate fermentation inhibitors.

Keywords: sugarcane bagasse; steam explosion; enzymatic hydrolysis; fermentation; lactic acid

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1. Introduction

Lactic acid is a valuable chemical platform with applications in different industrial sectors such as food, cosmetics, textiles, pharmaceuticals, and chemical synthesis [1]. The global lactic acid market increased from 1220 kilotons in 2016 to 1960 kilotons in 2025. This represents a revenue of USD 11.51 billion globally [2,3]. The production of polylactic acid (PLA), a biodegradable polymer, accounts for about 50% of the lactic acid demand [4]. The other half is mainly used as an acidulant, preservative, flavoring, emulsifier, and pH regulator in the food industry [5].

Lactic acid has two types of enantiomers (L or D). The pure enantiomers have greater value than the racemic mixture because they are used for special industrial applications. For instance, the L isomer is preferable for food, beverages, and pharmaceuticals because it is metabolized more rapidly by the human body than the D isomer. L-Lactic acid is used to synthesize poly-L-lactic acid (PLLA), while D-lactic acid is used to produce poly-D-lactic acid (PDLA). Both are semi-crystalline bioplastics, while PDLLA, made with the racemic mixture, is amorphous and relatively easy to break down, ideal for developing drug delivery systems. However, due to its biocompatibility and high mechanical strength, the L isomer predominates in biomedical applications, including bone fixation supports and biodegradable sutures [6]. Food packaging, injection molding, and additive manufacturing (resins for 3D printing) are other applications in which PLA has been widely used [7].

Optically pure lactic acid can be produced by fermentation, while chemical synthesis leads to racemic mixtures [8]. Fermentation is also advantageous because it uses renewable resources and mild process conditions [9]. Several bacteria, fungi, and yeasts can produce high optical purity lactic acid in high yields [10]. However, lactic acid bacteria (LAB) such as *Lactobacillus delbruckii*, *Lb. rhamnosus*, *Lb. casei*, and *Lb. plantarum*, and bioengineered bacteria, such as *Escherichia coli* and *Corynebacterium* spp., are the most widely used for lactic acid fermentation [2,11]. The process is performed by submerged fermentation, and the substrate accounts for almost 70% of the production cost. Starch is the predominant raw material for industrial manufacturing, with about 90% of globally traded lactic acid being produced from corn [12]. Thus, identifying cheaper and widely available substrates is pivotal to reduce process costs [13].

Agro-industrial residues such as sugarcane bagasse (SCB) are lignocellulosic materials with a high potential to reduce industrial costs. However, before fermentation, plant cell wall polysaccharides such as cellulose and hemicelluloses must be converted to simple sugars (primarily glucose and xylose). Pretreatment techniques such as hydrothermolysis, steam explosion, acid-catalyzed organosolv, and dilute acid hydrolysis can provide high yields of fermentable sugars in the form of C5 and C6 streams [14]. C5 sugars are obtained in pretreatment acid hydrolysates, while C6 sugars are derived from enzymatic hydrolysis of pretreated cellulosic materials. However, the use of high pretreatment temperatures (>200 °C) or long residence times result in partial dehydration of pentoses and hexoses, causing the release of fermentation inhibitors such as furfural and 5-(hydroxymethyl)furfural (5-HMF) that reduce process yields [15,16]. Such aromatic aldehydes are known to inhibit key enzymes of microbial carbon metabolism [17]. Other inhibitors such as low molar mass phenolic compounds may also be released from lignin. Also, mild pretreatment severities are enough to release inhibitory acetic acid (pKa = 4.76) from hemicellulose *O*-acetyl groups [16]. Weak acids diffuse through the cell membrane and lower the intracellular pH, affecting cell growth due to their effect on the proton transport activity of the plasma membrane [18,19].

Steam explosion uses saturated steam at high pressures to produce pretreated cellulosic materials with high accessibility to enzymatic hydrolysis [20]. While enzymatic hydrolysates of water-washed steam-exploded materials are easy to ferment, the C5 fraction typically contains inhibitory concentrations of organic acids (primarily acetic) and furan compounds (mostly furfural) [21]. Oligosaccharides released from hemicelluloses can also act as inhibitors for enzymatic hydrolysis [22], and in both situations, the release of inhibitory compounds will largely depend on pretreatment conditions and feedstock composition. High pretreatment severities will release more fermentation inhibitors by carbohydrate dehydration and lignin hydrolysis. At the same time, oligosaccharides will prevail at low severities, particularly when pretreatment is carried out without an exogenous acid catalyst [23].

Several detoxification techniques have been used to reduce the inhibitory effect of biomass acid hydrolysates. Furans can be removed by physical adsorption [24], liquid–liquid extraction [25], evaporation [26], freeze-drying [27], enzymatic treatments using laccases and other oxidative enzymes [28], or overliming [29] while reducing acetic acid to non-inhibitory concentrations may be more problematic. The most widely used detoxification techniques are adsorption on activated carbon powder and overliming. However, their efficiency depends on the type and concentration of fermentation inhibitors released in pretreatment liquors [30]. Physical adsorption was chosen in this work for simplicity, efficiency, and selectivity toward fermentation inhibitors. Furthermore, physical adsorption would not dilute the sugar stream while bringing these chemicals to non-inhibitory levels.

Bacillus sp. strains are more tolerant to inhibitory compounds. In a hydrolysate broth containing 4.01 $g \cdot L^{-1}$ acetic acid, 0.08 $g \cdot L^{-1}$ formic acid, 0.05 $g \cdot L^{-1}$ furfural, and 0.08 $g \cdot L^{-1}$ 5-HMF, *B. coagulans* IPE22 converted 96% of sugars into LA [31]. Also, *B. coagulans* JI12 could tolerate up to 20 $g \cdot L^{-1}$ acetic acid and 4 $g \cdot L^{-1}$ furfural by metabolizing it to 2-furoic acid [32], while *Bacillus* sp. P38 was tolerant to 10 $g \cdot L^{-1}$ furfural and 6 $g \cdot L^{-1}$ vanillin or

acetic acid [33]. This indicates that *Bacillus* spp. may be promising organisms to produce L-LA from biomass hydrolysate without a robust detoxification step. No information was found in the literature about the tolerance and inhibitory levels of *B. coagulans* DSM2314 to the organic acids and furan compounds listed above.

Second-generation lactic acid can be produced by C5 plus C6 fermentation or by co-fermentation of C5/C6 mixtures. For acid pretreatments such as steam explosion, C6 sugars are mainly produced from enzymatic hydrolysis of water-washed pretreated materials. By contrast, C5 sugars are recovered in pretreatment liquors (C5 streams) that must be detoxified before fermentation. *B. coagulans* has become one of the most popular organisms due to its capacity to metabolize C5 sugars via the pentoses phosphate (PP) pathway and produce optically pure L-LA with high yields [34]. Enzymatic hydrolysis and fermentation can be performed separately or simultaneously. Based on this, different bioprocessing strategies have been designed to produce biobased materials such as separate hydrolysis and fermentation (SHF), separate hydrolysis and co-fermentation (SHCF), simultaneous saccharification and fermentation (SSF), and simultaneous saccharification and co-fermentation (SSCF). SHF and SHCF involve enzymatic hydrolysis of the polysaccharides and subsequent fermentation of the sugars released. By contrast, SSF and SSCF are one-pot methods where enzymatic hydrolysis and microbial fermentation occur simultaneously. Combining these operations results in lower capital cost and higher productivity since enzymes perform better due to lower levels of end-product inhibition and sugars are released and readily consumed [33–40].

Michelson et al. [41] compared the performance and yield of two LA producers, *Lb. delbrueckii* ssp. *lactis* DSM 20,073 and *B. coagulans* SIM-7. The latter strain achieved final LA concentrations of 91.5 $g{\cdot}L^{-1}$ and 91.6 $g{\cdot}L^{-1}$ in batch and fed-batch cultivations for 23 and 21 h, respectively. The LA concentration in 10 h was already 56 $g{\cdot}L^{-1}$, whereas comparable results (52 $g{\cdot}L^{-1}$) were achieved only in 24 h by DSM 20073. The maximal production rates of SIM-7 and DSM 20,073 strains were 9.9 and 5.6 $g{\cdot}L^{-1}{\cdot}h^{-1}$, respectively.

Different enzymatic hydrolysis and fermentation conditions were used in this work to produce LA from steam-exploded SCB. Hydrolysis was performed with Cellic CTec3 (Novozymes, Bagsværd, Denmark) cellulases in the absence and presence of Cellic CTec3 hemicellulases at relatively high total solids (TS), using water-washed and centrifuged-but-never-washed steam-exploded materials. Fermentation inhibitors were removed from C5 streams using physical adsorption on activated carbon powder, while fermentation was carried out with *B. coagulans* DSM2314 using both SHF and SHCF protocols.

2. Materials and Methods

The overview of the experimental setup is given in Figure 1, in which the complexity and interrelationship of the main activities (chemical characterization, pretreatment, hydrolysis, and fermentation) are observed. Also, the sequence in which the experiments are performed is inferred by step connectors, while red and blue circles indicate processes of intermediates and final products, respectively. Further details about the experimental setup are given below.

2.1. Sugarcane Bagasse (SCB) Pretreatment and Characterization

Fresh SCB was kindly donated by Raízen (Piracicaba, SP, Brazil). Pretreatment was carried out by steam explosion at 195 °C for 7.5 and 15 min using a 10 L stainless-steel high-pressure steam reactor and SCB with a moisture content of 50 wt% [27]. Pretreatment slurries (20–25 wt% total solids) were centrifuged inside a cotton fiber bag to remove water-soluble hemicellulose sugars and low molar mass lignin components (C5 stream). Half of the unwashed centrifuged fiber cake (SEB7.5-UW and SEB15-UW) was reserved for enzymatic hydrolysis. The other half was water washed at 5 wt% TS for 1 h at room temperature (~25 °C) under constant mechanical stirring, followed by centrifugation to recover the water-washed fiber cake (SEB7.5-WW and SEB15-WW) for its subsequent characterization and enzymatic hydrolysis. Both SEB-UW and SEB-WW substrates, with

35–40 wt% total solids after centrifugation, were stored in vacuum-sealed plastic bags at 4 °C before chemical characterization and enzymatic hydrolysis.

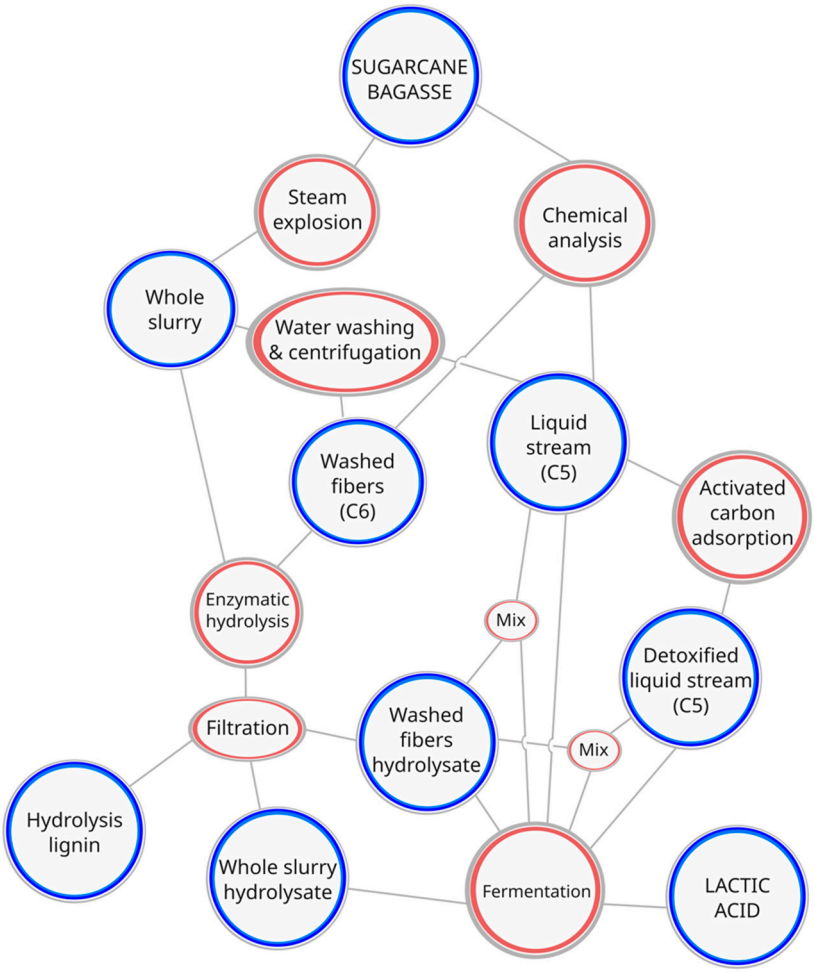

Figure 1. Experimental setup for lactic acid production from sugarcane bagasse.

The composition of untreated and pretreated materials was characterized following National Renewable Energy Laboratory (NREL, Golden, CO, USA) protocols for total moisture (drying at 105 °C until constant mass) [42], ash (calcination at 575 °C) [43], total extractives (exhaustive Soxhlet extractions with water and ethanol 95%) [44], and carbohydrates plus total lignin content (acid-soluble lignin and acid-insoluble lignin) following a two-stage sulfuric acid hydrolysis [45]. All reagents and solvents were obtained in analytical grade from Labsynth (Diadema, Brazil) and used as received. Chromatographic standards (>98% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mass balances and process yields were calculated according to the above-mentioned standard procedures. To this end, theoretical conversion factors were considered to express recovery yields concerning each raw SCB macromolecular component.

The pretreatment liquor (C5 fraction) was detoxified over activated carbon [46]. Detoxification was performed in 250 mL Erlenmeyer flasks that were loaded with 100 mL of liquid and 10 g of activated carbon powder (Neon, Suzano, Brazil), having a surface area of 507.9 $m^2 \cdot g^{-1}$ and an average pore size of 1.29 nm. The flasks were covered with Parafilm M to prevent evaporation and placed on a shaker incubator at 25 °C and 120 rpm for 10 min. Then, the suspension was centrifuged at 2500 rpm for 15 min, and aliquots were removed from the supernatant, filtered through a 0.22 µm PVDF filter (Millipore, Burlington, MA, EUA), and analyzed by high-performance liquid chromatography (HPLC) to quantify furfural, 5-HMF, acetic acid, formic acid, xylose, glucose, and arabinose, using

the chromatographic conditions that are described in Section 2.4. Then, the supernatant was passed through a 0.1-µm ash-less quantitative filter paper (Whatman® (Maidstone, UK)) to remove any remaining suspended solids. The detoxification process was repeated three times to ensure that acetic acid, furfural, and 5-HMF were brought to non-inhibitory concentrations.

2.2. Enzymatic Hydrolysis

The commercial enzymes used for hydrolysis were provided by Novozymes Latin America (Araucária, SP, Brazil). Cellic CTec3 is a commercial cellulase preparation while Cellic HTec3 contains hemicellulase activity predominantly [20]. Enzyme loading was always based on the wet weight of the commercial enzyme preparation that was added to the reaction system for enzymatic hydrolysis.

Enzymatic hydrolyses of SEB-UW and SEB-WW substrates were performed at 50 °C and 150 rpm for 96 h in acetate buffer (50 mmol·L^{-1}, pH 5.2) using 250 mL Erlenmeyer flasks in a shaker incubator (Ecotron, Infors HT). The best condition was derived from a factorial design that was based on three independent variables in two levels (2^3): substrate TS (10 and 20 wt%), Cellic CTec3 loading (20 to 60 mg g^{-1} TS), and Cellic HTec3 addition in a 10% mass ratio (wet basis) to Cellic CTec3 (2 to 6 mg·g^{-1} TS) [20]. Four quadratic polynomial equations were obtained using the R Studio® 3.4.3 software to describe the mathematical relationship between glucose release (g·L^{-1}) and the selected process variables. The goodness-of-fit of the models was evaluated by determining their adjusted R^2. For yield calculations, aliquots were collected at different incubation times and analyzed using HPLC to quantify cellobiose, glucose, and xylose using the chromatographic conditions described in Section 2.4. Hydrolysis yields were determined in percentage by expressing the total glucose release (glucose equivalents) in relation to the total glucose content (quantified as glucans) of the pretreated solids. Xylose was not considered in yield calculations because it was always found in very low quantities.

Enough substrate hydrolysate (C6 stream) for fermentation was obtained by performing the best hydrolysis conditions from the factorial design in a 3.6 L Labfors bioreactors (Infors HT, Bottmingen-Basel, Switzerland). Enzymatic hydrolyses of SEB-UW and SEB-WW substrates were performed at 50 °C and 150 rpm for 72 h in acetate buffer (50 mmol·L^{-1}, pH 5.2) using 20 wt% TS and 60 mg g^{-1} TS of Cellic CTec3, with and without addition of Cellic HTec3 (6 mg·g^{-1} TS). The total volume of this reaction system was 1000 mL. Aliquots were collected once again at different incubation times and analyzed using HPLC, and hydrolysis yields were determined as described above.

2.3. Microorganism and Fermentation

B. coagulans DSM2314 was acquired as a freeze-dried stock from the Germany Collection of Microorganisms and Cell Cultures (DSMZ, Leibniz Institute, Germany). Cells were grown on Man, Rogosa, and Sharpe (MRS) agar medium (HiMedia, Mumbai, India) and transferred to 50 mL flasks of MRS medium to be cultured for 16 h at 50 °C. The media were pre-sterilized for 15 min at 121 °C. When the optical density measured at 660 nm reached two, the pre-culture was added as inoculum to the fermentation, which was carried out at 50 °C and 150 rpm for 24 h in Multifors 2 bioreactor (Infors HT) that were pre-sterilized empty for 20 min at 121 °C.

Both SHF and SHCF fermentation experiments were carried out in duplicate in the Multifors 2 bioreactor using a working volume of 300 mL for a total volumetric capacity of 500 mL. SCB hydrolysates (C5, C6 and C5/C6 mixtures) were transferred to the bioreactor vessel and mixed with 1% yeast extract (Kasvi®, Conda Laboratories, Madri, Spain) and 10% (*v*/*v*) inoculum (30 mL). Temperature and agitation were set at 50 °C and 150 rpm, respectively, and dilute NaOH (5 mol L^{-1}) was used to maintain the fermentation broth at pH 6.0 during the entire reaction course. Fermentation ran for 24 h under anaerobic conditions using continuous N_2 purging. Aliquots were obtained at different times and analyzed using HPLC for carbohydrates and LA as described below. LA yields were

determined as percentage in relation to the theoretical amount of LA that could have been produced from the fermentable sugars available in substrate hydrolysates.

2.4. Chromatographic Analysis

C5 (from pretreatment) and C6 (from enzymatic hydrolysis) streams and fermentations broths were analyzed at 65 °C using a Shimadzu HPLC, LC-20AD series, and a Rezex RHM column (Phenomenex, 300 × 7.8 mm) that was preceded by a Carbo H guard column (300 × 7.8 mm). The column was eluted with 5 mmol L^{-1} H_2SO_4 at a flow rate of 0.6 mL min^{-1}. Sample injection (20 µL) was performed using a Shimadzu SIL-10AF autosampler. Quantitative analyses were carried out by external calibration using differential refractometry (Shimadzu RID-10A) for carbohydrates and organic acids, while UV spectrophotometry (Shimadzu SPD-M10AVP) at 280 nm was used to quantify furfural and 5-HMF. HPLC calibration curves were based on analyzing six independent primary standard solutions, and the corresponding linear regression coefficients (R^2) were always around 0.99.

2.5. Statistical Analysis

The Tukey's Test ($p \leq 0.05$) was applied to evaluate the statistical significance of the experimental data, and the experimental design was validated with analysis of variance (ANOVA) using the R Studio® 3.4.3 software [47]. Hydrolysis and fermentation yields were expressed as averages with their corresponding standard deviations for experiments carried out in two or three replicates.

3. Results

3.1. SCB Pretreatment and Characterization

The chemical composition of SCB before and after pretreatment is shown in Table 1. The untreated material had glucans (mainly cellulose), hemicelluloses (mostly xylans), total lignin, total extractives, and ash contents like those already reported elsewhere [20,21,27,31]. Variations in SCB chemical composition are attributed to its source and maturation stage upon harvesting, as well as the edaphoclimatic conditions used for cultivation and the technology used for its industrial processing [48].

Table 1. Chemical composition (%) of untreated and water-insoluble steam-exploded SCB and the corresponding mass recovery (%) of the main SCB components after pretreatment.

Component	Untreated	195 °C, 7.5 min		195 °C, 15 min	
		Content	Recovery	Content	Recovery
Glucans [1]	37.8 ± 0.7	54.7 ± 2.4	89.7 ± 1.8	55.5 ± 0.7	82.5 ± 1.1
Xylans [2]	21.0 ± 0.8	3.0 ± 0.4	5.9 ± 0.1	1.2 ± 0.1	3.8 ± 0.1
Arabinosyl residues [2]	1.3 ± 0.3	bdl [3]	-	-	-
Acetyl groups [2]	2.6 ± 0.1	bdl [3]	-	bdl [3]	-
Hexoses identified as 5-HMF [4]	1.1 ± 0.2	bdl [3]	-	bdl [3]	-
Pentoses identified as furfural [4]	0.6 ± 0.1	bdl [3]	-	bdl [3]	-
Total lignin [5]	22.8 ± 0.7	30.5 ± 0.8	92.2 ± 1.9	31.2 ± 0.2	96.6 ± 0.3
Acid-soluble lignin	5.0 ± 0.1	5.7 ± 0.2	70.4 ± 1.5	5.9 ± 0.2	78.5 ± 0.2
Acid-insoluble lignin	19.1 ± 0.6	27.8 ± 0.8	96.3 ± 0.9	28.9 ± 0.1	101.4 ± 0.3
Ash	4.0 ± 0.1	6.1 ± 1.5	93.7 ± 1.9	6.8 ± 1.1	113.8 ± 0.3
Total	98.2	94.3		94.7	

[1] Present as β-(1→4)-D-glucans (cellulose). [2] Present as heteroxylan components (hemicelluloses). [3] bdl, below the detection limit of the method. [4] 5-HMF and furfural are released in pretreatment liquors by hexose and pentose dehydration, respectively. [5] Summation of acid-soluble and ash-free acid-insoluble lignin.

Steam explosion at 195 °C for 7.5 and 15 min reduced the SCB hemicellulose content by 85.7 and 94.3%, respectively, with a corresponding rise in both glucans and total lignin content (Table 1) [49]. Hemicelluloses were almost entirely depleted of their arabinosyl

residues and acetyl groups because HPLC did not detect arabinose and acetic acid in sulfuric acid hydrolysates. Pentoses and hexoses were partly detected as furfural and 5-HMF due to dehydration, but in both situations, the reported values were not added to the corresponding polysaccharide quantification because their actual source was not elucidated. Hence, SCB hemicellulose content would be the summation of xylans, arabynosyl residues, and pentoses identified as furfural and acetyl groups, totaling 25.5%. Likewise, the total glucan content in Table 2 should be estimated at 38.9%, even though some 5-HMF may have come from hemicelluloses as well. The 5-HMF formation was higher in sulfuric acid hydrolysates because furfural was partially involved in side-reactions producing humins, while furans were not formed after acid hydrolysis of steam-treated materials because their hemicellulose content was very low [50,51].

Table 2. Chemical composition of pretreatment liquors before and after detoxification.

Component ($g \cdot L^{-1}$)	SEB7.5 [1]		SEB15 [1]	
	Untreated	**Detoxified**	**Untreated**	**Detoxified**
Glucose	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
Xylose	11.2 ± 0.2	11.1 ± 0.2	4.3 ± 0.1	4.3 ± 0.2
Arabinose	0.6 ± 0.1	0.60 ± 0.04	0.5 ± 0.1	0.4 ± 0.1
HMF [2]	1.6 ± 0.2	0.02 ± 0.02	1.6 ± 0.1	0.03 ± 0.01
Furfural [2]	0.8 ± 0.1	0.04 ± 0.02	1.4 ± 0.3	0.03 ± 0.01
Acetic acid [3]	9.7 ± 0.3	4.4 ± 0.5	12.1 ± 0.5	5.2 ± 0.2

[1] SEB7.5 and SEB15, steam explosion of cane bagasse (SEB) at 195 °C for 7.5 and 15 min; [2] 5-HMF and furfural are released in pretreatment liquors by hexose and pentose dehydration; [3] Acetic acid is released in pretreatment liquors by hemicellulose deacetylation.

Table 1 reveals the total recovery of SCB components in water-washed steam-exploded materials, in which the mass recovery of pretreated solids was also considered. Glucan recovery was around 90% when steam explosion was carried out at 195 °C for 7.5 min but doubling the pretreatment time to 15 min decreased this value by roughly 9%. The hemicellulose content (mostly xylans) in steam-exploded materials was very low and some lignin condensation may have occurred mainly at the highest pretreatment severity, in which lignin recovery was above 100%. Finally, ash recoveries above 100% may have indicated partial corrosion or abrasion of reactor walls [21,27].

3.2. Chemical Composition of Pretreatment Liquors before and after Detoxification

Pretreatment liquors were characterized using HPLC for their carbohydrate, acetic acid, 5-HMF, and furfural contents before and after detoxification with activated carbon powder (Table 2). Post-hydrolysis of these fractions with dilute sulfuric acid revealed xylose concentrations of 13.2 ± 0.4 and 10.5 ± 0.5 $g \cdot L^{-1}$ for pretreatments carried out for 7.5 and 15 min, respectively. The presence of acetic acid in pretreatment liquors was due to hemicellulose deacetylation, and its concentration was higher when pretreatment was carried out at more drastic conditions ($p < 0.05$). Acetic acid coming from *O*-acetyl groups is partially responsible for the auto-hydrolysis effect, which converts hemicelluloses to mono and oligosaccharides even without an exogenous acid catalyst. Furfural and 5-HMF derived from pentose and hexose dehydration, respectively, were also detected in pretreatment liquors in concentrations that increased with pretreatment severity. Accumulating organic acids, furans, and phenolic compounds in pretreatment liquors is undesirable due to their inhibitory effect on hydrolysis and fermentation [52,53].

Controlling microbial inhibition is essential for maximizing biomass conversion. Du et al. [54] identified and quantified 40 potentially toxic compounds after pretreating three different lignocellulosic materials with eight different pretreatment techniques. Fockink et al. [20] demonstrated that the autocatalytic steam explosion of SCB at 205 °C induced the accumulation of formic, levulinic, and acetic acid in the C5 fraction. Concerning furan compounds, furfural was present in higher concentration, while the accumulation of

5-HMF and 5-methyl-furan (5-MF) was higher at the highest pretreatment temperatures. This clearly indicates that a detoxification stage must be carried out for pretreatment liquors in which the presence of fermentation inhibitors is unavoidable [20,21].

The data presented in Table 2 showed that, by treating SCB pretreatment liquors with activated carbon powder, furfural and 5-HMF concentrations were reduced by 97–98% ($p < 0.05$), regardless of conditions used for pretreatment. Acetic acid removals were 55.2 and 57.2% for pretreatments carried out at 195 °C for 7.5 and 15 min, respectively. By contrast, no sugar losses were observed after adsorption on activated carbon powder at ambient temperature.

In our work, detoxification with activated carbon was highly efficient at room temperature, while other studies required heating to be effective. Lu, Dong, and Yang [55] reported using 2% ($m \cdot v^{-1}$) commercial activated carbon to remove 80% and 87.9% of furfural and 5-HMF present in wood chips acid hydrolysates, respectively. Adsorption was performed at 90 °C for 30 min under constant stirring (150 rpm). Miura, Suzuki, and Aoyama [56] detoxified wood acid hydrolysates using adsorption on 10% ($m \cdot v^{-1}$) activated carbon powder at 30 °C for 1 h. Around 83% of furfural and other low molar mass phenolic compounds were removed, while carbohydrate and acetic acid concentrations remained practically unaltered. Better detoxification efficiencies may have been due to the better textural properties of the activated carbon used in our studies.

3.3. Enzymatic Hydrolysis

Enzymatic hydrolysis of unwashed (SEB-UW) and water-washed (SEB-WW) substrates was investigated using an experimental design that was based on the following variables: substrate TS, Cellic CTec3 loading, and Cellic HTec3 supplementation. The latter variable was introduced because pretreatment liquors contained oligosaccharides that are known to inhibit total cellulase activity [57,58]. The results obtained after 96 h of hydrolysis were subjected to multiple linear regression analyses to generate mathematical models that could describe trends to the response function, which corresponded to the release of glucose equivalents in the reaction environment (in $g \cdot L^{-1}$). This was the only response function treated statistically because of its relevance for LA production since high concentrations of fermentable sugars are desirable to achieve high fermentation yields. Table 3 shows the mathematical equations that were generated to fit the experimental data for both SEB-UW and SEB-WW enzymatic hydrolyses, whereas Table 4 presents their corresponding analysis of variance (ANOVA). The quadratic models developed to adjust the enzymatic hydrolysis data were generally adequate, with adjusted regression coefficients (R^2) always above 0.99.

Table 3. Mathematical models for glucose release (g/L) from enzymatic hydrolysis experiments performed in shake flasks for 96 h.

Substrate	Equation	R^2
SEB7.5-WW	$Glc = -17.36(x1)^2 + 11.35x1 + 4.24x2 + 3.32x3 + 2.36x1x2 + 0.79x1x3 + 2.27x2x3 + 52.32$	0.9986
SEB15-WW	$Glc = -10.84(x1)^2 + 17.84x1 + 10.95x2 + 2.29x3 + 5.79x1x2 - 0.48x1x3 + 2.02x2x3 + 57.65$	0.9982
SEB7.5-UW	$Glc = -18.36(x1)^2 + 11.54x1 + 4.43x2 + 2.86x3 + 4.22x1x2 + 0.17x1x3 + 3.32x2x3 + 50.73$	0.9969
SEB15-UW	$Glc = -0.30(x1)^2 + 16.78x1 + 10.08x2 + 4.86x3 + 6.30x1x2 - 1.47x1x3 + 3.04x2x3 + 36.58$	0.9998

Table 4. Analysis of variance (ANOVA) of the mathematical models presented in Table 3 at a confidence level of 95% ($p < 0.05$).

Substrate	Conditions	Degrees of Freedom	RSR *	Adjusted R^2	F-Value	*p*-Value
SEB-WW	195 °C, 7.5 min	10	0.5219	0.9986	1702	1.459×10^{-14}
	195 °C, 15 min	10	0.8164	0.9969	779	7.208×10^{-13}
SEB-UW	195 °C, 7.5 min	10	0.9194	0.9982	1337	4.867×10^{-14}
	195 °C, 15 min	10	0.9317	0.9980	1207	8.156×10^{-14}

* Residual standard error.

The highest glucan conversion (74.4%) was obtained from SEB15-WW in the presence of Cellic HTec3 for a final glucose concentration of 84.5 g·L^{-1} after 96 h or hydrolysis (Table 5). This value was 25% higher than that obtained with the same substrate in the absence of Cellic HTec3 (67.2% glucan conversion for a 78.25 g·L^{-1} glucose concentration). In fact, Cellic HTec3 improved the hydrolysis performance of Cellic CTec3 in all reaction configurations. Therefore, residual xylans that were retained in steam-exploded materials seemed to have a role in limiting cellulose accessibility. Also, for SEB-UW substrates, additional hemicellulase activity helped converting water-soluble xylo-oligomers to fermentable xylose, justifying the achievement of slightly higher xylose recoveries. However, it is worth noticing that Cellic HTec3 contains some residual cellulase activity (~5 FPU g^{-1}), which may have been partially responsible for its boosting effect over Cellic CTec3.

Table 5. Glucose and xylose release after enzymatic hydrolysis of both SEB-UW and SEB-WW in shake flasks for 96 h using Cellic CTec3 in the absence and presence of 10% Cellic HTec3 (wet basis).

Parameter	SEB-WW [1]		SEB-UW [2]	
	CTec3	CTec3/HTec3	CTec3	CTec3/HTec3
Steam explosion at 195 °C for 7.5 min				
Glucose concentration (g·L^{-1})	46.17 ± 0.13	59.65 ± 0.33	45.61 ± 0.30	59.50 ± 0.37
Cellobiose concentration (g·L^{-1})	1.50 ± 0.12	2.52 ± 0.04	1.53 ± 0.12	2.25 ± 0.16
Glucan conversion (%)	39.26 ± 0.07	51.23 ± 0.19	38.27 ± 0.17	50.15 ± 0.22
Xylose concentration (g·L^{-1})	2.59 ± 0.15	3.00 ± 0.09	6.24 ± 0.12	6.89 ± 0.11
Xylan conversion	40.52 ± 0.32	46.9 ± 0.19	97.5 ± 0.16	97.7 ± 0.25
Steam explosion at 195 °C for 15 min				
Glucose concentration (g·L^{-1})	78.25 ± 0.28	84.52 ± 0.31	63.72 ± 0.13	75.14 ± 0.11
Cellobiose concentration (g·L^{-1})	3.09 ± 0.12	3.40 ± 0.06	1.51 ± 0.11	2.63 ± 0.12
Glucan conversion (%)	67.02 ± 0.16	74.44 ± 0.20	52.93 ± 0.08	63.15 ± 0.10
Xylose concentration (g·L^{-1})	1.78 ± 0.09	2.16 ± 0.12	2.88 ± 0.07	3.30 ± 0.02
Xylan conversion	27.8 ± 0.17	33.7 ± 0.11	45.0 ± 0.31	51.6 ± 0.47

[1] Water-washed steam-exploded sugarcane bagasse. [2] Unwashed steam-exploded sugarcane bagasse.

SEB-WW was better than SEB-UW for enzymatic hydrolysis, but the difference was minor for substrates produced at the lowest severity (195 °C, 7.5 min). Comparing the total processing time and the resulting glucose concentration in Table 5, water washing seems to be dispensable when pretreatment was carried out for 7.5 min, but mandatory when pretreatment was carried out for 15 min. This suggests that, by doubling the reaction time, the accumulation of potential hydrolysis inhibitors in the C5 stream became critical and this could not be attributed to XOS because these oligos tend to be almost completely hydrolyzed under more drastic pretreatment conditions. On the other hand, in both scenarios, better substrates for hydrolysis were produced when steam explosion was carried out for a longer reaction time or higher pretreatment severity.

Enzymatic hydrolysis of pretreated cellulosic materials was subsequently scaled-up by a factor of 10. Selected hydrolysis conditions (20 wt% TS and 60 mg g^{-1} TS of Cellic CTec3 plus 6 mg g^{-1} TS of Cellic HTec3) were used in the Infors-HT bioreactor to produce enough substrate hydrolysate for fermentation [20,22,59]. Figure 2 shows the hydrolysis profile of both SEB-UW and SEB-WW, with the results given in glucose release in g·L^{-1}. The effects of pretreatment severity, water washing, and Cellic HTec3 supplementation on hydrolysis efficiency were the same as those observed in shake flasks. Cellobiose was always within 1 and 3% of the total glucose release, meaning that the b-glucosidase activity of Cellic CTec3 was high enough to keep it below inhibitory levels. Xylan conversion was higher for SEB7.5-UW, which may have been caused by the xylose concentration of the C5 stream that was retained in the steam-exploded material after centrifugation.

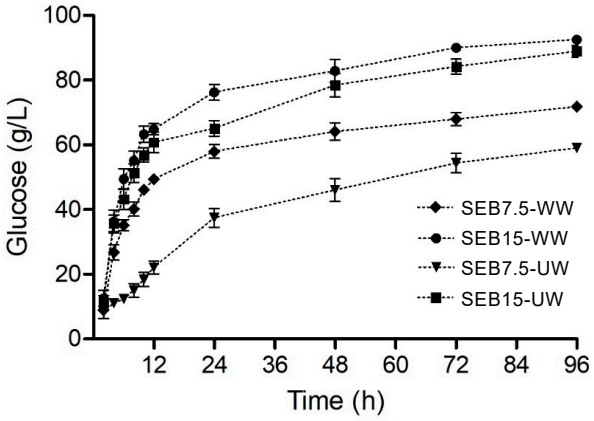

Figure 2. Glucose released (in g·L^{-1}) during the enzymatic hydrolysis of steam-exploded substrates for experiments that were carried out in Infors HT bioreactors.

3.4. Fermentation of SEB Pretreatment Liquors

SEB pretreatment liquors and enzymatic hydrolysates were fermented either alone or in combination using SHF and SHCF protocols. Initially, both fractions were fermented separately to evaluate the influence of detoxification on fermentation yields. There was a visual difference in the turbidity of the media (increase in cell biomass) and a significant difference in LA production between undetoxified and detoxified fractions ($p < 0.05$). Table 6 compares the fermentation profiles of SEB7.5 and SEB15 pretreatment liquors.

Table 6. Lactic acid fermentation of the pretreatment liquors before and after detoxification with a commercial activated carbon powder.

Parameter	SEB7.5 Liquor		SEB15 Liquor	
	Non-Detoxified	Detoxified	Non-Detoxified	Detoxified
Initial Xyl (g·L^{-1}) [1]	11.2 ± 0.2	11.1 ± 0.2	4.3 ± 0.2	4.3 ± 0.1
Initial Glc (g·L^{-1}) [1]	2.2 ± 0.2	2.3 ± 0.1	2.2 ± 0.1	2.1 ± 0.2
Initial Ara (g·L^{-1}) [1]	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.4 ± 0.1
Lactic acid (g·L^{-1}) [2]	2.3 ± 0.6	13.4 ± 0.5	1.7 ± 0.4	6.6 ± 0.6
$Y_{p/s}$ (g·g^{-1}) [3]	0.17 ± 0.03	0.96 ± 0.02	0.25 ± 0.10	0.97 ± 0.12
$Y_{x/s}$ (g·g^{-1}) [4]	0.19 ± 0.03	0.16 ± 0.04	0.09 ± 0.05	0.11 ± 0.07
P_{LA} (g·L^{-1}·h^{-1}) [5]	<0.01	0.54 ± 0.02	<0.01	0.24 ± 0.03
OD_{600} [6]	1.0 ± 0.3	5.9 ± 0.1	1.6 ± 0.4	3.8 ± 0.6

[1] Xyl, Glc, Ara—xylose, glucose, and arabinose. [2] Lactic acid produced by fermentation. [3] Lactic acid yield (gram of product per gram of substrate). [4] Biomass yield (gram of biomass per gram of substrate). [5] Lactic acid productivity (gram of product per liter per hour). [6] Optical densities at 600 nm.

After fermentation, LA contents in non-detoxified pretreatment liquors were lower than those obtained from detoxified fractions for both pretreatment conditions. LA production from non-detoxified media was 2.3 and 1.7 g·L^{-1} for SEB7.5 and SEB15, respectively. These values are statistically equal at a 95% confidence level, meaning that pretreatment liquors without detoxification have similar fermentation performances. By contrast, LA contents from detoxified samples were 13.4 and 6.6 g·L^{-1}, respectively. Lower residence times into the steam reactor led to a lower level of carbohydrate degradation and lower release of fermentation inhibitors in pretreatment hydrolysates. By contrast, the poor fermentation performance of non-detoxified samples was attributed to the presence of inhibitory compounds such as furans, organic acids, and phenolic compounds.

Oliveira et al. [60] used a synthetic media containing xylose and glucose to produce LA, as well as SCB hemicellulose hydrolysates that were obtained by acid hydrolysis at 10% TS using 0.5% (*v*/*v*) HCl at 140 °C for 15 min. This acid hydrolysate, containing 48 g·L^{-1} xylose, 7.86 g·L^{-1} glucose, 0.08 g·L^{-1} 5-HMF, and 0.01 g·L^{-1} furfural, was

supplemented with 20 $g \cdot L^{-1}$ yeast extract and fermented with *B. coagulans* 14–300 to produce 56 $g \cdot L^{-1}$ lactic acid for a theoretical yield of 87%. However, an evaporation step was added to concentrate the acid hydrolysate, which may have reduced its already low concentration of inhibitory compounds [21,27].

Ahorsu et al. [61] used *B. coagulans* DSM2314 to ferment nutshell hydrolysates obtained by microwave-assisted autohydrolysis. The experiments were carried out at 190 °C for up to 25 min in an equipment configuration that allowed five reactions to occur simultaneously. An LA productivity of 0.2 $g \cdot L^{-1} \cdot h^{-1}$ was obtained, which corresponded to a 93% xylose conversion (6 $g \cdot L^{-1}$ of LA). Furthermore, 0.45 $g \cdot L^{-1}$ furfural and 2.42 $g \cdot L^{-1}$ acetic acid did not affect the *B. coagulans* DSM2314 fermentation performance. However, the presence of xylo-oligosaccharides was not investigated, although probably present in pretreatment liquors, while fermentation times were up to 48 h.

van der Pol et al. [62] pre-cultured *B. coagulans* DSM2314 in the presence of fermentation inhibitors using glucose and xylose as carbon source. Inhibitors were found in the following concentrations: 1.6 $g \cdot L^{-1}$ furfural, 0.2 $g \cdot L^{-1}$ HMF, and 3.1 $g \cdot L^{-1}$ acetic acid. Although an increase in LA production was observed in the presence of furfural, fermentation trials were only carried out in synthetic media that simulated the carbohydrate composition of hemicellulose hydrolysates. Furthermore, the observed increase in productivity was not directly associated with furfural consumption or conversion.

A possible explanation for the albeit small formation of LA in the non-detoxified environment (Table 6, Figure 2) would be that, despite the toxicity of furfural being mainly caused by the formation of reactive oxygen species [63,64], several *B. coagulans* genes are known to encode for enzymes such as superoxide dismutase and catalase that can reduce these reactive species to less inhibitory compounds such as 2-furoic acid [65]. Since the DSM2314 strain is catalase-positive, this mechanism may be involved in its tolerance to the presence of furfural [62]. Ye et al. [66] used *B. coagulans* JI12 to ferment cellulosic and hemicellulosic hydrolysates from oil palm empty fruit bunches that contained glucose and xylose in different proportions (1:10 and 1:1). The strain was able to ferment both hydrolysates in the presence of 4 $g \cdot L^{-1}$ furfural, which was partially metabolized to 2-furoic acid.

3.5. Fermentation of SEB-UW and SEB-WW Enzymatic Hydrolysates

Table 7 presents the fermentation profile of both SEB-UW and SEB-WW enzymatic hydrolysates. C6 (glucose) fermentation predominated in both systems, but the former involved more C5/C6 co-fermentation because the substrate retained part of the pretreatment liquor (C5 stream) after centrifugation. Carbohydrates-to-LA conversions ($Y_{p/s}$) of 0.88 and 0.93 $g \cdot g^{-1}$ were achieved for SEB7.5-WW and SEB7.5-UW, while these values decreased to 0.61 and 0.78 $g \cdot g^{-1}$ for SEB15-WW and SEB15-UW, respectively. Therefore, UW hydrolysates were not inhibitory to LA fermentation and microbial growth (see OD_{600} values in Table 7). Also, *B. coagulans* DSM2314 consumed both glucose and xylose indistinctively, as demonstrated by the percentages of residual sugar detected after fermentation. On the other hand, lower $Y_{p/s}$ values for both SEB15-WW and SEB15-UW hydrolysates were probably due to the higher osmotic stress caused by applying higher initial glucose concentrations in the fermentation media.

For SHCF co-fermentation experiments, the C5/C6 ratio was based on van der Pol et al. [62], which was achieved by combining pretreatment liquors (C5 stream) and SEB-WW enzymatic hydrolysates to achieve 10% C5 and 90% C6. For this, pretreatment liquors were used without any further treatment (e.g., filtration or rotary evaporation) or after physical adsorption on activated carbon power to eliminate most fermentation inhibitors (detoxification). Table 8 presents the fermentation profile for SEB-WW enzymatic hydrolysates to which non-detoxified or detoxified C5 streams were added. Adding non-detoxified pretreatment liquor to the corresponding SEB7.5-WW enzymatic hydrolysate decreased fermentation efficiency ($Y_{p/s}$) by ~90%, from 0.88 ± 0.01 to 0.07 ± 0.02 $g \cdot g^{-1}$. This fact was observed by the low conversion of carbohydrates in the medium and the

low cell density at the end of fermentation. Likewise, $Y_{p/s}$ decreased from 0.93 ± 0.01 to 0.07 ± 0.01 g·g^{-1} after adding the untreated C5 stream to SEB7.5-WW hydrolysates. Nevertheless, *B. coagulans* DSM 2314 produced 4.6 g·L^{-1} LA in the presence of fermentation inhibitors, demonstrating its ability to adapt to relatively high concentrations of furanic compounds such as furfural. It is important to notice that a possible elimination of the washing stage decreased LA production from 64.2 g·L^{-1} to 48.8 g·L^{-1} LA for SEB7.5, nearly 24% of reduction. SEB15 also showed a decrease in LA, from 56.9 g·L^{-1} to 52.4 g·L^{-1}, nearly 8%. In fact, these results can be explained by the superior hydrolysis performance of water-washed substrates, which also impacted the subsequent fermentation yields.

Table 7. Lactic acid fermentation of enzymatic hydrolysates derived from water-washed (SEB-WW) and unwashed (SEB-UW) pretreatment solids.

Parameter	SEB7.5-WW	SEB7.5-UW	SEB15-WW	SEB15-UW
Initial Xyl (g·L^{-1}) [1]	2.1 ± 0.1	6.4 ± 0.3	0.9 ± 0.7	4.9 ± 0.9
Initial Glc (g·L^{-1}) [1]	71.0 ± 0.4	59.8 ± 0.3	93.0 ± 1.8	89.9 ± 1.6
Residual Xyl (g·L^{-1}) [1]	0.2 ± 0.1	2.1 ± 0.3	0.13 ± 0.07	2.4 ± 0.8
Residual Glc (g·L^{-1}) [1]	17.1 ± 1.2	14.3 ± 1.6	34.6 ± 1.7	42.0 ± 1.1
Lactic acid (g·L^{-1}) [2]	64.2 ± 1.3	48.8 ± 0.5	56.9 ± 1.2	52.4 ± 0.8
$Y_{p/s}$ (g·g^{-1}) [3]	0.88 ± 0.01	0.74 ± 0.01	0.61 ± 0.01	0.55 ± 0.02
$Y_{x/s}$ (g·g^{-1}) [4]	0.09 ± 0.02	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.3
P_{LA} (g·L^{-1}·h^{-1}) [5]	2.68 ± 0.08	2.03 ± 0.03	2.37 ± 0.07	2.19 ± 0.05
OD_{600} [6]	14.6 ± 0.8	15.3 ± 0.9	14.1 ± 0.3	15.9 ± 0.4

[1] Xyl, Glc —xylose and glucose. [2] Lactic acid produced by fermentation. [3] Lactic acid yield (gram of product per gram of substrate). [4] Biomass yield (gram of biomass per gram of substrate). [5] Lactic acid productivity (gram of product per liter per hour). [6] Optical densities at 600 nm.

Table 8. Lactic acid fermentation of enzymatic hydrolysates derived from water-washed (SEB7.5-WW) pretreatment solids with the addition of non-detoxified and detoxified pretreatment liquors (C5 stream) for a C5/C6 ratio of 1:10.

Parameter	SEB7.5-WW Hydrolysates Containing:		SEB15-WW Hydrolysates Containing:	
	Non-Detoxified C5	Detoxified C5	Non-Detoxified C5	Detoxified C5
Initial Xyl (g·L^{-1}) [1]	6.9 ± 0.7	6.4 ± 0.3	8.9 ± 0.9	5.5 ± 0.6
Initial Glc (g·L^{-1}) [1]	54.4 ± 0.9	59.8 ± 0.3	87.2 ± 0.9	91.0 ± 1.4
Residual Xyl (g·L^{-1}) [1]	4.4 ± 0.9	1.1 ± 0.5	6.2 ± 0.6	1.2 ± 0.3
Residual Glc (g·L^{-1}) [1]	51.9 ± 0.3	3.8 ± 0.7	85.7 ± 0.4	26.4 ± 0.2
Lactic acid (g·L^{-1}) [2]	4.6 ± 0.3	61.4 ± 1.8	4.1 ± 0.5	76.7 ± 1.4
$Y_{p/s}$ (g·g^{-1}) [3]	0.07 ± 0.02	0.93 ± 0.02	0.04 ± 0.02	0.78 ± 0.02
$Y_{x/s}$ (g·g^{-1}) [4]	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.01	0.08 ± 0.01
P_{LA} (g·L^{-1}·h^{-1}) [5]	0.19 ± 0.06	2.55 ± 0.11	0.17 ± 0.03	3.20 ± 0.08
OD_{600} [6]	3.7 ± 0.2	16.2 ± 0.4	3.3 ± 0.2	16.0 ± 0.7

[1–6] See legends in Table 6.

Organic acids and phenolic acids are toxic to the bacteria because they can cross the cell membrane, decreasing the intracellular pH and causing damage to cell functions. Upon inhibition, the metabolic energy is spent to maintain homeostasis instead of being used for cell growth. Protein denaturation, metabolism inhibition, and cell death may also occur in the presence of potent inhibitory compounds [67–69].

Cubas-Cano et al. [34] studied the effect of inhibitory compounds on lactic acid fermentation using *B. coagulans* A162 and DSM2314 strains. Acid hydrolysates from garden plant waste acid-catalyzed steam explosion (180 °C for 10 min plus 60 mg·g^{-1} H_2SO_4) were used as carbon source. After pretreatment, the hemicellulose hydrolysate presented the following composition: 5 g L^{-1} glucose, 15 g L^{-1} xylose, 3.4 g L^{-1} arabinose, 0.20 g L^{-1} furfural, 0.23 g L^{-1} 5-HMF, 1.15 g L^{-1} acetic acid, and 0.23 g L^{-1} formic acid. The highest

LA productivity of 2.4 $g \cdot L^{-1} \cdot h^{-1}$ was attained by the A162 strain, which was tolerant to the presence of fermentation inhibitors at these concentrations.

van der Pol et al. [70] applied pSSF for LA production using H_2SO_4-impregnated steam-exploded SCB (170 °C for 15 min using 0.72 vol% H_2SO_4 in relation to SCB dry mass). The substrate was pre-saccharified for 4 to 6 h with an enzyme cocktail containing both xylanase and cellulase activities (15 $FPU \cdot g^{-1}$ Genencor GC220 in relation to the substrate dry mass), when an inoculum (5%, *v*/*v*) that was pre-cultured in the presence of furfural was added to the medium. LA production reached 70.4 $g \cdot L^{-1}$ in 68 h, representing 89.7% carbohydrate conversion and an estimated productivity of 0.98 $g \cdot L^{-1} \cdot h^{-1}$. LA concentrations above 90 $g \cdot L^{-1}$ have already been reported in other studies for fermentation times up to 50 h [71].

4. Conclusions

Sugarcane bagasse steam explosion was successfully used to produce second generation LA by SHF and SHCF under anaerobic conditions. The pretreatment liquor (C5 stream) was shown to be highly inhibitory to *B. coagulans* DSM2314. This problem was circumvented by adsorption of fermentation inhibitors on activated carbon powder without causing noticeable sugar losses. Furan compounds such as furfural and 5-HMF were almost completely removed, while acetic acid was decreased by nearly 50%. The presence of xylo-oligosaccharides in substrate hydrolysates reduced hydrolysis efficiency, but their effect on fermentation was negligible. SHF and SHCF produced 64.2 and 61.4 $g \cdot L^{-1}$ LA from materials pretreated for 7.5 min, while samples pretreated for 15 min produced 56.9 and 76.7 $g \cdot L^{-1}$, respectively. However, lactic acid yields were better for pretreatment at 195 °C for 7.5 min, reaching $Y_{p/s}$ values of 0.88 and 0.93 $g \cdot L^{-1}$ for SHCF with and without adding the detoxified C5 stream. Doubling the pretreatment time caused a decrease in $Y_{p/s}$ to 0.78 $g \cdot g^{-1}$, but the corresponding LA productivity from SHCF with the detoxified C5 stream reached 3.20 $g \cdot L^{-1} \cdot h^{-1}$. For pretreatment at 195 °C for 7.5 min, elimination of substrate water washing seemed feasible, while the use of longer pretreatment times made it mandatory to eliminate fermentation inhibitors.

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