Optimization of second generation lactic acid from corn stover by alkaline catalysis in subcritial water reaction medium

Candela Helena, Illera Alba Ester, Barea Pedro, Ruiz María Olga, Beltrán Sagrario, Sanz María Teresa[1]*

[1]Department of Biotechnology and Food Science, Faculty of Science, University of Burgos. Plaza Misael Bañuelos s/n. 09001 Burgos. Spain.

E-mail: tersanz@ubu.es

Abstract

The optimization of hydrothermal lactic acid (LA) production from corn stover using $Ca(OH)_2$ as catalyst was investigated. Initial studies with a 9 g/L glucose solution, and different $Ca(OH)_2$ concentrations, in the range of 0.025 to 0.1 M, identified 0.075 M as the optimal concentration. $Ca(OH)_2$ acted both as catalyst and reactant, neutralizing LA, and other organic acids produced, primarily acetic and formic acids. Excess of $Ca(OH)_2$ was required to maintain the alkaline environment necessary for LA production, achieving 3.5 g LA/L with a 40 % yield.

LA production from corn stover was studied at temperatures ranging from 90 to 260 ºC with 0.075 M $Ca(OH)_2$, and a biomass loading of 5 wt %. Higher temperatures increased LA production reaching 5.9 g LA/L with 15.1 % of yield at 260 ºC. The lower value of the yield compared to that obtained from glucose, was attributed to the lower OH-/sugar

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* Corresponding author

monomer molar ratio, which was 3 for glucose, but only 0.65 for corn stover. To counteract organic acid formation, $Ca(OH)_2$ concentrations up to 1.5 M were tested. The OH^-/sugar monomer molar ratio emerged as a key design parameter, with an optimum value of 2.61, resulting in 12.4 g LA/L and 32 % yield. This ratio compensates $Ca(OH)_2$ consumption in neutralization reactions and minimizes excess base, which primarily remained in the solid residue due to the low solubility of $Ca(OH)_2$ in water. This approach also achieves minimal mass intensity, with 5.8 kg of reactants/kg of LA, with unnecessary reactant consumption.

Keywords

Alkaline catalysis, subcritical water, lactic acid, corn stover, molar ratio OH-/sugar monomers

1 Introduction

Lactic acid (LA) is an increasing important chemical commodity, particularly for the production of biodegradable lactic acid polymers (polylactic acid, PLA).[1] It has also a broad range of applications as flavor enhacer in the food industry, as moisturizing agent in the cosmetic industry, and in the pharmaceutical industry.[2,3] But LA is also an important platform molecule to synthesize other chemicals.[3] The global LA market size was estimated at USD 3.37 billion in 2023 and is expected to grow at a compound annual growth rate (CAGR) of 8.0% from 2024 to 2030 (https://www.grandviewresearch.com/industry-analysis/lactic-acid-and-poly-lactic-acid-market#). LA is currently produced either through chemical synthesis or fermentation processes, with more than 90 % of LA production via biological fermentation.[4] Both production methods have inherent challenges. Chemical sysnthesis involves the use of toxic and expensive feedstocks.[5,6] On the other hand, fermentation requires long operation time (2-8 days), leading to low productivity. Moreover, large-scale industrial fermentation typically uses first generation biomass as source of carbohydrates, owing to the absence of contaminants and ease of processing.[7] However, this creates competition with food supplies as the available land, water and resources should be used for food production. Additionally, the cultivation of crops for industrial chemicals can lead to deforestation and loss of biodiversity, contributing to environmental degradation.[7]

Various agricultural harvesting residues have demonstrated significant potential as carbon sources for producing a wide range of chemicals, offering an opportunity to use these residues that would otherwise be discarded.[7] These residues generally present similar chemical composition with approximately 15-20 % lignin, 30-45 % cellulose and 20-25 % hemicellulose.[8] In a recent study assessing the potential of agroforesty residues for the bioeconomy within the European Union, wheat straw was identified as the most promising lignocellulosic material, followed by corn stover. The value of residue-to-crop ratio of corn stover can reach values as high as 1.3, with an average value of 1.13, as nearly 50 % of the external corn plant is stover.[8,9] Given this residue-to-crop ratio and its chemical composition, corn stover emerges as a promising raw material, offering an alternative that avoids competition with food production and addresses sustainatiblity concerns.[8]

The use of lignocelluloic biomass as raw material requires a different pre-treatment process compared to first-generation biomass to facilitate the fractionation into its components. Hydrothermal treatment has emerged as a sustainable and green alternative for producing a variety of second-generation chemicals from lignocellulosic materials.[10] Under subcritical water conditions, water exhibits unique properties, becoming an effective catalyst and reaction medium. However, hydrothermal treatment of pure sugar monosaccharides, such as glucose and xylose, without any external catalyst, yields negligible amounts of the targeted chemical of this work, LA, yielding furfural and hydroxymethyl furfural (HMF) as the main degradation products from xylose and glucose, respectively.[11] Previous studies have shown that the addition of lanthanides as Lewis acid catalyst increased LA yields up to 39.5 and 42.9 % from xylose and glucose, respectively. [12] When using corn stover as the raw material, slightly lower LA yields were observed (approximately 30.3 %).[12] Although these results were promising, the use of rare earth metals as catalyst still presents limitations to large-scale applications due to the high energy and capital cost involved.[13] To enable large-scale production, more affordable and readily available catalyst must be explored.

In the literature, it has been described that the addition of alkali catalysts can improve LA yields from different biomass-derived sugars.[14–18] However, only a limited number of studies focus on LA production from real biomass. Sánchez et al.[5,6] explored LA production via alkaline hydrothermal treatment using two different biomass sources: corn cobs and bread residues. For both raw materials, $Ca(OH)_2$ was identified as a suitable catalyst to produce LA observing a different trend regarding the influence of $Ca(OH)_2$ concentration on LA yield for each material. For corn cobs, the optimal catalyst concentration was determined to be 0.7 M $Ca(OH)_2$ (approximately 2.1 g $Ca(OH)_2$ per g of corn cob) at 300 ºC and 30 min, with a LA yield of 44.76 % (corresponding to 7.38 g LA/L). On the other hand, when using bread residues, a continuous increase in LA yield was observed with $Ca(OH)_2$, reporting that 3.5 M of $Ca(OH)_2$ (6.5 g $Ca(OH)_2$ per g of bread residue) led to a LA yield of 72.9 % (concentration of 29.2 g LA/L).[5] The higher LA yield from bread residues could be closely related to its chemical composition, with approximately 75 % of starch content.

In this work, several steps are proposed to try to shed light for LA production from lignocellulosic biomass, focusing on optimizing the concentration of $Ca(OH)_2$ to minimize its dosage, but obtaining high LA yields and concentration in the reaction medium. The goal is also to minimize the large quantities of acid needed to neutralize the excess of alkaline solution normally used in different literature studies after treatment, while evaluating the mass LA productivity at different $Ca(OH)_2$-to-biomass ratios as sustainability parameter.

Initially, glucose was used as a model compound to evaluate the effects of temperature and $Ca(OH)_2$ concentration. Once optimal conditions were determined, corn stover was treated to produce LA, considering the effect of temperature, $Ca(OH)_2$ concentration and ratio catalyst:biomass on the efficiency of the process to reduce costs and shorten the production time of lactic acid compared to microbial fermentation. Special attention was given to pH values throught the treatment and to the organic acid profile generated during the process to better understand the optimal conditions.

2 Experimental section

2.1 Raw material and chemicals

Corn stover was kindly provided by a local farmer from Saldaña (Palencia, Spain), consisting of leaves and stems. It was milled using a Retsch SM100 mill with a 2 mm aperture size for subsequent use. Its moisture content was determined to be 6.2 ± 0.6 wt %. The composition of the corn stover and the solid residue after alkaline treatment were analyzed following the National Renewable Energy Laboratory (NREL).[19]

2.2 Alkaline catalysis in subcritical water reaction medium

The treatment was carried out using a laboratory-assambled batch system with a 0.5 L capacity reactor (p_{max} = 60 bar). The reactor was covered with a ceramic heating jacket (230 V, 4000 W, ø 95 mm, 160 mm height) to achieve and maintain the desired working temperature. The system was pressurized by using nitrogen and pressure was fixed at 55 bar. A Pt100 sensor placed inside the reactor and connected to a PID system enabled temperature control and monitoring throughout the treatment.

In a typical run, 200 mL of the sugar monomer or corn stover solution with the required amount of $Ca(OH)_2$ were charged into the reactor. A magnetic stirring bar was placed at the bottom of the reactor to enhance homogeneity fixing the stirring rate at 700 rpm.

A needle valve (Autoclave Engineers) connected to a cooling system enabled the withdrawal of samples (2-3 mL) to follow the reaction progress. Collected samples were kept refrigerated until their analysis to follow the LA production kinetics. After a specified reaction time, the vessel was cooled and depressurized when the temperature was below 90 °C. Due to the low solubility of $Ca(OH)_2$ in water, any excess remains in the solid residue generated after treatment of corn stover. To remove the $Ca(OH)_2$ from the solid, approximately 6 mL of HNO_3 (20 wt %) were added per gram of solid residue. The pH of the solution was determined to ensure an acidic environment and the formation of the highly soluble salt $Ca(NO_3)_2$. The final solid residue was washed, dried, and weighed, and subsequently analysed to determine its chemical composition. The variability of the hydrothermal treatment was estimated to be less than 10 %.

First, LA production from glucose, the most abundant biomass-derived sugar from corn stover, was used as the raw material. An initial glucose solution of 9 g/L was prepared, and hydrothermal alkaline treatment was conducted at two different temperature levels, 90 ºC and 190ºC, with $Ca(OH)_2$ concentrations ranging from 0.025 M to 0.1 M. These values correspond to ratios from 0.206 to 0.82 g of $Ca(OH)_2$ per g of glucose (0.5 to 2 moles of $Ca(OH)_2$ per mol of glucose).

Afterwards, LA production from corn stover was studied at the optimal $Ca(OH)_2$ concentration determined for glucose, at different temperatures ranging from 90 ºC to

260 ºC. Further optimization, regarding $Ca(OH)_2$ concentration and the mass ratio of catalyst to biomass, was also conducted. A total of 28 treatments were performed, including 10 for pure biomass-derived sugars and 18 for corn stover under different experimental conditions. All treatments conducted in this work have collected in Table 1 for better clarity.

2.3 Analytical methods

2.3.1 Carbon content

The carbon content of the corn stover and the solid residues generated after different hydrothermal alkaline treatments was determined by an organic elemental micro-analyzer equipment (Thermo Scientific Model Flash 2000).

2.3.2 Quantification of lactic acid, sugars and other degradation products

Monomeric sugars, lactic acid and other sugar-derived compounds were determined by HPLC as described by Alonso-Riaño et al. [20] according to the NREL protocols.[21] The HPLC system was equipped with a Biorad Aminex HPX-87H column, its corresponding pre-column, and two detectors: a variable wavelength detector (VWD, fixed at 210 nm) and a refractive index detector (RID). Both, the column and the refractive index detector were maintained at 40 ºC. Samples (10 µL) were injected after being filtered through a 0.2 µm syringe filter. The mobile phase consisted of 0.005 M sulfuric acid at a flow rate of 0.6 mL/min. Total analysis time was 60 min. Calibration was performed using pure standards.

Standards for L(+)-lactic acid solution (40 wt % in water), glucose (99 %), xylose (99 %), furfural (99 %), glyceraldehyde (98 %) and glycoaldehyde (98 %) were purchased from Sigma-Aldrich. Manose was purchased from Apollo Scientific (99.5 %). Standards for 5-HMF (97%) were obtained from Alfa Aesar, formic acid (98%) from Fluka, acetic acid

(99.8 %) from VWR, glycolic acid (98 %) and propionic acid (99 %) from TCI, 1,3-dihydroxyacetone (95 %) from Fluorochem and hydroxyacetone (95 %) from Thermoscientific. Peak identification was done by comparing the retention times of the sample peaks with those of pure standard compounds. Relative standard deviations for RID and VWD detectors were lower than 2 % for each identified compound.

The D-/L-Lactic Acid (D-/L-Lactate) assay kit by Megazyme (Neogen) was used for the specific concurrent measurement of L-lactic acid (L-lactate) and D-lactic acid (D-lactate).

2.4 Parameters in lactic acid production

The C-yield of LA was assessed by considering the initial C content in the feed solution coming from the saccharide fraction and the C content in the lactic acid produced:

$$Lactic\ acid\ yield\ (\%) = \frac{moles\ of\ C\ in\ lactic\ acid_{(t)}}{moles\ of C\ in\ the\ feed\ from\ saccharides_{(0)}} * 100 \qquad [1]$$

A similar yield percentage was also evaluated for other organic compounds produced during the reaction, such as organic acids or aldoses.

3 Results and discussion

3.1 Corn stover characterization

The chemical composition of the corn stover is presented in Table 2, expressed as a weight percentage on a dry basis (6.2 wt % of moisture content). The total carbohydrate fraction accounted for 67.9 wt % (excluding the acetyl groups in the hemicellulose fraction), with 39.2±0.9 wt% corresponding to glucan, 24.5±0.7 wt% to xylan, and 4.2±0.2 wt% to arabinan. In addition to carbohydrates, corn stover contains a significant amount of acid insoluble lignin, at 15.3±0.4 wt%. Minor amounts of protein, lipids, and ashes were also present. These values are consistent with the ranges previously reported for lignocellulosic agricultural harvesting residues, as mentioned in the introduction section.

3.2 Lactic acid production from glucose

3.2.1 Effect of $Ca(OH)_2$ concentration at two different temperature levels

Figure 1a presents the LA concentration and yield at 190 ºC at four different $Ca(OH)_2$ molar concentrations: 0.025 M, 0.05 M, 0.075 M and 0.1 M. A glucose concentration of 9 g/L (0.05 M) was chosen based on previous studies by other authors who investigated the catalytic action of $Ca(OH)_2$ using glucose as raw material and summarized in Table 3. In these studies, the initial glucose concentration ranged from 0.0056 M to 0.5 M. An intermediate value of 0.05 M was selected in this work to facilitate comparisons with previous results presented in Table 3. Zero time was defined as the moment when the reactor reached the target temperature, with an initial non-isothermal period lasting approximately 25-30 min in each experiment. It can be observed that LA production from glucose at 190 ºC was fast, with the majority of LA being produced during the heating period. The lowest LA concentration and yield were observed at 0.025 M $Ca(OH)_2$ (0.21 g of $Ca(OH)_2$/g of glucose = 1 mol OH^-/mol of glucose) yielding 1.54 g of LA/L corresponding to a production yield of 17.1 %. Similar final LA concentrations and yields were achieved for the rest of molar concentrations essayed (around 3.5 g of LA/L, corresponding to a production yield close to 40 %); although a slower initial reaction rate was observed at 0.05 M $Ca(OH)_2$.

Due to the fast glucose consumption at 190ºC, the production of LA from glucose was explored at 90 ºC using the same $Ca(OH)_2$ concentrations, from 0.025 M to 0.1 M. This temperature was chosen to work with water in its liquid state, but at a temperature below its saturation value at atmospheric pressure. The results are shown in Figures 1b. LA production from glucose at 90 ºC was also fast, with similar kinetic trends to those observed at 190 ºC, although slightly lower LA concentrations and yields were obtained at 90 ºC. The use of moderate reaction conditions for lactic acid production from pure sugar-derived biomass when using $Ca(OH)_2$ as catalyst is advantageous. The possibility to operate at moderate temperatures and ambient pressure reduces limitations related to the materials used in process equipment. This fact has been also highlighted in the study by Li et al.[22] on the catalytic activity of $Ba(OH)_2$ for lactic acid formation from glucose. These authors reported a LA yield as high as 95.4 % using an initial glucose concentration of 0.025 M with a 0.25 M of $Ba(OH)_2$ at 25 ºC after 48 h of reaction under a nitrogen atmosphere at 1 bar of total pressure. Their findings also showed a decrease in LA yield with increasing glucose concentration, with values dropping below 1 % at 1.0 M of glucose due to fructose formation via isomerization.

Based on these results, it can be concluded that a molar ratio higher than 3 moles of OH^- per mol of glucose (0.075 M of $Ca(OH)_2$ in 9 g/L of glucose or 5.6 g $Ca(OH)_2$ per g of glucose) was needed to achieve the optimal LA yield and reaction rate.

The kinetic profiles of the other degradation compounds obtained at 0.075 M $Ca(OH)_2$ at temperatures of 190 ºC and 90 ºC identified in this work have been plotted in Figure 2a and 2b, respectively. Among the main compounds identified in this study, two different groups can be distinguished: aldoses and organic acids. Some hexoses such as fructose and mannose were identified, but their concentration decreased rapidly. Glyceraldehyde (triose) was also identified, being this compound an intermediate in LA production through the dehydration to form pyruvaldehyde, that can subsequently be converted into LA via a 1,2-hydride shift, as detailed in the literature.[16]

Other organic acids were also detected (Figure 2a and 2b). In this regard, it is important to highlight that $Ca(OH)_2$ acts both as a catalyst and as a reactant, with organic acids neutralization resulting in a reduction of $Ca(OH)_2$ concentration in the medium, potentially leading to a decrease in the pH medium. This behaviour was clearly observed by determining the pH during treatment at 90 ºC at the different molar concentrations of $Ca(OH)_2$ along with the total molar concentration of carboxylic groups evaluated as the sum of the carboxylic groups derived from the organic acids quantified in this work during treatment. Results have been only plotted for 0.025 and 0.075 M $Ca(OH)_2$, including also the initial molar concentration of OH^- ions for each $Ca(OH)_2$ concentration tested (Figures 2c and 2d). At 0.025 M, the pH continuouly decreased until reaching a neutral value (pH values of 7.5-7.2). At this concentration (Figure 2c), most of the initially $[OH]^-$ ions (0.05 M) were neutralized with the carboxylic groups of organic acids and the pH was insufficiently basic to facilitate the conversion of pyruvaldehyde to LA via the 1,2-hydrdride shift reaction under alkaline conditions. In contrast, 0.075 M $Ca(OH)_2$ (3 moles OH^- per mole of glucose) seems to be enough to maintain a basic pH through the entire treatment, despite the formation of organic acids and the consumption of the catalyst in forming the corresponding calcium salts (Figure 2d).

These findings suggest that the molar ratio OH^- to glucose is a key parameter to optimize LA production via alkaline treatment. While an excess of base is necessary to compensate its consumption due to neutralization reactions with the organic acids formed, excessive base should be avoided.

Other literature results reporting LA production from glucose have been summarized in Table 3, where results were standardized to express the initial concentration as mol of OH^- per mol of glucose. For instance, Yan et al.[16] found that increasing $Ca(OH)_2$ concentration from 0.08 to 0.32 M (0.16 to 0.64 M OH^-) improved the LA yield to a maximum of 20%, under reaction conditions of 300 ºC and 60 s (molar ratio OH^- to glucose of 5.7). Li et al.[23] observed a maximum LA yield at molar ratio OH^- to glucose ratio of 1.22 at 220 ºC and 180 min, followed by a decrease with further $Ca(OH)_2$ addition. They attributed the decline to the decomposition of LA at higher $Ca(OH)_2$ concentrations. However, in both, the present work and Yan et al.[16] no evidence of LA decomposition was observed at higher molar ratios of OH^- to glucose, being calcium lactate rather stable.

The lower LA yields obtained in this work, compared to the values reported by Esposito et al. [14] and Li et al [23] could be attributed to the lower reaction temperature employed in this work, 190 ºC, and higher glucose concentration in the medium. Li et al. [23] studied the effect of glucose concentration, observing that increasing the glucose concentration from 5.6 to 8.4 mmol/L (with a catalyst dosage of 25 wt% relative to glucose) led to a decrease in LA yield from 87 % to 69.4 %. These authors suggested that higher glucose concentrations promote the formation of intermediate species such as glyceraldehyde and dihydroxyacetone, while excessive glucose molecules would overlap the catalyst surface resulting in a decrease in LA formation. An increase of glucose concentration led to lower LA yields. [4,16,24]

Based on the data from glucose conversion studies, an initial $Ca(OH)_2$ concentration of 0.075 M was selected for testing LA production from corn stover.

3.3 Lactic acid production from corn stover

3.3.1 Effect of temperature

The production of LA from the polysaccharide fraction of corn stover was evaluated in a batch reactor using 0.075 M $Ca(OH)_2$ as catalyst and an initial biomass loading of 5 wt % in 200 mL of water. The treatment was initially performed at the two previously tested temperatures, 90 ºC and 190 ºC. To evaluate the LA yield from corn stover, all the carbohydrate fraction was considered as potential carbon source (equation 1). For supporting this assumption, prior test using xylose as the starting monomeric sugar was carried at 190 ºC and 0.075 M $Ca(OH)_2$. This test yielded up to 41 % LA yield, a value similar to that obtained from glucose, with similar profile for the main degradation products (Figure 3).

At 90 ºC, the concentration and yield of LA were very low, contrasting with the results from pure glucose, concluding that the complex structure of lignocellulosic biomass likely hindered the reaction (Figure 4). On the other hand, at 190 ºC, water under subcritical conditions provided the required reaction medium; however, the LA concentration and yield at 190 ºC (2.6 g/L, 6.7 % yield), after 210 min of isothermal treatment time, were still lower than those obtained with pure glucose (3.5 g/L, 39 % yield). These results could be explained considering the initial molar ratio OH^- to monomeric sugars available in the initial raw material, evaluated as 0.65, which is lower than the minimum OH^- to glucose ratio used in earlier experiments with glucose (Table 3). According to the results presented in Table 3, low molar ratios OH^- to glucose could be offset by increasing the reaction temperature. Therefore, experiments were conducted at higher temperatures, up to 260 ºC (Figure 4). The maximum values for LA were obtained at 260 ºC (the maximum temperature essayed in this work), with 5.8 g LA/L and a yield of 15.1 %. To compare the LA yield from pure glucose with that from corn stover at the highest temperature essayed in this work, 260 ºC, an additional experiment was conducted at 260 ºC with 0.075 $Ca(OH)_2$. Figure 1c illustrates the kinetic profile of LA production at 260 ºC, along with profiles at 190 ºC and 90 ºC for enhanced comparison. It can be observed that raising the temperature also led to an increase in LA concentration and yield when starting from pure glucose. However, the increase was less significant than when starting with corn stover. For example, at 190 ºC, the LA concentration and yield from pure glucose were 3.7 g/L and 41.0 %, respectively, while at 260 ºC they were

4.1 g/L and 45.7 %, indicanting an 11 % increase. In contrast, for corn stover, the concentration and yield at 190 ºC were 2.6 g/L and 6.7 %, and at 260 ºC thy were 5.6 g/L and 14.74 %, resulting in a 120 % increase in yield. This greater increase was due to the low yield obtained at 190 ºC with corn stover due to its chemical composition. The polysaccharides must be first hydrolyzed into monomer units before being converted into LA. In this context, the properties of subW play an important role with an increase in its hydrolytic power with temperature, faciliting the release of monomers.The effect of temperature on water under subcritical conditions has been widely reported in the literature. The ionic product of water, K_w, increases with temperature, leading to higher concentrations of hydronium (H_3O^+) and hydroxide (OH^-) ions from water autoionization. This phenomenon could promote biomass hydrolysis and facilitates the release of sugar monomers from the polysaccharides.[25]

The experiments carried out at different temperatures enabled us to evaluate the apparent activation energy of the process, assuming an Arrhenius temperature dependence for the initial reaction rates:

$$r_o = A \cdot \exp(-Ea/RT) \qquad [2]$$

where r_o is the initial LA formation rate, T is the absolute temperature, A is the pre-exponentail factor, E_a is the molar activitation energy, and R is the universal gas constant. An activiation energy value of 34.67 kJ/mol was obtained ($\ln r_o = -4.3262 \cdot (1000/T(K)) + 5.2923$, $R^2 = 0.9107$). This value is lower than those typically reported for reactions in acidic media [11,26] supporting that different catalytic pathways are involved. In alkaline conditions, LA is likely formed via pyruvaldehyde, whereas acidic conditions favour sugars dehydration towards furfural and hydroxymethyl furfural. The activation energy value, although slightly lower, is comparable to the activation energy reported by Oefner et al.[27] for the alkaline (0.1 M NaOH) degradation of D-xylose, which produced a variety of organic acids (including pyruvic, formic, glycolic, lactic and acetic acids) in yields ranging from 10 to 23 % over a temperature range of 180-220 ºC, with an activation energy value of 63.7 kJ/mol.

It is also worth noting the high stability of the produced lactic acid in the alkaline medium, as calcium lactate. Wang et al. also reported higher thermal stability of calcium lactate during LA production from glucose using $Ca(OH)_2$ as catalyst.[4] In contrast, previous studies using Yb^{3+} (a Lewis lanthanide) as a catalyst under subcritical water conditions showed that LA suffered degradation at temperatures above 220 ºC.[12]

The profiles of other components quantified at the end of the treatment are presented in Figure 5. The most abundant degradation compounds were organic acids, accounting for more than 72 %, consistent with glucose studies, mainly acetic and formic acids. C3 and C2 aldoses (primarily glyceraldhyde, hydroxyacetone, dihydroxyacetone) were also detected in the final reaction medium, but only small amounts of C6 sugars could be identified at the end of the treatment, which are not included in Figure 5, but account for the remainder up to 100 %.

The composition of the solid residues collected after treatment with 0.075 M $Ca(OH)_2$ at different temperatures is listed in Table 2. An increase in temperature resulted in higher hydrolysis yield of the corn stover charged into the reactor, from 49.9 % at 190 ºC up to 81 % at 260 ºC. Hemicelluloses (xylose + arabinose) were more easily hydrolyzed than glucans. At 190 ºC only 9.5 ± 0.3 % of the initial hemicellulose fraction remained in the solid residue, while at temperatures above 240 ºC, no hemicelluloses were detected in the solid. In contrast, over 80 % of the initial glucans remained in the solid residue at 190-200 ºC; but at 240 ºC, only 7.9 ± 0.4 % of the initial glucan remained in the solid, with no detectable glucans at 260 ºC (note that Table 2 reports compositions). Regarding lignin, its insoluble fraction primarily remained in the solid phase.

The percentage of C content in the solid residue increased compared to the initial corn stover, with higher values observed at high temperatures (Table 2). The CHNO elemental composition values were used to calculate the molar H:C and O:C ratios (Table 2). The molar H:C ratio followed the order: corn stover > $\text{solid}_{190\,^{\circ}\text{C}}$ > $\text{solid}_{200\,^{\circ}\text{C}}$ > $\text{solid}_{220\,^{\circ}\text{C}}$ > $\text{solid}_{240\,^{\circ}\text{C}}$ > $\text{solid}_{260\,^{\circ}\text{C}}$, ranging from 1.60 to 1.06, indicating that higher temperatures result in more aromaticity of the solid fractions. This trend aligns with lignin-enriched composition of the solid residue at higher temperatures which could have valuable applications for further biobased chemicals production [28] (Table 2). The molar O:C ratio followed a similar trend as the H:C ratio, decreasing from 0.8 in the untreated corn stover to 0.38 at 260 ºC. This decrease in the O:C ratio is associated with a decrease in the content of hydroxyl (OH), carboxyl (COOH), and carbonyl (C=O) groups. [20]

LA yield increased with temperature using 0.075 M of $Ca(OH)_2$; however, the yield remained lower than from pure sugar monomers. This lower yield was attributed to the presence of organic acids generated during treatment, since maintaining alkaline conditions during LA production is a critical factor. Figure 6a shows the final pH after treatment at 260 ºC, which was 4.88. This indicates an underestimation of the initial $Ca(OH)_2$ concentration required to maintain an alkaline medium throughout the treatment. The final molar concentration of carboxylic groups was approximately 0.28 moles of COOH/L, much higher than the initial 0.15 M $[OH^-]$ in the reaction medium (Figure 6a). Figure 6b shows the kinetic profile of total carboxylic groups evaluated from the identified organic acids during treatment at 190 ºC and 260 ºC. At the selected working conditions, all $[OH^-]$ ions were consumed in neutralization reactions with organic acids.

3.3.2 Effect of alkaline concentration.

Based on the final pH value at 0.075 M $Ca(OH)_2$, experiments were conducted at 260 ºC using higher $Ca(OH)_2$ concentrations, from 0.2 to 1.5 M, resulting in higher LA concentrations and yields (Figure 7a). This Figure also includes the results obtained when treating corn stover in the absence of any catalysts added to the medium.

The distribution percentage of the main components quantified at the end of the treatment are also presented in Figure 5, please note that for 0.4 M $Ca(OH)_2$ no kinetic was performed but only composition at the end of treatment. As in previous experiments at different temperatures and 0.075 M $Ca(OH)_2$, organic acids were the most abundant compounds, representing 76 -91 % of the total identified compounds in the final reaction mixture, while aldoses (C3 and C2), mainly glyceraldehyde, contributed between 9 % (at 1.5 M $Ca(OH)_2$) and 24 % (at 0.075 M $Ca(OH)_2$). The ratio of LA to other organic acids identified in this work was 1.95 g of LA per g of other organic acids, accounting formic and acetic acids more than 78 % of the other organic acids. It is important to note the notable low concentration of LA achieved without $Ca(OH)_2$ as catalyst. Furthermore, a slight reduction in LA concentration was observed by the end of the treatment during the cooling process, due to lower stability of LA compared to calcium lactate (during the cooling, the LA concentration dropped from 1.98 g/L to 1.87 g/L). The reduced stability of organic acids was evident from the low levels of formic acid observed during treatment with no catalyst (reaching zero concentration by the end of the treatment); in contrast acetic acid did concentration was kept at similar levels at those determined for treatment in the presence of $Ca(OH)_2$. The most notable difference in the component profile was the presence of typical dehydration products of glucose and xylose in the medium, HMF and furfural, respectively. Although, these were found in much lower concentrations compared to previous studies conducted at lower temperatures ranging from 150 to 200 ºC [11], due to their degradation at high temperatures.

Sánchez et al. [6] studied LA production from corn cobs (23.13 ± 3.40 lignin, 36.75 ± 0.54, cellulose and 29.98 ± 3.6 hemicelluloses) in a bath reactor (1:40 biomass to solvent mass ratio, 1.23 g of corn cobs in 50 mL of disolution) with $Ca(OH)_2$ as the catalyst at 275 ºC. They found that organic acids were also the main degradation products; but contrary to this work, significant levels of sugar monomers, particularly arabinose, were reported.

The effect of higher $Ca(OH)_2$ concentrations on final pH values is illustrated in Figure 6a, where the final pH of the final reaction mixture is plotted along with the initial $[OH^-]$ and the total molar concentration of carboxylic groups (ranging between 0.24 and 0.27). At concentrartions of $Ca(OH)_2$ higher than 0.3 M, the initial $[OH^-]$ seemed to be sufficient to counteract its comsumption during neutralization reactions (final pH of 8.45). Further analysis was done by plotting the LA yield as a function of the hydroxide group-to-monomeric sugar molar ratio (Figure7b), observing an increase until reaching a plateau at ratios between 2.6 and 3.5, with no further increase. This trend was similar to the one observed for glucose, although higher yields were obtained for glucose, approximately 39% compared to corn stover, 34 % (results are also plotted in Figure 7b). Therefore a certain value of the molar ratio OH-/monomeric sugars is required to counteract catalyst comsumption during neutralization reactions.

On the contrary, Sánchez et al. [6] in their study on LA production from corn cobs covered a concentration range between 0.32 M and 1.05 M of $Ca(OH)_2$ observing a maximum at 0.7 M $Ca(OH)_2$ (275ºC, 30 min) with a LA yield of 41 ± 2 % (6.73 g/L). Theses results have been also included in Figure 7b, after expressing their results as molar OH^- to monomeric sugars ratio.

The optical purity of final LA product obtained at 260 ºC and 0.3 M $Ca(OH)_2$ was determined with the D-/L- LA kit resulting in a racemic mixture of 61 ± 6 % of D-LA. Although hydrothermal process assisted by $Ca(OH)_2$ resulted in shorter reaction times compared to microbial production, the racemic mixtures is not so valuable as the high pure isomers. For instance, in polylactic acid production, the mixture of L-LA and D-LA will result in amorphous and unstable polymers.[29]

Mass intensity (MI) was evaluated to compare the outcomes at different molar concentrations of $Ca(OH)_2$:[30]

$$Mass\ Instensity\ (MI) = \frac{total\ mass\ in\ reaction\ vessel\ (kg)}{mass\ of\ product\ (kg)} \qquad [2]$$

In the ideal scenario MI would approach to 1. Total mass includes all components introduced into the reaction vessel (solvents, catalyst and reagents), excluding water, since water as itself generally does not constitute a significant environmental impact.[30] A minimum MI was determined at a 2.61 molar ratio of OH^- to initial monomeric sugars available in the raw material with 5 % biomass loading (Figure 7b). Higher or lower OH^- /sugar molar ratio resulted in increased MI values, indicating lower mass efficiency of the process. Figure 7b also includes the MI values evaluated for the work of Sanchez et al. [6] with higher MI values likely due to the lower biomass loading (and therefore reduced availability of monomeric sugar for LA production), resulting in lower LA concentrations in their reactor. Consequently, another important factor to consider is the biomass loading into the reactor.

3.3.3 Effect of biomass concentration

To asses the effect of biomass concentration, three additional biomass loadings were tried, 3 wt %, 6.2 wt%, and 7.5 wt%. In these experiments, no kinetics were followed, and samples were analysed after 3 h of isothermal treatment at 260 ºC. Two different sets of experiments were conducted: (1) a fixed $Ca(OH)_2$ molar concentration of 0.3 M, the optimum concentration for 5 % biomass loading (2) a fixed molar ratio OH^-/sugar of 2.61, the optimum value corresponding to 0.3 M $Ca(OH)_2$ for a 5 % of biomass loading.

The results are presented in Table 4, which also includes the values obtained at 5 % biomass loading. At biomass loadings higher than 5 %, and 0.3 M $Ca(OH)_2$, lower LA yields were obtained. This outcome was linked to lower final pH in the reaction medium (5.41 and 5.78), as the carboxylic acids generated neutralize the $Ca(OH)_2$ when working at lower ratios than the suggested optimal 2.61 OH^-/sugar ratio. The unexpected lower LA yields obtained when working at the optimum 2.61 OH^-/sugar molar ratio (0.45 M and 0.37 M $Ca(OH)_2$ for 7.5 % and 6.25 % biomass loadings respectively), specially at 7.5 % biomass, may be attributed to the stirring difficulties. To confirm this assumption, some essays were conducted at room temperature to determine the water-swelling capacity of corn stover. At 8 wt %, corn stover was able to retain 5.8 ± 0.1 g water/ g dry corn stover, making it difficult to create a homogeneous reaction medium. Furthermore, mass transfer limitations could be significant, as the reaction medium is highly heterogeneous due to the presence of solid biomass and the excess of $Ca(OH)_2$.

At 3 wt% biomass loadings, both experiments conducted to good LA yield according to the non-acidic final pH values (Table 4). However, at low biomass loading, lower LA concentration was obtained due to the initially reduced availability of monomeric sugars. The MI at the optimum 2.61 OH^-/sugar molar ratio and 3 % of biomass was similar to that obtained at 5 % of biomass, but increased when using $Ca(OH)_2$ molar concentrations higher than the corresponding 2.61 OH^-/sugar molar ratio identified as the design parameter to be considered instead of molar concentration of $Ca(OH)_2$, as it has been demonstrated.

Structural composition of the solid residue after treatment for biomass loading of 6.25 %, 5 % and 3 % at the optimum OH-/sugar mole ratio of 2.61 is also presented in Table 2, after carefully removing the excess of $Ca(OH)_2$ with HNO_3, due to the limiting solubility of $Ca(OH)_2$ in water. Similar composition was obtained for three solids with no hemicelulloses and glucans composition ranging from 8.7 to 11.1 % and high insoluble lignin content, agreeing with the C/H and O/H ratios.

Conclusions

Alkaline treatment by using $Ca(OH)_2$ as an affordable and ready available catalyst in subW reaction medium has been shown as a promising green technology to produce LA from corn stover, selected as an agricultural harvesting residue to replace first-generation biomass.

Basic studies conducted with biomass-derived sugars, primarily glucose but also xylose, indicated that by using $Ca(OH)_2$ as catalyst, LA was the main chemical produced. However, some aldoses (such as glyceraldehyde, identified as LA precursor) and other organic acids were also identified in the medium. $Ca(OH)_2$ acts both as a catalyst and a reactant, neutralizing the organic acids formed. Therefore, an excess of $Ca(OH)_2$ is needed to maintain the necessary basic reaction medium.

Optimization of LA production from corn stover was carried out at different temperatures and $Ca(OH)_2$ concentrations. Increasing temperature led to an increase in the initial reaction rate, and a maximum LA concentration of 5.9 g/L (15.1 % LA yield) at the highest temperature studied 260 ºC. A concentration $Ca(OH)_2$ high enough was necessary to mantain the basic conditions required for LA production and to compensate the organic acid production, including LA and other organic acids, mainly formic and acetic acids. These findings suggest that the molar ratio OH^- to sugar monomer in the raw material is a key parameter for optimizing LA production via alkaline treatment. An excess of base is necessary to compensate for its consumption due to neutralization reactions, but excessive base should be avoided. Due to the low solubility of $Ca(OH)_2$ in water, any excess will primarily remain in the solid residue, reducing its value and leading also to high mass intensity values with unnecessary higher consumption of reactants.

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