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Jimenez Gutierrez, J.M.

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Pretreatment of lignocellulosic biomass for acetic acid co-valorization

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by

Jose Maria JIMENEZ GUTIERREZ

Master of Science in Biotechnology

Wageningen University and Research, the Netherlands

born in Granada, Spain

This dissertation has been approved by the

Promotor: Dr. ir. A.J.J. Straathof

Promotor: Prof. dr. ir. L.A.M. van der Wielen

Composition of the doctoral committee:

Rector Magnificus	Chairperson
Dr. ir. A.J.J. Straathof	Delft University of Technology
Prof. dr. ir. L.A.M. van der Wielen	Delft University of Technology

Independent members:

Prof. dr. ir. A.A. Kiss	Delft University of Technology
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Prof. dr. I. Cornet	University of Antwerp, Belgium
Prof. dr. S.I. Mussatto	DTU, Denmark
Prof. dr. M.J. Barbosa	Wageningen University & Research

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"Once you carry your own water,
you will learn the value of every drop"
(African proverb)

A la LUz que me guía,
al KORAzón que me mueve
y por quienes sonRÍO cada día.

Summary

The use of renewable resources is nowadays a well-established practice and a general policy to address the fossil fuel depletion, as well as the continuous increase in greenhouse gas emissions. Several approaches have been adopted, with a growing trend toward developing new technologies that target efficiency, sustainability and feasibility. Because it closes the carbon cycle, biomass has a significant potential as renewable source, and not exclusively for the production of energy. Thus, similar to the traditional refinery, the fractionation and conversion of sources to generate, separate and purify different products is also applicable to biomass. Hence, the concept of biorefinery enables the use of renewable feedstocks to obtain bio-based fuels, chemicals and materials in a greener and eco-friendlier manner. Moreover, lignocellulosic biomass (LCB) used as second generation feedstock, encompasses plenty opportunities due to features such as availability, price and versatility.

However, the transformation of LCB into added-value products involves certain complexities, with the biomass recalcitrance being one of the main challenges to tackle. With the purpose of disrupting the LCB structure, increasing the surface area and improving the subsequent steps, pretreatment is considered crucial stage within the biorefinery process. Among the different techniques that have been extensively studied and applied, liquid hot water (LHW) pretreatment is regarded as a promising method due to the moderate operational conditions and the lack of extra chemicals addition. Furthermore, LHW not only has the benefit of improving the digestibility of LCB and providing a sugar platform for the subsequent enzymatic and microbial conversion, but also the possible generation of useful side-products, such as acetic acid (HAc).

During LHW pretreatment process, most of the hemicelluloses in the biomass are solubilised, including the hydrolysis of acetyl groups present in their structure, releasing therefore acetic acid. This thesis aims to develop a process to improve the hydrothermal pretreatment of lignocellulosic biomass, with special focus on the possible recovery of the acetic acid fraction before the enzymatic hydrolysis and fermentation steps.

Carbon dioxide is generally perceived as the primary greenhouse gas emitted by burning fossil fuels. However, it also has the potential of becoming an eco-friendly catalyst for the pretreatment of lignocellulosic biomass. Pressurized CO_2 reacts with water producing carbonic acid, lowering the pH of the system and consequently enhancing the acid hydrolysis of hemicelluloses. In **chapter 2**, the use of subcritical CO_2 during LHW pretreatment was explored, using the generation of acetic acid as a progress marker of hemicellulose degradation. It was found that the CO_2 has no additional effect on the HAc release during LHW pretreatment of hardwood, at the conditions applied. Results indicated also that deacetylation of LCB can be achieved by LHW pretreatment regardless the pressure or gas type used (CO_2 and N_2), as the presence of carbonic acid does not contribute significantly to acidification during the process.

Chapter 3 evaluates the scalability of the LHW pretreatment from laboratory to pilot plant by a factor of 500. Equivalent conditions were employed, regarding pretreatment temperatures (180-188 °C) and processing times (30 – 240 min). Moreover, special interest was given in applying similar heating profiles to both scales, obtaining analogous results at lab and pilot scales for the LHW of hardwood. Besides, the liquid and solid fractions obtained after the pretreatment process were analysed, indicating that it is possible to achieve a complete deacetylation of poplar wood.

To describe the LHW pretreatment methodology, a simplified model is developed in **chapter 4**, using the acetyl hydrolysis reaction as the progress indicator. Since this reaction is temperature-dependent and catalysed by released acetic acid, the model includes the heating, cooling, thermal expansion and pH of the reaction mixture. Very good fit was observed for the experimental data of HAc generation with the simulated model, while the experimental values of pH did not fit that well, indicating that multiple factors affect the buffering capacity of the system and therefore, an improvement of the releasing method for the suggested single buffer component approach might be required.

To conclude the investigation, **chapter 5** compares dilute acetic acid to LHW as an alternative pretreatment process of LCB, obtaining similar release of HAc in both techniques for longer processing times. However, the initial addition of HAc enhanced the release of glucose and xylose at the beginning of the pretreatment, as there is no need to wait for the hydrolysis of acetyl groups. Consequently, this initial low pH also accelerated the production of degradation compounds, such as furfural and 5-hydroxymethylfurfural (HMF). In this chapter, further experiments explored the possible recirculation of the liquid fraction after the LHW pretreatment, considering it therefore as a potential method to increase the concentration of HAc.

Chapter 6 gives an overview of the potential recovery of the acetic acid from the biomass hydrolysate obtained after LHW pretreatment of LCB. Different separation and purification techniques have been evaluated and compared, concluding its feasibility as long as sufficient HAc concentration is provided from the pretreatment step. Besides, co-production of other side-compounds such as furfural, might improve the viability of the whole biorefinery process, with a more sustainable and efficient approach. However, the existence of multiple azeotropes due to the presence of other degradation compounds makes the separation and purification processes very challenging. Several options to increase the HAc concentration are also exposed in this chapter and additionally, alternative pretreatment methodologies to LHW such as steam explosion or supercritical CO_2, are discussed too. In conclusion, LHW pretreatment offers a potential additional value from the recovery and co-production of side products, and especially acetic acid, apart from the benefits of disrupting the LCB structure and achieving a certain degree of hemicellulose depolymerisation.

Sammenvatting

Het gebruik van hernieuwbare bronnen is tegenwoordig standaardbeleid om de uitputting van fossiele grondstoffen en de gestage toename van de uitstoot van broeikasgassen aan te pakken. Dit heeft geleid tot een groeiende trend in de richting van het ontwikkelen van nieuwe technologieën die gericht zijn op efficiëntie, duurzaamheid en haalbaarheid. Omdat het de koolstofkringloop sluit, heeft biomassa veel potentieel als hernieuwbare bron, en niet uitsluitend voor de productie van energie. In analogie met de traditionele olieraffinaderij , maakt bioraffinage van hernieuwbare biogrondstoffen het mogelijk om biogebaseerde brandstoffen, chemicaliën en materialen te verkrijgen op een groenere en milieuvriendelijkere manier. Bovendien biedt lignocellulosebiomassa (LCB), gebruikt als grondstof van de tweede generatie, tal van mogelijkheden dankzij kenmerken als ruime beschikbaarheid, marktprijs en veelzijdigheid.

Echter, de transformatie van LCB in producten met toegevoegde waarde brengt bepaalde complexiteiten met zich mee, waarbij de recalcitrantie van biomassa een van de belangrijkste uitdagingen is om aan te pakken. Met als doel de LCB-structuur te verbreken, het oppervlak te vergroten en de daaropvolgende stappen te verbeteren, wordt voorbehandeling beschouwd als een cruciale fase binnen het bioraffinageproces. Van de verschillende technieken die uitgebreid zijn bestudeerd en toegepast, wordt de voorbehandeling met warm water (LHW) beschouwd als een veelbelovende methode vanwege de milde operationele omstandigheden en het ontbreken van extra toevoeging van chemicaliën. Bovendien heeft LHW niet alleen het voordeel dat het de omzetbaarheid van LCB verbetert en een suikerplatform biedt voor de daaropvolgende enzymatische en microbiële omzetting, maar ook de mogelijke vorming van nuttige bijproducten, zoals azijnzuur (HAc).

Tijdens het LHW-voorbehandelingsproces worden de meeste hemicellulose structuren in de biomassa opgelost, inclusief de hydrolyse van acetylgroepen die in hun structuur aanwezig zijn, waardoor azijnzuur vrijkomt. Dit proefschrift heeft tot doel een proces te ontwikkelen om de hydrothermische voorbehandeling van lignocellulosebiomassa te verbeteren, met speciale aandacht voor de mogelijke terugwinning van de azijnzuurfractie vóór de stappen van enzymatische hydrolyse en fermentatie.

Koolstofdioxide wordt over het algemeen gezien als het primaire broeikasgas dat wordt uitgestoten door de verbranding van fossiele brandstoffen. Het heeft echter ook het potentieel om een milieuvriendelijke katalysator te worden voor de voorbehandeling van lignocellulose-biomassa. Onder druk, lost CO_2 op in water en produceert koolzuur, waardoor de pH van het systeem wordt verlaagd en bijgevolg de zure hydrolyse van hemicellulosen wordt bevorderd. In **hoofdstuk 2** werd het gebruik van subkritisch CO_2 tijdens LHW-voorbehandeling onderzocht, waarbij de vorming van azijnzuur werd gebruikt als een indicator van de afbraak van hemicellulose. Er is geconstateerd dat de CO_2 geen additioneel effect heeft op de HAc-afgifte bij LHW-voorbehandeling van

hardhout, bij de toegepaste condities. De resultaten gaven ook aan dat deacetylering van LCB kan worden bereikt door LHW-voorbehandeling, ongeacht de druk of het gebruikte gastype (CO_2 en N_2), aangezien de aanwezigheid van koolzuur niet significant bijdraagt aan verzuring tijdens het proces.

Hoofdstuk 3 evalueert de schaalbaarheid van de LHW-voorbehandeling van laboratorium naar proeffabriek met een factor 500. Er werden gelijkwaardige omstandigheden gebruikt, met betrekking tot voorbehandelingstemperaturen (180-188 °C) en verwerkingstijden (30-240 min). Bovendien was er speciale aandacht voor het toepassen van vergelijkbare verwarmingsprofielen op beide schalen, waardoor analoge resultaten op laboratorium- en pilootschaal werden verkregen voor het LHW van hardhout. Daarnaast werden de vloeibare en vaste fracties verkregen na het voorbehandelingsproces geanalyseerd, waaruit blijkt dat het mogelijk is om een volledige deacetylering van populierhout te bereiken.

Om de LHW-voorbehandelingsmethodiek te beschrijven, wordt een vereenvoudigd model ontwikkeld in **hoofdstuk 4**, waarbij de acetylhydrolysereactie als voortgangsindicator wordt gebruikt. Omdat deze reactie temperatuurafhankelijk is en wordt gekatalyseerd door vrijgekomen azijnzuur, omvat het model de verwarming, afkoeling, thermische uitzetting en pH van het reactiemengsel. Er werd een zeer goede fit waargenomen voor de experimentele gegevens van HAc-generatie met het gesimuleerde model, terwijl de experimentele waarden van pH niet zo goed pasten aan, wat aangeeft dat meerdere factoren de buffercapaciteit van het systeem beïnvloeden en daarom, een verbetering van de afgiftemethode voor de voorgestelde benadering met een enkele buffercomponent kan nodig zijn.

Om het onderzoek af te ronden, vergelijkt **hoofdstuk 5** verdund azijnzuur met LHW als een alternatief voorbehandelingsproces van LCB, waarbij een vergelijkbare afgifte van HAc in beide technieken wordt verkregen voor langere verwerkingstijden. De initiële toevoeging van HAc verbeterde echter de afgifte van glucose en xylose aan het begin van de voorbehandeling, aangezien er niet gewacht hoeft te worden op de hydrolyse van acetylgroepen. Bijgevolg versnelde deze aanvankelijk lage pH ook de productie van afbraakverbindingen, zoals furfural en 5-hydroxymethylfurfural (HMF). In dit hoofdstuk hebben verdere experimenten de mogelijke recirculatie van de vloeibare fractie na de LHW-voorbehandeling onderzocht, waarbij het daarom wordt beschouwd als een mogelijke methode om de concentratie van HAc te verhogen.

Hoofdstuk 6 geeft een overzicht van de mogelijke terugwinning van het azijnzuur uit het biomassahydrolysaat verkregen na LHW-voorbehandeling van LCB. Verschillende scheidings- en zuiveringstechnieken zijn geëvalueerd en vergeleken, waarbij de haalbaarheid ervan is geconcludeerd zolang er voldoende HAc-concentratie wordt geleverd vanaf de voorbehandelingsstap. Bovendien zou de coproductie van andere nevenverbindingen, zoals furfural, de levensvatbaarheid van het hele bioraffinageproces kunnen verbeteren, met een duurzamere en efficiëntere aanpak. Het bestaan van meerdere azeotropen vanwege de aanwezigheid van andere afbraakverbindingen maakt de scheidings- en zuiveringsprocessen echter zeer uitdagend. Verschillende opties worden ook besproken om de HAc-concentratie te verhogen in dit hoofdstuk en daarnaast, worden alternatieve voorbehandelingsmethoden voor LHW, zoals stoomexplosie of superkritisch CO_2, besproken. Concluderend biedt LHW-voorbehandeling een potentiële toegevoegde waarde uit de terugwinning en coproductie van nevenproducten, en met name azijnzuur, afgezien van de voordelen van het verstoren van de LCB-structuur en het bereiken van een zekere mate van hemicellulose-depolymerisatie.

Resumen

Hoy día el uso de recursos renovables es una práctica bien establecida y una política general que aborda el agotamiento de los combustibles fósiles, así como el continuo aumento en las emisiones de gases de efecto invernadero. Varias estrategias han sido adoptadas, con una tendencia creciente hacia el desarrollo de nuevas tecnologías que abogan por la eficiencia, la sostenibilidad y la viabilidad. No siendo exclusivo para la producción de energía, la biomasa tiene un considerable potencial como recurso renovable, ya que cierra el ciclo del carbono. Por tanto, al igual que en la refinería tradicional, el fraccionamiento y conversión de recursos para generar, separar y purificar diferentes productos también es aplicable a la biomasa. De este modo, el concepto de biorefinería permite el uso de materias primas renovables para obtener biocombustibles, productos bioquímicos y biomateriales de una manera más ecológica y respetuosa con el medio ambiente. Además, la biomasa lignocelulósica (LCB) como materia prima de segunda generación, ofrece muchas oportunidades debido a características como su disponibilidad, precio y versatilidad.

Sin embargo, la transformación de la LCB en productos con valor añadido implica ciertas complejidades, siendo la resistencia de la biomasa uno de los principales retos a afrontar. Con el propósito de romper la estructura de la lignocelulosa, aumentar el área superficial y así mejorar las subsiguientes operaciones, el pretratamiento se considera una etapa crucial dentro del proceso de biorefinería. Entre las diferentes técnicas que han sido extensamente estudiadas y aplicadas, el pretratamiento con agua caliente (LHW) se considera un método prometedor debido a las moderadas condiciones de operación y a que no se añaden productos químicos adicionales. Además, este pretratamiento no solo tiene el beneficio de mejorar la digestibilidad de la LCB y proporcionar una fuente de azúcar para la posterior conversión enzimática y microbiana, sino que también ofrece la posibilidad de generar productos secundarios, como el ácido acético (HAc).

Durante el proceso de pretratamiento LHW, la mayoría de las hemicelulosas de la biomasa se solubilizan, incluyendo los grupos acetilo presentes en su estructura, liberando así ácido acético. Esta tesis tiene como objetivo desarrollar un proceso para mejorar el pretratamiento hidrotérmico de la lignocelulosa, con atención especial a la posible recuperación de la fracción de ácido acético antes de las etapas de hidrólisis enzimática y fermentación.

El dióxido de carbono es considerado generalmente como el principal gas de efecto invernadero emitido por la quema de combustibles fósiles. Sin embargo, también tiene el potencial de convertirse en un catalizador ecológico en el pretratamiento de lignocelulosa. El CO_2 comprimido reacciona con agua produciendo ácido carbónico, bajando el pH del sistema y consecuentemente mejorando la hidrólisis ácida de las hemicelulosas. En el **capítulo 2**, se aborda el uso de CO_2 subcrítico durante el pretratamiento LHW, utilizando el ácido acético liberado como indicador del progreso de la degradación de la hemicelulosa. Se observó que el CO_2 no tiene ningún efecto

adicional sobre la generación de HAc durante el pretratamiento LHW de madera dura en las condiciones aplicadas. Los resultados también indicaron que la des-acetilación de la LCB se puede conseguir mediante el pretratamiento LHW independientemente de la presión o el tipo de gas usado (CO_2 y N_2), ya que la presencia de ácido carbónico no contribuye significativamente a la acidificación durante el proceso.

El **capítulo 3** evalúa la escalabilidad del pretratamiento LHW desde laboratorio hasta planta piloto con un factor de 500. Se emplearon condiciones equivalentes con respecto a las temperaturas de pretratamiento (180-188 °C) y los tiempos de procesamiento (30-240 min). Además, se puso especial interés en aplicar perfiles de temperatura similares en ambas escalas, obteniendo resultados análogos a escala de laboratorio y planta piloto para el procesado LHW de madera dura. Además, se analizaron las fracciones líquidas y sólidas obtenidas tras el proceso de pretratamiento, indicando que es posible conseguir una des-acetilación completa de la madera de álamo.

Con el objetivo de describir la metodología del pretratamiento LHW, en el **capítulo 4** se desarrolla un modelo matemático simplificado, que usa la reacción de hidrólisis del grupo acetilo como indicador de progreso. Dado que esta reacción depende de la temperatura y es catalizada por el ácido acético generado, el modelo incluye el calentamiento, el enfriamiento, la expansión térmica y el pH de la mezcla de reacción. Se observó un ajuste muy bueno de los datos experimentales de HAc con la simulación del modelo, mientras que los valores experimentales de pH no se ajustaron tan bien, lo que revela que son múltiples factores los que afectan la capacidad tampón del sistema y, por lo tanto, se hace necesario una mejora en el método de generación del componente tampón, sugerido como único.

Para concluir la investigación, el **capítulo 5** compara el uso de ácido acético diluido con el proceso LHW como alternativa de pretratamiento de la LCB, obteniendo una liberación de HAc similar en ambas técnicas para tiempos de procesamiento largos. Sin embargo, la adición inicial de HAc mejoró la generación de glucosa y xilosa al comienzo del pretratamiento, ya que no es necesario esperar a la reacción de hidrólisis del grupo acetilo. En consecuencia, este bajo pH inicial también aceleró la producción de compuestos de degradación, como el furfural y el 5-hidroximetilfurfural (HMF). En este capítulo, mediante otros experimentos se plantea la posible recirculación de la fracción líquida obtenida después del pretratamiento LHW, considerándolo consecuentemente como un potencial método para incrementar la concentración de HAc.

El **capítulo 6** ofrece una descripción general de la potencial recuperación del ácido acético obtenido en el hidrolizado de biomasa después del pretratamiento LHW de la LCB. Diferentes técnicas de separación y purificación son evaluadas y comparadas, concluyendo su viabilidad siempre y cuando se proporcione suficiente concentración de HAc después del pretratamiento. Además, la coproducción de otros compuestos secundarios, como el furfural, podría mejorar la viabilidad del proceso global de biorefinería, con un enfoque más sostenible y eficiente. Sin embargo, la existencia de múltiples azeótropos debido a la presencia de otros compuestos de degradación aumenta la complejidad de los procesos de separación y purificación. En este capítulo

también se exponen varias opciones para aumentar la concentración de HAc, además de analizar otras técnicas de pretratamiento alternativas al LHW, como la explosión de vapor o el uso de CO_2 supercrítico. En conclusión, el pretratamiento LHW ofrece un potencial valor adicional en la recuperación y cogeneración de productos secundarios, especialmente de ácido acético, además de los beneficios de romper la estructura de la LCB y lograr cierto grado de despolimerización de la hemicelulosa.

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Chapter 1

General Introduction

1. From oil-based to bio-based

Measures needs to be taken. The global population is expected to increase over 25% in the next 30 years, reaching 9.7 billion by 2050. Consequently, the growing of global energy demand and the intrinsic scarcity feature predict an exponential increase in fossil fuels prices (IEA 2021). Reducing the greenhouse gas emissions seems to be crucial to reach, or at least aim, some of the 17 sustainable development goals adopted by the United Nations (UN) in their 2030 Agenda for Sustainable Development (UN 2015). In their recent "Net Zero by 2050", the International Energy Agency (IEA) described a path towards that reduction in CO_2 emissions, future perspectives and the importance of cooperation for international organizations, governments and companies in the private sector (IEA 2021).

During the past years, advances and innovation in new technologies have led, not only the energy sector but many other industrial sectors, to a transition towards the use of renewable sources. Switching from a traditional oil-based refinery to an eco-friendly biorefinery is considered a possible solution to mitigate the levels of CO_2 associated to the use of fossil fuels (Katakojwala and Mohan 2021). From this perspective, the biorefinery concept has gained certain relevance, as the production of bioenergy and biofuels in combination with bio-based chemicals and materials (Figure 1.1).

Fractionation of biomass to generate useful products depends directly on the characteristics of the source and the end product. Several technologies have been designed and applied for the conversion of biomass, which could be categorized under either thermochemical or biological methods (Abdurrahman 2020).

Thermochemical conversion of biomass includes processes such as combustion, gasification, torrefaction, pyrolysis, and liquefaction (Ong et al. 2019). On the other hand, biochemical decomposition of biomass involves sequential steps, including biological conversion to obtain products and intermediates by using different microorganisms or enzymes (Abdurrahman 2020). This process provides a platform to obtain fuels and chemicals such as biogas, hydrogen, ethanol, butanol, acetone and a wide range of organic acids (Rajesh Banu et al. 2021). Compared to other conversion methodologies, biochemical decomposition technologies have a larger potential to be clean because of the selectivity of conversions.

2. Biorefinery concept

Mostly considered for the production of biofuels, such as bioethanol (Verardi et al. 2020) or biogas (Hernández-Beltrán et al. 2019), the biochemical conversion of biomass has been widely studied in the past decades, for a wide range of feedstock (Chandel et al. 2021). Furthermore, biorefinery has evolved towards an ampler concept aiming sustainable and effective utilization of all biomass fractions, considering not only different feedstocks but also process technologies and final products (Duque et al. 2021).

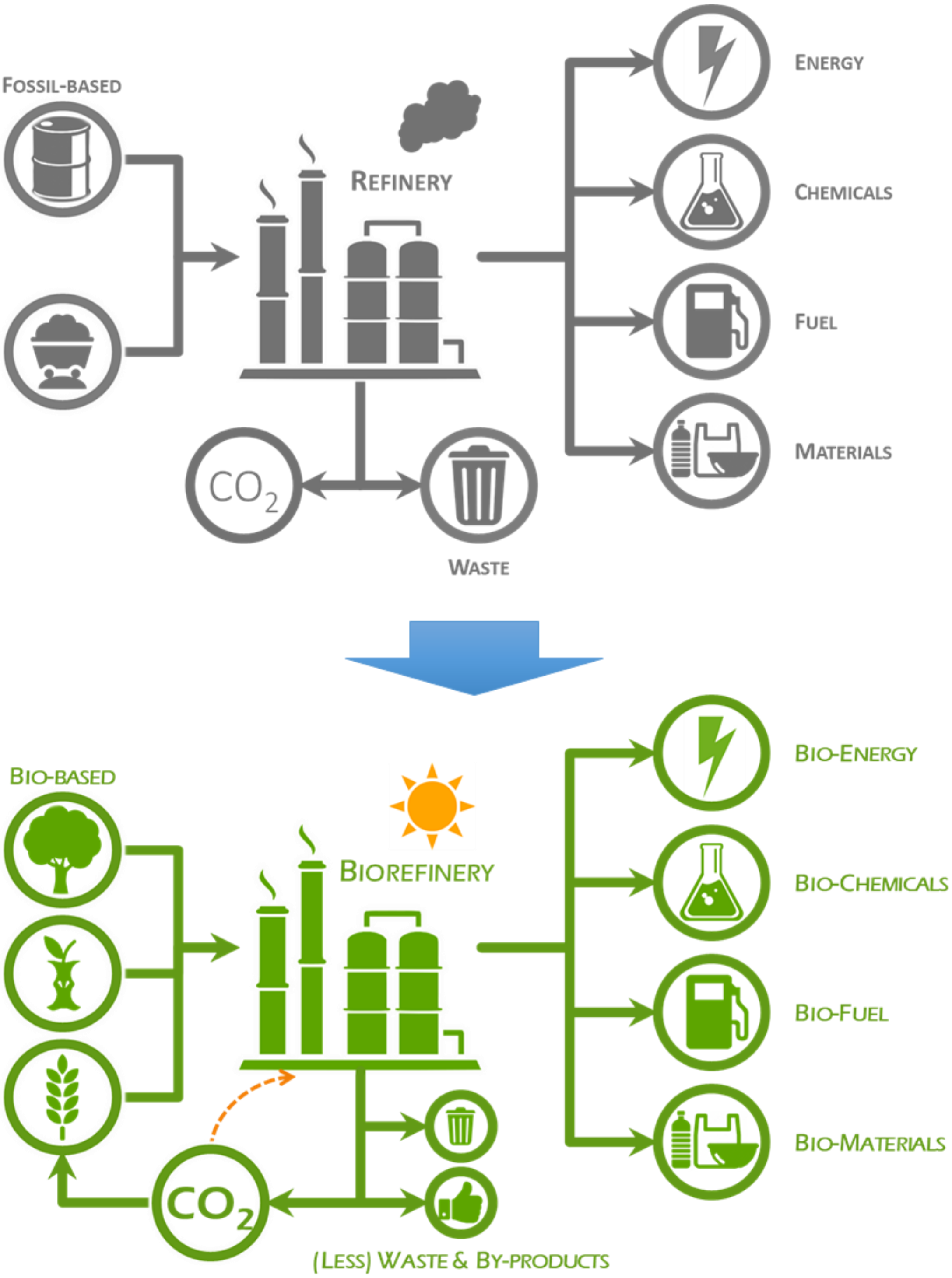

Figure 1.1. *Graphical representation of the transition from a general fossil-based refinery towards a biorefinery.*

A general biorefinery procedure involves mainly four steps (De Bhowmick et al. 2018):

- Pretreatment
- Enzymatic hydrolysis
- Fermentation
- Downstream processing

The purpose of the pretreatment is to disrupt the biomass structure, improve the surface area and enhance therefore its digestibility for the subsequent steps (Tan et al. 2020). The enzymatic hydrolysis aims to break down the polysaccharides and oligomers obtained from the pretreatment to smaller sugars that can be easily used for the microorganisms to produce the desired substances in the fermentation (Pino et al. 2018). Usually, the enzymatic hydrolysis and fermentation steps are coupled and performed either concurrently in the same reactor or separately (Abo et al. 2019). Thus, five different schemes can be derived:

- Separate Hydrolysis and Fermentation (SHF): separate reactors.
- Simultaneous Saccharification and Fermentation (SSF): same reactor.
- Pre-hydrolysis SSF (PSSF): same reactor with two stages of temperature, in order to favour pre-hydrolysis first and fermentation later.
- Simultaneous Saccharification and Co-Fermentation (SSCF): C6 and C5 sugars are consumed by the microorganisms.
- Consolidated Bioprocessing (CBP): combination of enzymes production, substrate hydrolysis and fermentation of C5 and C6 sugars, all in one step.

Depending on the main target of the bioprocess and the nature of the mixture of compounds obtained after the fermentation, the downstream process may involve several separation and purification steps, in order to meet the product requirements (Ramaswamy et al. 2013) (Sun et al. 2020).

Overall, the bioconversion process of biomass into useful products comprises certain complexity, where the pretreatment is considered the key step to cope with the biomass recalcitrance (Usmani et al. 2021).

3. Types of biomass

The raw ingredients and sources used for this biochemical conversion are categorized into four generations. First-generation (1G) biomass types are those from edible crops parts such as starchy, sugary products, and plant oils (Kumar et al. 2020) (Dutta et al. 2014). The second-generation (2G) feedstocks are lignocellulosic biomass (LCB), derived from agricultural residues, crop wastes, forest biomass, solid cattle manure, energy grasses, industrial wastes and municipal solid waste (Dutta et al. 2014) (Zabed et al. 2016). Algal biomass (both macro and microalgae) is the third-generation feedstock (3G). There is a fourth-generation (4G) biomass, though there is not full consensus about it yet. Some authors refer to algae which are manipulated genetically to boost their yield, (Singh et al. 2022), while others include genetically modified plants and microorganism with high carbon capture capacity (Kumar et al. 2020).

The use of edible sources as raw material (1G) brings land competition between energy and food production (Su et al. 2020). Therefore, 2G biofuels could contribute to reduce the soil competition, as residues and/or lignocellulosic biomass would be used instead of allocating crops for the creation of biofuels and bio-products (Bryngemark 2019). Besides, a wide variety of co-products can be extracted by different processing branches applied to 2G lignocellulosic materials (Pinales-Márquez et al. 2021).

Therefore, due to its environmental and economic benefits, using LCB as the second generation feedstock for the biorefinery activity is tremendously encouraged (Raj et al. 2022).

4. Structure of lignocellulosic biomass

Mainly made of polysaccharides, phenolic polymers and proteins, LCB has a complex, non-uniform and three-dimensional spatial structure (Yousuf et al. 2020) which consists of aligned bundles of partly crystalline and partly amorphous cellulose fibrils embedded in a disordered matrix of hemicellulose and lignin (see Figure 1.2) (Petridis and Smith 2018). The composition of LCB is largely diverse and may vary considerably depending on the different species, variety, climate, and soil fertility (Das et al. 2021). Hence, on averaged ranges, the three major constituents of LCB are cellulose (40 – 60 %), hemicellulose (20 – 40%) and lignin (10 – 25%), with other minor components, such as minerals and extractives (Ashokkumar et al. 2022).

4.1. Cellulose

Cellulose is linear polysaccharide composed of glucose units linked through β–(1→4) glycosidic bonds. Hydrogen bonds and Van der Waals interactions form the crystalline structure of cellulose chains, which are consequently aggregated into micro-fibrils and then fibres (Zheng et al. 2022). This polymer is insoluble in water unless at extremely low or high pH levels (Baruah et al. 2018).

4.2. Hemicellulose

Hemicellulose is an amorphous and variable highly branched heteropolymer that consists of short chains of different polysaccharides (such as xylan, galactomannan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan) that are held together by β-(1→4)- and/or β-(1→3)-glycosidic bonds (Baruah et al. 2018) (Scheller and Ulvskov 2010). Therefore, its composition includes C5 (xylose and arabinose) and C6 (mannose, glucose, galactose, rhamnose and fructose) monomer units, uronic acids and acetyl groups (Ashokkumar et al. 2022).

Hemicellulose is cross-linked with either cellulose or lignin, contributing then to strengthening the cell wall (Zheng et al. 2022) (Scheller and Ulvskov 2010). Hemicelluloses are mostly acetylated to various degrees, with the acetyl groups attached randomly with ester linkages to the hydroxyl groups of sugars. The presence of acetyl group and the branched structure of hemicellulose are considered to cause its lack of crystalline structure (Ashokkumar et al. 2022). Thus, the low degree of polymerization and non-crystalline nature cause the readily degradability of hemicelluloses into monosaccharides, unlike the cellulose (Baruah et al. 2018).

4.3. Lignin

Lignin is a complex, three-dimensional cross-linked polymer that consist of three types of phenylpropanoid monomers: 4-propenyl phenol, 4-propenyl-2-methoxyphenol, and 4-propenyl-2,5-dimethoxy phenol (del Río et al. 2020). As the third major component of LCB, lignin is covalently linked to cellulose and hemicellulose, contributing to the rigidity and compactness of the plant cell wall (Kang et al. 2019).

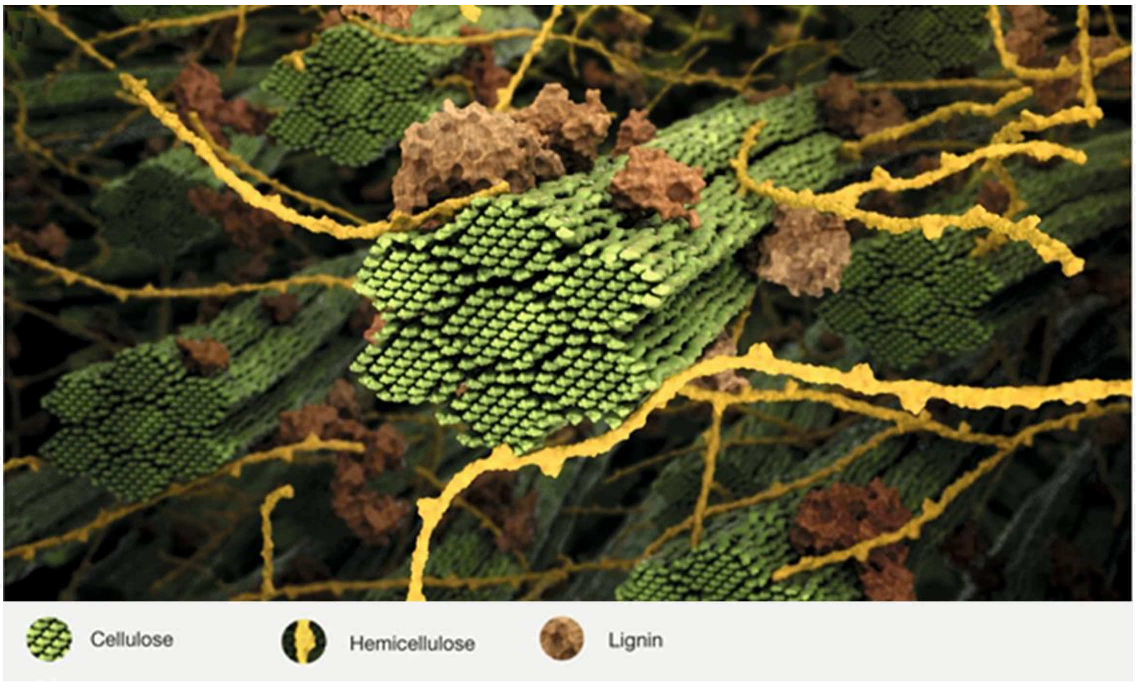

***Figure 1.2**. Representation of lignocellulosic biomass structure, including cellulose (green, with seven micro-fibrils depicted as forming a fibre), hemicellulose (yellow) and lignin (brown) (Adapted with permission from Petridis and Smith (2018)).*

The crystallinity of cellulose, the hydrophobicity of lignin, and the encapsulation of cellulose by the lignin–hemicellulose matrix re the main factors that contributes to robustness or recalcitrance of lignocellulose (Ashokkumar et al. 2022).

5. Poplar biomass

Among all possible species of LCB, poplar is one of the most promising sources and it has been extensively studied and applied for the production of biofuels and value-added products (Dou et al. 2017) (Vera et al. 2015) (Morales-Vera et al. 2020). As the genus *"Populus"* contains a large number of species, it seems difficult to find its specific composition. Besides, substantial variations occur not only for different species but also on the same species (Sakamoto et al. 2015) (Templeton et al. 2016). Thus, most data in literature referred to averages with significant standard deviations, to a certain extent. Disregarding differences between species and individuals, a typical composition is ~65% holocellulose (which includes cellulose and hemicellulose), ~25% lignin, and ~10% other compounds.

In this hardwood, the main hemicellulose is *O*-acetyl-4-*O*-methyl-glucuronoxylan. Its backbone consists of β-(1→4)-linked D-xylopyranosyl residues substituted with one α-(1→2)-linked 4-*O*-methyl-D-glucuronic acid per approximately every 10th xylose residue. The xylose residues are partially acetylated at their C-2 or C-3 position (Johnson et al. 2017). Acetylation seems to decrease the solubility of the glucuronoxylan in hot water, and increase its solubility in hydrophobic solvents (Gröndahl et al. 2003). The level of acetylation can vary widely even for a single type of measurement. For instance, Johnson et al. (2017) found acetate levels ranging between 3.56% w/w and 6.00% w/w, with an average of 5.18%.

6. Dealing with biomass recalcitrance

In general, LCB has natural resistance to chemical and biological breakdown, known as biomass recalcitrance. At the micro level, biomass recalcitrance has been previously defined as *"multiple resistance of plant material to microbial and enzymatic degradation"* (Himmel 2008). Essentially, the biomass recalcitrance can be attributed to two aspects: physical barriers and chemical barriers, although several factors might be considered responsible, such as the crystalline structure of cellulose, the degree of lignification and the structural heterogeneity and complexity of the plant cell-wall (Guerriero et al. 2016).

Therefore, the recalcitrant nature of lignocellulosic biomass presents a technical challenge for releasing fermentable sugars from LCB. To overcome this, pretreatment methodologies have been design to disrupt the LCB structure, resulting in removal of lignin barrier, degradation of hemicellulose and reduction in crystallinity and degree of polymerization of cellulose (Petridis and Smith 2018) (Bhatia et al. 2020).

7. Pretreatment technologies

Pretreatment is therefore carried out to increase the surface area and provide efficient dissolution and fractionation of LCB into components that are easily accessible for enzymes and microorganisms (Chen et al. 2017). Hence, this initial step is considered crucial for a highly efficient chemical bioconversion of LCB within the biorefinery process (Mankar et al. 2021).

Microstructure, macrostructure, and chemical composition of LCB change during the pretreatment process. Consequently, the conditions applied have direct effect on the characteristics of the substrate obtained (Bajpai 2016).

In general, an ideal pretreatment process should overcome LCB recalcitrance, decrease cellulose crystallinity and ensure maximum recovery of sugars and relevant bioproducts (Mankar et al. 2021). According to some authors (Mankar et al. 2021), an effective pretreatment method relies on its ability to meet the following criteria:

1) Remove lignin without much alteration in its native structure
2) Low energy consumption
3) Cost-effective operation
4) Reduce the crystallinity of cellulose
5) Reduce the particle size to increase the surface area for improved enzymatic hydrolysis
6) Applicable to different types of LCB feedstocks
7) Avoid inhibitors production
8) Use eco-friendly chemicals

Based on their mechanisms of action, the pretreatment approaches are typically classified into four categories: physical, chemical, physicochemical and biological (see Figure 1.3). Several reviews in literature describe most of them more in detail (Ashokkumar et al. 2022) (Zheng et al. 2022) (Mankar et al. 2021) (Tu and Hallett 2019) (Hassan et al. 2018).

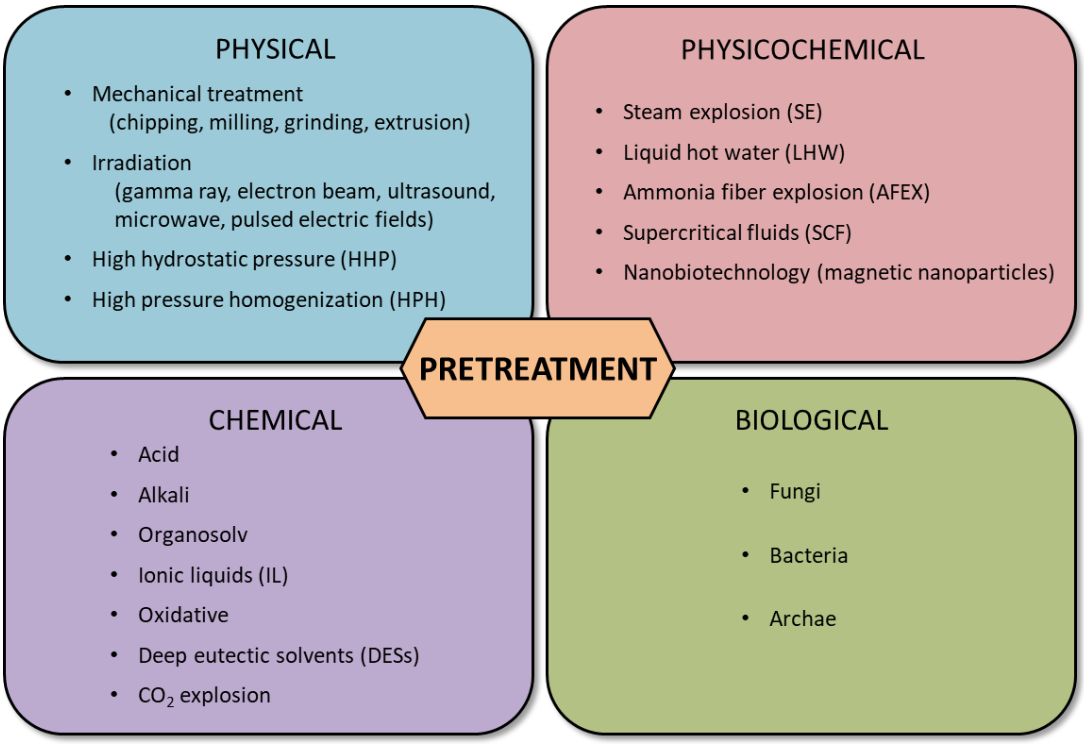

Figure 1.3- *Pretreatment methodologies, classified based on literature (Sources: (Ashokkumar et al. 2022) (Zheng et al. 2022) (Mankar et al. 2021) (Tu and Hallett 2019) (Hassan et al. 2018))*

The differences in composition of each lignocellulosic feedstock entail their potential for biofuel, chemical and/or material production. The ratio of cellulose, hemicellulose, and lignin may vary depending on the type of plant, species, and even within species. Consequently, pretreatment methods might be more suited for certain feedstock, as well as the optimal conditions for pretreatment may also differ depending on the lignocellulosic source (Tu and Hallett 2019). Regarding pretreatment conditions, while low pH may remove almost all hemicelluloses, mainly lignin is dissolved at high pH methods, leaving the solid fraction composed on cellulose and hemicellulose (Galbe and Wallberg 2019).

8. Fundamentals of LHW

Liquid Hot Water (LHW, also called autohydrolysis) pretreatment is nowadays one of the most promising techniques for the disruption of the lignocellulosic biomass structure, because of the relative moderate operational conditions and the lack of chemical addition (Chen et al. 2022), leading to environmental benefits and lower operational cost. This technique consists of pretreating the LCB with water at elevates temperatures (160 – 240 °C) in a closed system, keeping the pressure correspondingly high to maintain the water in liquid state (Zhuang et al. 2016) (Michelin and Teixeira 2016). Under these conditions, the auto-ionization of water generates hydronium ions that act as primary catalysts for the hydrolysis of biomass, initiating hemicellulose depolymerisation and cleavage of acetyl groups (Michelin and Teixeira 2016) (Lu et al. 2016). Consequently, organic acids (such as uronic acid and most importantly acetic acid) are released from hemicelluloses, causing further hydrolysis and generation of oligosaccharides and monomeric sugars, such as glucose and xylose (Mosier et al. 2005) (Martín-Lara et al. 2020). Despite it is considered a physicochemical pretreatment, the LHW mechanism of action is similar to an acid pretreatment due to the acetic acid generation, involving mostly the dissolution of the hemicellulose, with limited cellulose hydrolysis and partial removal and relocation of lignin (Li et al. 2017). As only water and heat is used during LHW, it is usually termed as hydrothermal pretreatment, together with steam explosion (SE), which uses steam instead of water (Xiao et al. 2017).

Depending on the conditions applied during LHW pretreatment of LCB, mostly depolymerisation of hemicellulose occurs, although at the expenses of side reactions. This may produce sugar degradation compounds, such as furfural from xylose and HMF from glucose, as well as other carboxylic acids, such as formic and levulinic acid, besides phenolic compounds from lignin (Steinbach et al. 2017). Those side compounds, including acetic acid from the acetyl hydrolysis of hemicellulose, are considered to have an inhibitory effect on the following enzymatic hydrolysis and fermentation (Jönsson and Martín 2016).

9. Side-products and inhibitors production

Among all the degradation compounds present in the biomass hydrolysate after pretreatment, acetic acid shows the higher concentration regardless the conditions applied. Despite the toxicity of acetic acid to bacteria and yeast used for ethanol production has been proved to be relatively low compared to other components (Klinke et al. 2004), its relative high concentrations after LHW pretreatment makes it a relevant inhibitor. When comparing the inhibitory effect of formic, acetic and levulinic acids, at the same concentrations, on *S. cerevisiae* during fermentation of spruce hydrolysate, the first one showed the highest, followed by levulinic acid and lastly acetic acid (Larsson et al. 1999). It was suggested that the smaller molecular size of formic acid may facilitate its diffusion through the cell membrane, while levulinic acid might be more inhibitory than acetic acid due to its greater lipophilicity. However, acetic acid concentrations lower than 10 g/L inhibited partly or totally the ethanol production during fermentation with different feedstocks, microorganisms and conditions (Klinke et al. 2004) (Taherzadeh et al. 1997) (Ranatunga et al. 1997).

The influence of acetic acid on the fermentation step depends on several process factors, such as type of microorganism involved, cell mass concentrations, operational conditions and even the presence of another inhibitory compounds (Palmqvist and Hahn-Hägerdal 2000). In fact, a decrease in the apparent specific growth rate and ethanol yield from glucose was observed on *S. cerevisiae* when acetic acid and furfural were present (Palmqvist et al. 1999), outcome that was stronger when both compounds were together than the sum of their inhibitory effect individually.

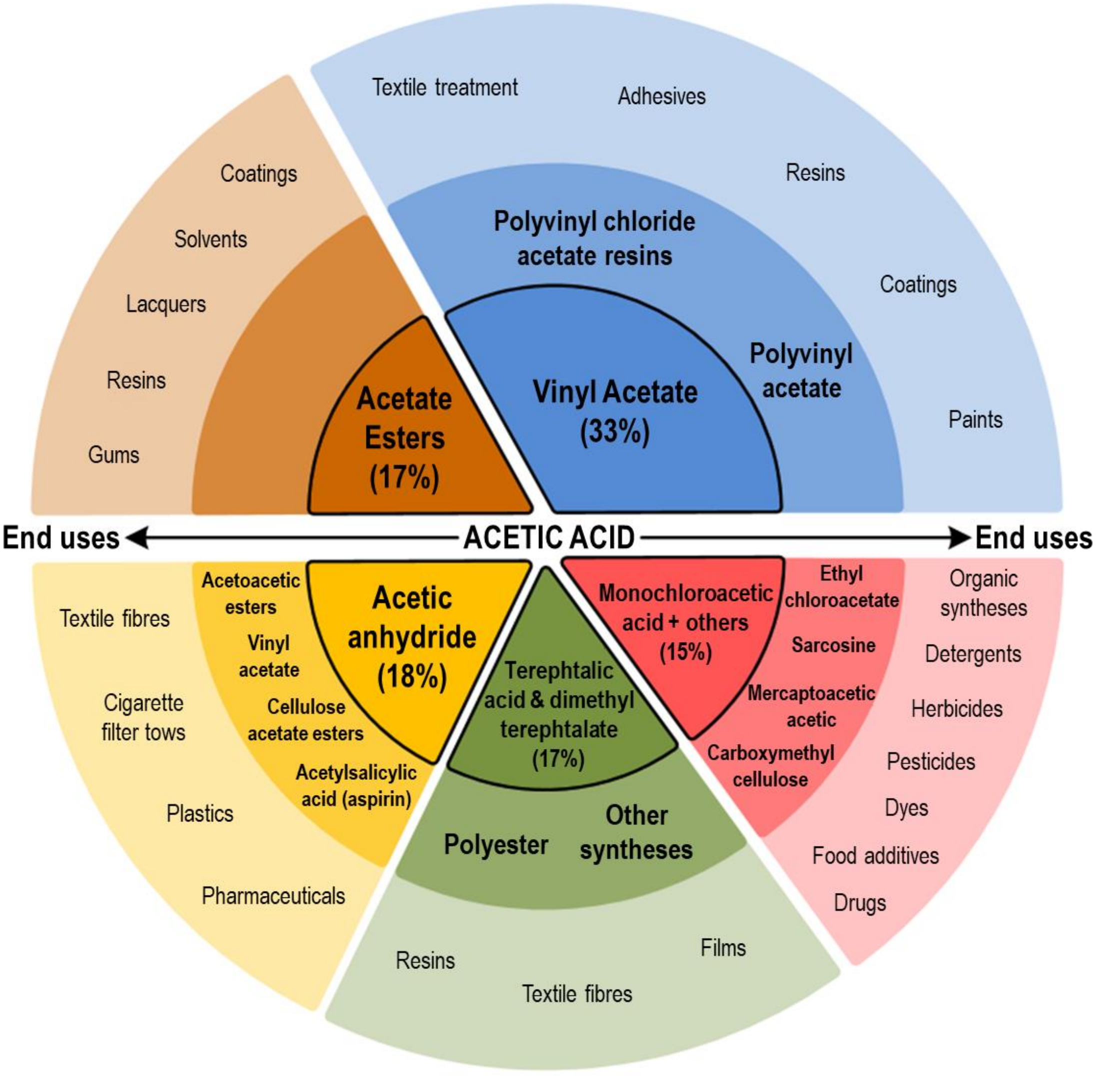

Figure 1.4. Breakdown of end uses of acetic acid worldwide. Own compilation based on 2010 data (Le Berre et al. 2014).

10. Acetic acid

As discussed previously, the production of acetic acid through hemicellulose depolymerisation during biomass pretreatment seems unavoidable. Therefore, an opportunity may rise by considering acetic acid as a potential side-product from the LCB fractionation.

Used directly or as a feedstock for the production of derivatives, acetic acid is a widely employed organic acid (Cheung et al. 2011). The largest applications (see Figure 1.4) of this weak acid are in the manufacture of vinyl acetate and acetic anhydride, as well as a process solvent for the production of terephthalic acid (Gunjan and Haresh 2020).

The global acetic acid market has reached a volume of 9.07 million tonnes in 2020, with a growing expectation of 30% by 2026 (EMR 2020). This continuously increasing trend contributes remarkably to consider acetic acid as an interesting and potential side-product to be obtained from an integrated biorefinery, improving the conventional biomass conversion process by creating this additional value (Agbor et al. 2011) (Stuart and El-Halwagi 2013).

11. Scope of this thesis and outline

Focusing on biobased processes and biorefinery, with sustainability and circular economy as driven forces, the present work aims to develop a process to improve the hydrothermal pretreatment of lignocellulosic biomass that allows recovery of acetic acid before enzymatic hydrolysis and fermentation.

In **chapter 2**, the use of pressurized CO_2 during LHW pretreatment is explored. At subcritical conditions, carbon dioxide reacts with water producing carbonic acid *"in situ"*, which consequently might decrease the pH and hence increase the hemicellulose hydrolysis reaction rate. Through the hemicellulose depolymerisation that occurs during the hydrothermal pretreatment of lignocellulosic biomass, the acetyl groups present in the hemicellulose structure are cleaved, releasing acetic acid as a result. Hence, the acetic acid concentration is used as indicator of the progress in hemicellulose degradation.

One of the key challenges regarding the development of a biobased processes is the scaling up phase. In **chapter 3**, LHW pretreatment method is compared at laboratory and pilot plant scale at equivalent conditions, with extra attention in using the same temperature profiles.

Consequently, a simplified model to describe this LHW pretreatment is proposed in **chapter 4**, using acetyl hydrolysis reaction as the progress indicator, and including the heating, cooling, thermal expansion and pH of the reaction mixture.

Dilute acetic acid is considered as an alternative pretreatment process of lignocellulosic biomass in **chapter 5**. The recirculation of the liquid fraction after the LHW pretreatment is also explored for longer processing times, as a comparable process to dilute acetic acid pretreatment.

In the final **chapter 6**, the potential production and recovery of acetic acid after LHW pretreatment and prior the enzymatic hydrolysis and fermentation is covered. Additionally, alternative pretreatment methods are compared with the selected LHW, such as steam explosion or supercritical CO_2.

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Chapter 2

Subcritical CO_2 shows no effect on liquid hot water pretreatment of poplar wood

Abstract

Pretreatment of lignocellulosic biomass is required for many biorefinery processes. Previous studies have described hydrolysis of hemicelluloses by using liquid hot water (LHW) pretreatment. We evaluated the effect of carbonic acid originating from pressurized carbon dioxide during LHW pretreatment of poplar. The conditions applied covered temperatures from 120 to 200 °C, pretreatment times from 5 to 240 min and pressures from 1.0 to 2.2 MPa CO_2 or N_2. The pressure and the type of gas (CO_2 or N_2) did not have an effect on production of acetic acid, which functioned as a marker of progress of biomass hydrolysis. Results suggested that the presence of carbonic acid in the process does not significantly contribute to acidification. Deacetylation of lignocellulosic biomass can be achieved by LHW pretreatment irrespective of pressure and of gas type used, at the conditions tested.

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1. Introduction

Considerable efforts have been made in the past decades towards investigating options to solve the fossil fuels depletion in combination with the increasing energy consumption and CO_2 emissions by our society (Goldemberg 2007). This situation has led to a path where biorefineries are promising options, picturing a scenario with industrial activities using bio-based sources and biological operations as main trend (Hassan et al. 2019).

Potential biomass sources are side streams from well-established processes, waste and excess production from agricultural activities, forestry, and crops grown specifically for biorefinery purposes (Kim and Dale 2004). Lignocellulosic biomass has been investigated intensively, with special interest in the disruption of its structure in order to increase the digestibility of the compounds present in its matrix (Kim and Holtzapple 2006, Hendriks and Zeeman 2009, Kumar and Sharma 2017).

Lignocellulosic biorefinery processes typically consist of four main parts: pretreatment, enzymatic hydrolysis, fermentation and purification. Due to the complex matrix and structure of lignocellulosic biomass, the pretreatment and enzymatic hydrolysis steps usually produce derived substances, apart from the desired and expected sugars, which could limit the activity of the microorganisms and reduces the efficiency during the fermentation (Liu et al. 2019).

In this paper, we focus on pretreatment, which hydrolyses hemicelluloses and improves accessibility to cellulose for subsequent enzymatic hydrolysis (Kumar et al. 2009). Several pretreatment methods have been extensively studied, involving different technologies (Mosier et al. 2005, Alvira et al. 2010). By using high temperatures and pressures in liquid hot water (LHW) pretreatment, it is possible to hydrolyse and dissolve hemicelluloses, while partially solubilising the lignin with almost no influence on the cellulose fibres (Garrote et al. 1999, Liu and Wyman 2005, Zhuang et al. 2016). Optionally, acids or other catalysts can be applied to shorten reaction time or decrease temperature and pressure conditions (Esteghlalian et al. 1997, Hu and Ragauskas 2012, Cybulska et al. 2013). For a green process, one would like to take out and reuse such catalysts. However, acid catalysts usually have to be neutralised to allow subsequent enzymatic hydrolysis, leading to alkali costs and to waste salts that can hardly be recovered. Therefore, using carbon dioxide as volatile (and hence recyclable) acid catalyst has been proposed as a green alternative pretreatment method, due to decrease on pH promoted by the production of carbonic acid (Magalhães da Silva et al. 2014, Morais et al. 2015).

The use of supercritical CO_2 has been extensively studied for pretreatment purposes (Kim and Hong 2001, Alinia et al. 2010, Luterbacher et al. 2010, Narayanaswamy et al. 2011). It has been reported that supercritical CO_2 easily penetrates cellulose such that explosion caused by depressurization increases accessible surface area in cellulose for enzymatic hydrolysis. This is an alternative to steam explosion, but requires no increase in temperature (Zheng et al. 1995). Compared to inert gases (nitrogen, helium), carbon dioxide showed more effective glucose yield when pretreating cellulose (Zheng et al.

1998). However, the use of supercritical CO_2 entails certain limitations at industrial scale due to the level of pressure utilized (Agbor et al. 2011).

At pressures below critical (7.4 MPa), CO_2 use can be considered a modification of LHW pretreatment. In the literature, however, there is no agreement on the influence of subcritical CO_2. Rogalinski et al. (2008) found no change in the solubilisation of lignocellulosic material by using CO_2 during LHW treatment. In addition, McWilliams and van Walsum (2002) also reported no different outcome on xylose recovery when carbonic acid was present during LHW treatment of aspen wood. On the other hand, other authors noticed enhancement of corn stover hydrolysis from higher values of xylose and xylan oligomers obtained in presence of CO_2 (van Walsum and Shi 2004), and even a kinetic model has been proposed for the hydrolysis of wheat straw in water at high pressure CO_2 (Relvas et al. 2015). One reason for these discrepancies is that experimental conditions varied, including the type of biomass used, and another is that the performance of pretreatment is judged from the concentration of unstable reaction products or from the performance of subsequent enzymatic hydrolysis.

To understand better the influence of CO_2 on LHW pretreatment, we aim to quantify pretreatment on basis of acetic acid formation. Acetic acid originates from cleavage of acetyl groups in hemicelluloses, and undergoes no subsequent reaction. Most of the previous studies on LHW pretreatment are focused on the monosaccharides release (mainly xylose) as preeminent indicator of the degradation of hemicelluloses (Laser et al. 2002, Negro et al. 2003, Mosier et al. 2005, Pérez et al. 2008). In this work, acetic acid is considered as predominant indicator of hemicellulose hydrolysis. It is a stable compound, and the main compound contributing to acidity in conventional LHW pretreatment.

For the current study, poplar wood was selected as relevant lignocellulosic biomass type, because it has been widely studied already and has a high acetyl content. Release of acetyl groups from hemicelluloses and depolymerisation of hemicellulose typically occur at 170-190 °C, whereas cellulose hydrolysis occurs at temperatures above 200 °C (Weil et al. 1997, Thomsen et al. 2006). Focusing on hemicellulose hydrolysis, and therefore acetic acid release, the conditions applied in this work during this hydrothermal pretreatment cover temperatures between 120 and 200 °C, pretreatment times from 5 to 240 min and subcritical pressures of CO_2 or N_2 up to 2.2 MPa. Compressed nitrogen is designated as inert gas, in order to provide a comparable reference and assess the possible catalytic effect of carbon dioxide on acetic acid release during LHW pretreatment.

2. Materials and methods

2.1. Materials

Acetic acid (≥ 99.7%) was from Sigma-Aldrich. The lignocellulosic material (poplar wood with stems and bark) was provided by Woodoo GmbH & Co. KG, through the

Bioprocess Pilot Facility B.V. Samples were pre-milled in a bench mill and sieved to a mesh size of ~1 cm.

2.2. *Pressurized reactor*

A pressurized reactor was used, which consists of a stainless steel 100 mL vessel with an electrical heating and an agitation system. The reactor was connected to independent inlet lines for CO_2 and N_2, and to two outlet lines for gas, one of them with a safety relief valve set at 2.2 MPa. The reactor is depicted in Figure 2.1.

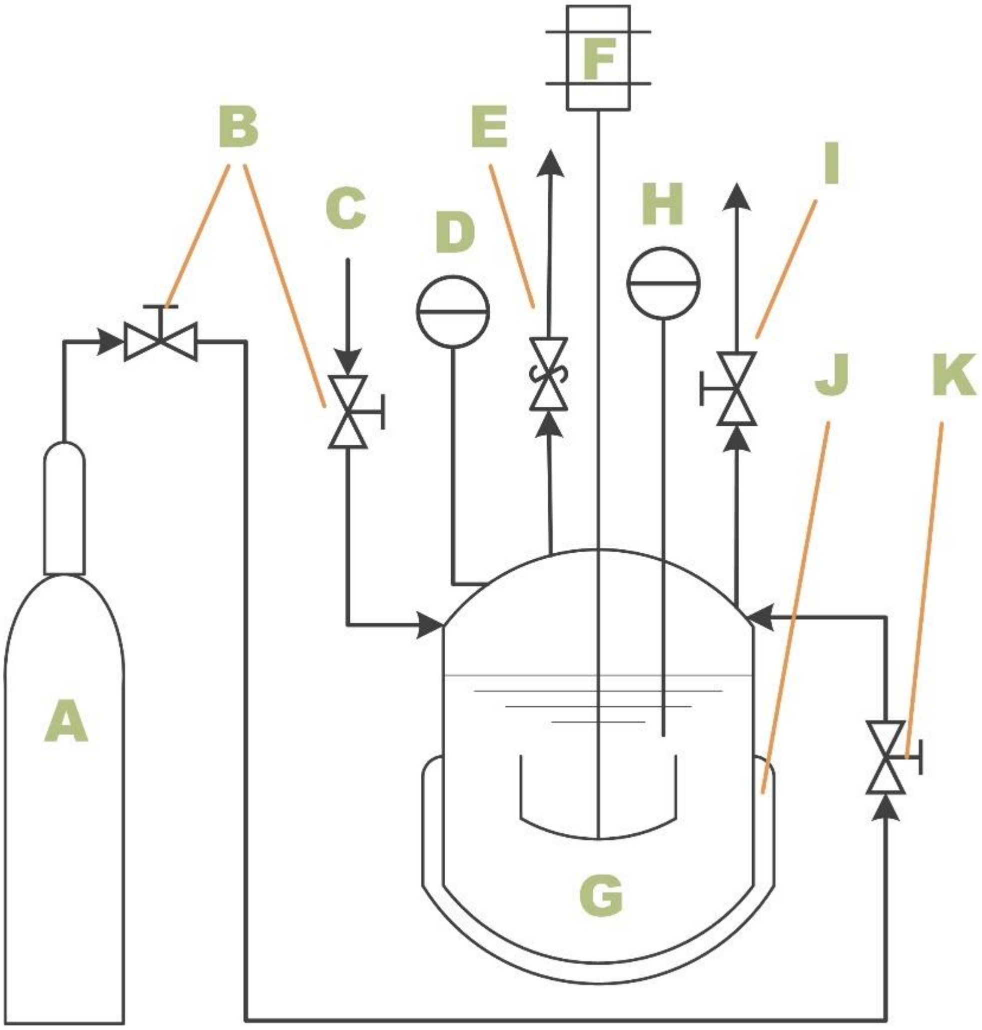

Figure 2.1. *Scheme of the pressurized reactor setup. A: Compressed CO_2; B: gas valves (for CO_2 and N_2); C: compressed N_2 line; D: pressure meter; E: pressure relief valve; F: motor for stirrer; G: pressurized reactor; H: temperature controller; I: outline gas valve; J: heating system; K: extra CO_2 valve.*

The reactor vessel was filled with 5 g of biomass (on wet basis) and 45 g of water. Insulation fabric was placed around. Before starting the experiments, all pipes and valves were shortly flushed with CO_2 or N_2, assuming negligible evaporation of water. Then, agitation was switched on and the pressure of gas (CO_2 or N_2) was progressively increased until the desired "initial pressure" was reached in the reactor vessel. After 30 min (estimated time to reach liquid/gas equilibrium), the heating system was switched on, increasing the temperature inside the vessel up to the set point, which increased the pressure, up to the "pretreatment pressure" value. The reactor was kept at the set temperature by the temperature controller during a time that will be indicated as "pretreatment time". Subsequently, the heating system was switched off and the insulating fabric was removed from the reactor, allowing a fan to speed up cooling of the vessel. Once the reactor was cooled to room temperature, the outline gas valve was opened to proceed with decompression. When ambient pressure was reached, the reaction suspension was centrifuged and filtered (Whatman 0.2 µm), and the pH of the liquid fraction was measured. Several liquid samples were taken and stored at -20 °C until further analysis by HPLC, while the solids were discarded.

2.3. *Analysis of samples*

Acetic acid was analysed on a Waters HPLC system with a Bio-Rad HPX-87H column (300 x 7.8 mm) at 59 °C. Phosphoric acid (1.5 mmol/L at 0.6 mL/min) was used as eluent. Quantification was by refraction index detection (Waters 2414) using external standards. Volumes of injection of standards and samples were 10 µL at 15 °C. All samples were analysed in duplicate for acetic acid content.

2.4. *Severity factor*

Overend et al. (1987) have proposed a severity factor (log R_0) to combine parameters applied to a pretreatment process:

$$R_0 = \exp\left(\frac{T - 100}{14.75}\right) \cdot t$$

T is the temperature in °C and t is the pretreatment time in minutes. This severity factor is used to represent the results of the present study.

3. Results and discussion

3.1. *Effect of severity*

During LHW treatment, in presence of CO_2 or N_2, different combinations of temperature and pretreatment time were applied to the lignocellulosic biomass, associated in the severity factor of the reaction conditions (see Table 2.1). Conditions applied covered severity values from 0.7 to 5.3 (log R_0). Figure 2.2A shows that with increasing severity the concentration of acetic acid increased up to approximately 5.3 g/L, regardless the gas used. This maximum acetic acid concentration was obtained at log R_0 of 5.17, corresponding to 200 °C and 170 min. For beech wood LHW pretreatment, Nitsos et al. (2013) reported a similar trend at a severity range (log R_0) between 2.0 and 5.0, obtaining up to 3.4 g/L acetic acid. Analogous results were also obtained by Morais et al. (2014) when processing wheat straw, although they included higher pressures of CO_2 (closer to the critical point) within a narrower range of severity.

Table 2.1. *Pretreatment conditions used during experiments for LHW of poplar.*

Experiment	Temperature, °C	Time, min	Pressure, MPa	Gas type	Severity (log R_0)
P10	180	50	1.75	CO_2	4.054
P11	180	40	1.80	CO_2	3.958
P12	180	100	1.64	CO_2	4.355
P13	180	130	1.26	CO_2	4.469
P14	180	140	2.08	CO_2	4.502
P15	180	110	1.07	CO_2	4.397
P16	180	120	2.05	CO_2	4.435
P17	180	70	1.88	CO_2	4.201
P18	180	30	1.99	CO_2	3.833
P19	180	107	0.95	CO_2	4.385
P20	180	90	0.97	CO_2	4.310
P21	200	90	1.51	CO_2	4.899
P22	200	240	1.50	CO_2	5.325
P23	200	170	1.50	CO_2	5.175
P24	120	10	0.38	CO_2	1.589
P25	140	5	0.48	CO_2	1.877
P26	160	5	0.67	CO_2	2.466
P27	144	54	0.48	CO_2	3.031
P28	100	5	0.28	CO_2	0.699
P29	159	5	0.66	CO_2	2.436
P30	160	140	0.75	CO_2	3.913
P31	160	25	0.74	CO_2	3.165
P32	140	20	0.52	CO_2	2.479
P12N	180	125	1.34	N_2	4.452
P13N	180	130	1.14	N_2	4.469
P14N	180	120	1.92	N_2	4.435
P15N	180	120	1.01	N_2	4.435
P16N	180	120	1.77	N_2	4.435
P17N	180	70	1.76	N_2	4.201
P18N	180	30	1.79	N_2	3.833
P19N	180	120	0.97	N_2	4.435
P20N	180	90	0.97	N_2	4.310
P21N	200	90	1.49	N_2	4.899
P22N	200	240	1.50	N_2	5.325
P23N	200	170	1.51	N_2	5.175
P24N	120	5	0.35	N_2	1.288
P25N	140	5	0.44	N_2	1.877
P26N	160	5	0.64	N_2	2.466
P27N	128	50	0.35	N_2	2.545
P28N	100	5	0.28	N_2	0.699
P30N	159	60	0.66	N_2	3.515
P31N	160	30	0.68	N_2	3.244
P32N	140	20	0.47	N_2	2.479

Furthermore, our results are in line with the absence of significant acetyl group release for LHW pretreatment of poplar below log R_0 values of 3.5 (Bouchard et al. 1991) and the trend observed for carbonated subcritical water pretreatment of switchgrass at higher temperatures (Dhamdere et al. 2012). Comparing results from this work with literature values of acetyl content of poplar (Kim et al. 2009) suggests complete deacetylation of poplar at values of log $R_0 > 5$. However, that was not confirmed since the structure and composition of the solid fraction of the pretreated biomass was not analysed in this work. At less severe conditions, some acetyl groups remained in the solid phase linked to hemicelluloses, while part of it was bound to xylo-oligosaccharides (Garrote and Parajó 2002, Kabel et al. 2007).

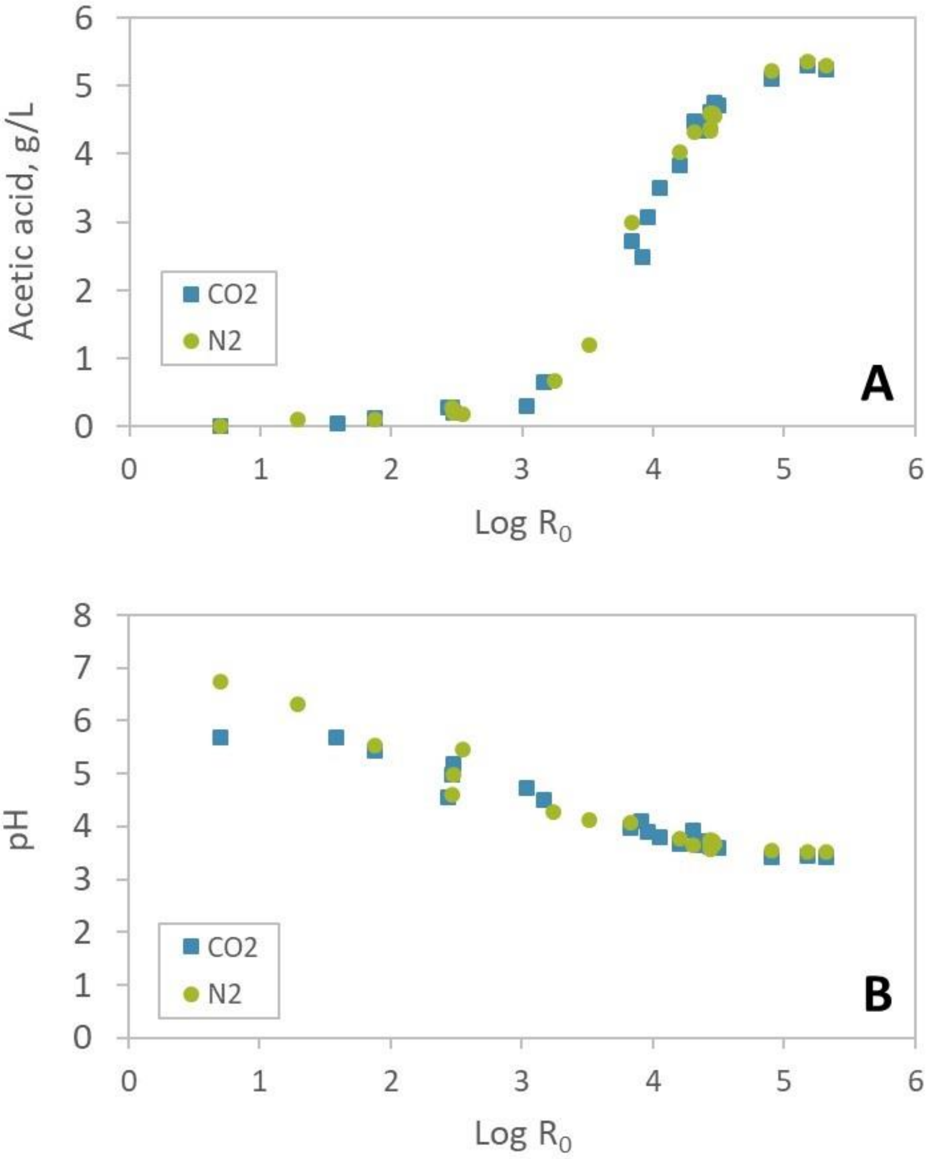

Figure 2.2. *Effect of severity on acetic acid release (A) and pH (B) for LHW pretreatment at pressures in the range from 1.01 to 2.08 MPa CO_2 or N_2.*

Like generally observed in studies on biomass pretreatment, higher severity promoted not only acetic acid release, but also release of other hydrolysis products such as xylose and glucose, and of their degradation products such as formic acid, furfural and 5-

hydroxymethylfurfural (Kim et al. 2009). Data for these other compounds are available in the supplementary material but will not be discussed, because the observed patterns are in line with those generally described in pretreatment literature and here. Still other components, such as lignin, were not measured, though.

3.2. *Effect of pressure and gas type*

Several initial subcritical pressure values of CO_2 and N_2 were applied (0.2, 0.4, 0.6, 0.8 and 1 MPa) at otherwise the same reaction conditions: 180 °C during approximately 2 h (log $R_0 \approx 4.4$). Pressures increased once the reactor was heated up, slightly more in case of CO_2 than in case of N_2, while the concentrations of acetic acid obtained in all these experiments were similar (see Figure 2.3A). This outcome was opposite to those of some other studies in which hydrolysis of biomass increased in the presence of CO_2, although for different biomass types and at higher but still subcritical pressure values (van Walsum and Shi 2004, Zhang and Wu 2013, Morais et al. 2015, Relvas et al. 2015). Figure 2.3A shows that the gas type (CO_2 or N_2) and its pressure during pretreatment have no significant effect on the hydrolysis of poplar.

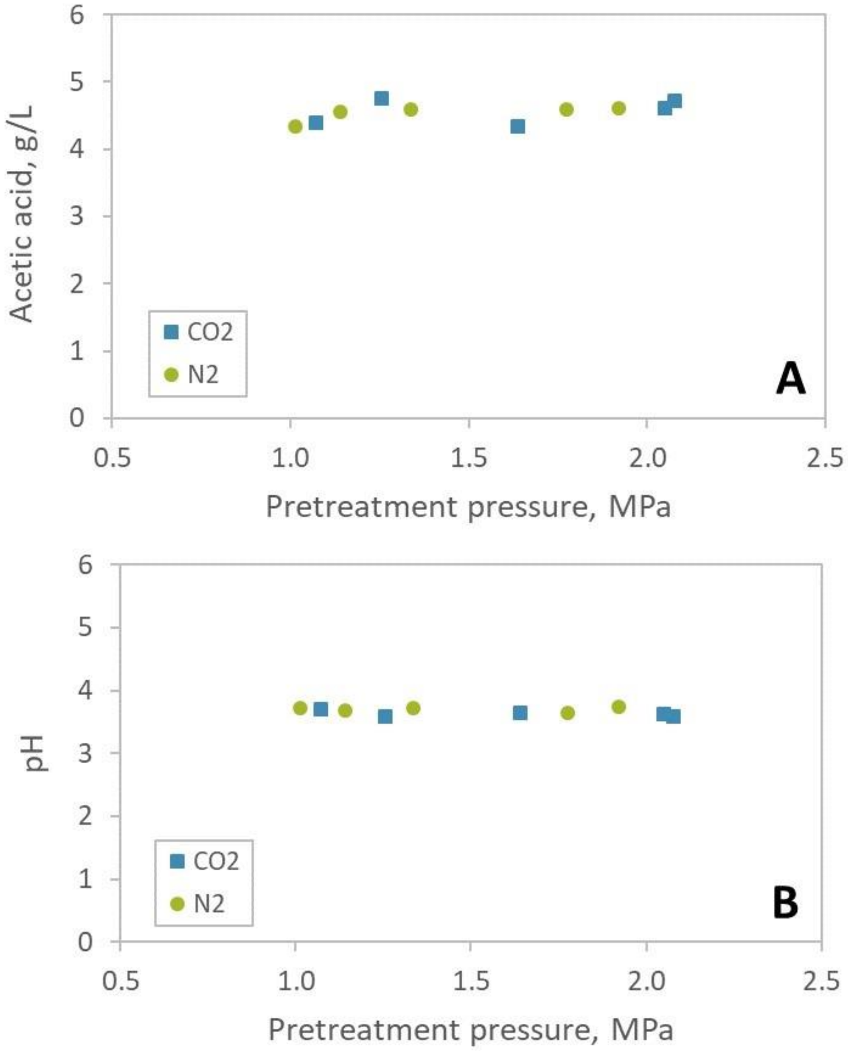

Figure 2.3. *Effect of pressure and gas type on acetic acid release (A) and pH (B) for LHW pretreatment performed at 180 °C and approximately 120 min (log* $R_0 \approx 4.4$*).*

3.3. *Effect on pH*

Figure 2.2B shows a decrease in pH with severity, as expected on basis of increased carboxylic acid amounts at higher severity (Jacobsen and Wyman 2002). These values of pH were measured at room temperature, after cooling and filtering the liquid samples. Again, no difference is observed between using carbon dioxide and nitrogen during pretreatment, and pressure has no effect (Figure 2.3B).

During LHW pretreatment of lignocellulosic biomass, the auto-ionization of water produces hydronium ions, which, in combination with the high temperatures, provokes hydrolysis of hemicelluloses, and therefore cleavage of acetyl groups, producing acetic acid. This acetic acid, and other carboxylic acids released such as uronic acids, contribute to the hydrolysis reaction, widely known as autohydrolysis (Garrote et al. 2002, Gírio et al. 2010). In addition, those carboxylic acids contribute to subsequent carbohydrate degradation towards other side-products (Mosier et al. 2005). Although acetic acid is not yet present in the beginning of pretreatment, in previous studies of hydrothermal treatment, the hydronium ions generated from acetic acid were considered more important than those of water origin (Garrote et al. 1999, Gírio et al. 2010). The use of carbon dioxide during this process has been mentioned to enhance the hydrolysis by further lowering the pH due to the formation of carbonic acid (Luterbacher et al. 2012, Magalhães da Silva et al. 2014, Morais et al. 2015). Some authors suggested that the autocatalysis due to release of acetyl groups has similar influence as the presence of carbonic acid (McWilliams and van Walsum 2002), while other researchers suggested that effect of dissociation of carbonic acid in water combined with the release of acetic acid from deacetylation of hemicelluloses is crucial for the hydrolysis of lignocellulosic biomass (Gurgel et al. 2014). At our conditions, CO_2 clearly plays no role in acidification.

At ambient conditions, an aqueous solution of 5 g/L acetic acid is at pH 3.0, according to $pK_a = 4.82$. The minimum pH in Figure 2B is 3.4, corresponding to 0.5 g/L acetic acid and thus indicating the presence of buffering compounds. Their source might be minerals or proteins originating from the biomass. Other authors (van Walsum and Shi 2004, Morais et al. 2014) found somewhat higher final values of pH, probably because their biomass contained less acetyl groups or more buffering compounds. At final (ambient) conditions, carbon dioxide does not contribute to low pH because of its low solubility. At 225 °C and 5.4 MPa initial CO_2 pressure, it has been calculated that the pH of an aqueous CO_2 solution is 3.77 (van Walsum and Shi 2004), suggesting that acetic acid is a stronger contributor to low pH than CO_2.

As a consequence, improvement of lignocellulosic biomass pretreatment should occur due to addition of acetic acid prior to LHW pretreatment. However, the literature does not report a clear picture on this (Laser et al. 2002). Therefore, further research regarding the effect on acetyl group cleavage on hemicelluloses by initial addition of acetic acid to LHW pretreatment of lignocellulosic biomass could provide deeper understanding of this methodology. Besides, systematic studies of different reaction conditions and different biomass types might reveal why different studies find different effects of CO_2.

4. Conclusions

Liquid hot water pretreatment at log $R_0 > 5$ is an efficient methodology for deacetylation of hemicelluloses from poplar. Acetic acid released was proportional with the severity of the process but it has no direct relation with the pretreatment pressure up to 2 MPa, nor with the gas used for achieving this pressure. At the conditions applied, subcritical CO_2 does not affect acetic acid formation. Its role in acidification, even associated to the initial phase of pretreatment, is negligible. At different reaction conditions and with different types of biomass than tested in this work, CO_2 might still improve pretreatment.

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Chapter 3

Liquid hot water pretreatment of lignocellulosic biomass at lab and pilot scale

Abstract

Liquid hot water pretreatment is considered to be a promising method for increasing biomass digestibility due to the moderate operational conditions without chemicals addition. A necessary step towards the scalability of this pretreatment process is performing pilot plant trials. Upscaling was evaluated with a scaling factor of 500, by using 50 mL in the laboratory and 25 L in a pilot plant batch reactor. Pretreatment times were varied from 30 to 240 min, and temperatures used were 180-188°C, while applying similar heating profiles at both scales. The initial mass fraction of poplar wood chips ranged from 10% to 16%. Liquid hot water pretreatment at laboratory and pilot scale led to analogous results. The acetic acid analysis of the liquid and solid fractions obtained after pretreatment indicated that complete deacetylation of poplar biomass can be achieved.

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1. Introduction

The continuous increase in the greenhouse gas emission and the depletion of fossil carbon sources reinforce a transition from oil-based towards bio-based production processes. This motivates extensive research on development of production of bio-chemicals, bio-energy and bio-materials from renewable sources (Gnansounou 2010). The use of lignocellulosic biomass as main source for these biorefinery processes is widely accepted, especially because second generation feedstock does not compete with the food supply chain and in many cases even increases sustainability of food production (Souza et al. 2015).

Pretreatment of biomass is one of the key technologies in bio-based industry, and it has been extensively studied due to the complexity of the internal structure of the lignocellulosic material (Hassan et al. 2018, Rezania et al. 2020). Different methods have been applied, which usually focus on enhancement of the availability of carbohydrates for subsequent enzymatic hydrolysis and fermentation to bio-fuels and bio-based chemicals (Ahmed et al. 2019).

Various compounds are released during the deconstruction of lignocellulosic biomass from pretreatment. Pentoses as well as hemicellulosic hexoses are obtained from hemicellulose hydrolysis. Depending on the pretreatment, cellulose- and lignin-derived monomers and degradation products are also found in significant concentration after the pretreatment step (Behera et al. 2014). Furfural and 5-hydroxymethylfurfural (HMF) are produced from degradation reactions of the C-5 and C-6 monosaccharides, respectively, while acetic acid is generated from the cleavage of acetyl groups, present in the hemicellulose structure up to 5% dry weight (Johnson et al. 2017). Whereas HMF can further degrade, to formic and levulinic acid, for example, furfural might also decompose, to formic acid and other molecules (Almeida et al. 2009). The severity of the pretreatment increases the extent of carbohydrate release but also the extent of these degradation reactions (Kabel et al. 2007, Pedersen and Meyer 2010).

Most of these side-products are considered inhibitors for a wide range of microorganisms during fermentation, although they are interesting building blocks for bio-based chemicals themselves (Ko et al. 2015). Therefore, knowing the presence and proportion of the most relevant side-products after pretreatment might help the design of an efficient bioprocess, as further strategic steps could be taken into account accordingly (Jönsson and Martín 2016).

Among the physical, chemical, physicochemical and biological pretreatment methods of biomass, liquid hot water (LHW) pretreatment is one of the most attractive procedures, because it involves no addition of chemicals and relative moderate operational conditions (Pérez et al. 2008). In contrast, large amounts of water and energy might be required, leading to diluted products and large pieces of equipment (Moreno et al. 2019).

This hydrothermal treatment, also known as autohydrolysis of biomass, involves addition of water and increase of temperature. This causes a decrease of pH in the system due to the ionization of water and the release of carboxylic acids (mainly acetic acid), in combination with the effect of the high temperature and pressure applied (Cybulska et al. 2013, Xiao et al. 2017). This autohydrolysis leads to the disruption of the lignocellulosic biomass structure, improving the accessible surface area, and therefore enhancing the subsequent enzymatic hydrolysis (Garrote et al. 2007, Kim et al. 2009, Lee et al. 2009, Tan et al. 2020) and fermentation steps (Kucharska et al. 2018).

1.1. Hydrothermal pretreatment at pilot scale

Most previous studies have focused on LHW pretreatment at laboratory scale, and generated a substantial amount of laboratory data (Hongdan et al. 2013, Li et al. 2017, Feng et al. 2019). Implementation of industrial biorefinery processes entails an appropriate scaling-up procedure, to achieve a successfully bioprocess design at industrial level, where a pilot plant is still a "must-have" step in the scalability of the process.

According to Harmsen et al. (2018), a pilot plant scale process might be performed for many different reasons, such as provision of reliable stream compositions and insight in realistic process conditions. Hydrothermal pretreatment methods have been previously studied and modeled at pilot scale, for a wide range of conditions and settings, including continuous tubular reactors (Petersen et al. 2009, Sievers and Stickel 2018, Rodríguez et al. 2019). Recently, Ruiz et al. (2020) reviewed hydrothermal pretreatment strategies at pilot scale, including biomass structure changes, reactor technologies and engineering from batch to continuous.

Rocha et al. (2015) compared pilot scale steam explosion (200 L) of sugarcane bagasse with dilute acid pretreatment, using 1% (w/v) H_2SO_4 in a 350-L batch reactor at 120 °C and 1:10 solid-liquid ratio during 10 min, the former method being more efficient at the tested conditions, with higher hemicellulose solubilization and further cellulose conversion. The same 350 L reactor was used for pilot alkaline pretreatment (Nakanishi et al. 2018) and also to obtain Enzymatic Hydrolysis Residue Lignin by hydrothermal pretreatment of sugarcane bagasse, processed at 190 °C for 10 min with 1:10 solid-liquid ratio (Menezes et al. 2016).

For the conversion of lignocellulosic biomass into biofuels and a potential scale-up at pilot plant, Rossner and Parra (2017) evaluated different pretreatment methods for eucalyptus wood at the laboratory. Autohydrolysis or hydrothermal pretreatment performed better than organosolv and steam explosion. They pretreated *Eucalyptus globulus* wood chips in an 88 L packed bed reactor at 190 °C with 20% solid, obtaining low concentrations of degradation products such as formic acid, levulinic acid, furfural and HMF (values not reported).

LHW pretreatment at pilot scale has been tested also by applying different temperatures (185, 190 and 195 °C) during 10 min to pretreat sugarcane bagasse in a 20 L batch reactor, leading to a high extent of solubilization of hemicellulose and providing a reduction in the recalcitrance of the lignocellulosic material (Silva et al. 2011).

Krátký et al. (2012) processed wheat straw, among other lignocellulosic materials, in an 8 L batch pressure vessel by applying LHW pretreatment with expansion for testing pilot biogas production. They obtained promising results in biogas production by increasing temperature and residence time during pretreatment, although there was no analysis of degradation compounds and their possible effect on the anaerobic digestion.

Nascimento et al. (2017) performed hydrothermal pretreatment at laboratory and pilot scale. They pretreated sugarcane bagasse at 190 °C for 10 min, but performed steam explosion at pilot and LHW pretreatment at laboratory, including significant differences during the heating phases. Mosier et al. (2005) compared continuous LHW pretreatment of corn fibers at 160 °C for 20 min at lab and pilot scales, using direct injection of steam as heating source at the laboratory, while using indirect heat exchanger instead at larger scale, to avoid dilution and reduce energy consumption. Aguilar et al. Aguilar et al. (2018) also evaluated the hydrothermal pretreatment of agave bagasse for bioethanol production, using the severity factor as scalability factor from 125 mL to 750 mL.

Thus, studies on LHW pretreatment process for lignocellulosic biomass have been performed at larger scale, but systematic comparison to lab scale, at similar composition and heating profile, has not been done yet.

In order to improve the comparability between scales, as mass and heat transfer depend on scale and stirring conditions, it is important to have similar temperature profiles.

In this work, we try to perform the LHW pretreatment process of poplar biomass at pilot plant scale and laboratory at equivalent conditions, with special interest in using the same temperature profile, in order to confirm the scalability of the process by a factor 500 and the possible optimization at laboratory scale. Poplar wood was selected as biomass source, as in a preceding study (Jimenez-Gutierrez et al. 2020). Typical conditions for LHW pretreatment include temperatures around 200 °C with a residence time of a few minutes (Lee et al. 2009). To obtain sufficient hemicellulose degradation, the selected pretreatment conditions in this work were in the temperature range of 180-200 °C and residence (contact) time range of 30-240 min.

2. Materials and Methods

2.1. Materials

Acetic acid (≥ 99.7%), furfural (≥ 99%), 5-hydroxymethylfurfural (HMF) (≥98%) and xylose (≥99%) were from Sigma-Aldrich. D-Glucose (≥99.5%) was from Merck. Formic acid (≥98%) was from Honeywell Fluka. The lignocellulosic material (poplar wood with stems and bark) was provided by Woodoo GmbH & Co. KG, Grunow-Dammendorf, Germany, through the Bioprocess Pilot Facility B.V, Delft, the Netherlands. Samples were pre-milled in a bench mill and sieved to a mesh size of ~1 cm (moisture mass fraction of 10.8%).

2.2. *Pilot plant pretreatment*

The pilot scale experiments were carried out at the Bioprocess Pilot Facility (BPF). The equipment consisted of a 50 L batch autoclave (Büchi AG, Uster, Switzerland) with approximate inner dimensions Ø 38 cm diameter and 45 cm height. It was provided with a thermic oil-filled jacket connected to an automatic temperature controller, an agitation system with anchor blade stirrer and an inlet line of compressed nitrogen gas. The temperature sensor was positioned parallel to the stirrer axis at 9 cm from the reactor wall and 15 cm from the reactor bottom.

The autoclave vessel was filled with 2.5 kg of poplar chips (including 10.8% moisture content) and then 22.5 kg of water were added (except for the experiment at higher biomass/water ratio, in which 2 kg of biomass were filled together with 10.5 kg of water). The mixture was stirred manually for few minutes and then the vessel was lifted and screwed to the autoclave base. Automatic stirring with the anchor impeller was then switched on at 130 rpm. The oil jacket was switched on to start the heating after flushing the reactor head space with N_2 gas three times (between 0.2-0.4 MPa), with the purpose of avoiding other hydrothermal mechanisms, such as wet oxidation (Palonen et al. 2004).

Temperatures of oil bath and the inside of the reactor were monitored and used by the automatic control system to follow the sequence described below. First, the oil bath was heated to its maximum (200 °C), to achieve the shortest heating phase possible (generally around 80-90 min). Once the inside of the vessel reached 180 ± 2 °C (except for the experiments performed at a pretreatment temperature of 188°C), the pretreatment phase was started by switching the control system to oil bath to keep the temperature inside the reactor at this setpoint. At the end of the pretreatment phase (after 30, 120, 180 or 240 min), the pressure was recorded and the oil bath temperature was automatically decreased, allowing the reactor to cool down. When room temperature was reached inside the reactor, the autoclave was opened. Then, the suspension was filtered in a pilot sieve, and the wet solids and liquid fractions were weighed. Samples of both fractions were stored at -20 °C until further analysis.

2.3. *Laboratory pretreatment*

At lab scale, a pressurized reactor was used as described in Jimenez-Gutierrez et al. (2020), which consists of a stainless steel 100 mL vessel with an electrical heating and an agitation system. The reactor was connected to an inlet line for N_2, and to two outlet lines for gas, one of them with a safety relief valve set at 2.2 MPa.

The reactor vessel was filled with 5 g of biomass, including its 10.8% moisture content, and 45 g of water (except for the experiment at higher biomass/water ratio, in which 5.25 g of biomass was used with 29.75 g of water). Insulation fabric was then placed around. Before starting the experiments, all pipes and valves were shortly flushed with N_2, assuming negligible evaporation of water. Then, the agitation and the heating system were switched on, increasing the temperature inside the vessel up to 180 °C (except for the experiment performed at pretreatment temperature of 188 °C), which increased the pressure, up to the "pretreatment pressure" value (see supplementary material). The reactor was kept at the set temperature by the controller during the pretreatment time (30, 120, 180 and 240 min). Subsequently, the heating system was switched off and the insulating fabric was removed from the reactor, allowing a fan to speed up the cooling of the vessel to room temperature. When ambient conditions were reached, the reaction suspension was filtered (Whatman 0.2 µm), and the pH of the liquid fraction was measured. Wet solid and liquid fractions were weighed to calculate the percentage of total mass loss due to handling (see Table 3.S3, supplementary material), as the difference from the total mass initially added to the reactor (biomass + water) and the total amount obtained after LHW pretreatment (solid + liquid fractions). Several liquid samples were then taken and stored at -20 °C until further analysis by HPLC. For the laboratory experiments, the solids were washed twice with 100 mL of demi-water, dried overnight in an oven at 105 °C and weighed. For the pilot scale, not all solids were used for the analytical procedure but only samples of the solids. Samples of the washing liquid, as well as the washed and dried solids were stored for further analysis.

2.4. *Analysis of samples*

Solid samples were analyzed following a slightly modified methodology based on the NREL (National Renewable Energy Laboratory) laboratory analytical procedure (LAP) for determination of structural carbohydrates in biomass (Sluiter et al. 2008). A two-step acid hydrolysis process was carried out. Firstly, 1.5 g of the dried, milled and sieved solids (180 µm mesh size) were incubated with a 72% w/w solution of H_2SO_4 during 60 min at 30 °C and then, after dilution to 4% w/w, the mixture was autoclaved for 1 h at 121 °C. Once room temperature was reached, calcium carbonate was added to an aliquot of the filtered liquid (Whatman 0.2 µm) to neutralize the acid until pH reached 6-7. The neutralized liquid samples were filtered again and analyzed by HPLC as described below. Lignin and ash content were not determined in this work.

Liquid samples were analyzed on a Waters HPLC system with a Bio-Rad HPX-87H column (300 x 7.8 mm) at 60 °C. Phosphoric acid (1.5 mmol/L at 0.6 mL/min) was used as eluent. Glucose, xylose, acetic acid, furfural and HMF were detected by refraction index (Waters 2414), while formic acid was detected using UV (Waters 2489) at 210 nm. Quantification was done using external standards. Volumes of injection of standards and samples were 10 µL at 15 °C. All samples were analyzed in quadruplicate. After statistical analysis of these data, in most of the cases, the values of each analysis showed less than 5% of relative standard deviation, and in many cases lower than 1%. The small error bars have not been displayed in the graphical representation of the results to facilitate readability.

3. Results and Discussion

3.1. *Heating profiles*

As previously indicated (Nabarlatz et al. 2004), the LHW pretreatment method follows a semi-isothermal profile. The temperature profile is divided in three parts: heating phase, pretreatment phase and cooling phase. The temperature profiles that we obtained at lab and pilot scale were equivalent during the pretreatment and cooling phase. Only small differences were observed during the heating phase (see Figure 3.1 and Figure 3.S1 in supplementary material). Hydrolysis of hemicellulose typically occurs at 170-190 °C, whereas cellulose hydrolysis typically occurs above 200 °C (Weil et al. 1997, Thomsen et al. 2006). Therefore, the major part of the hydrolysis is assumed to happen during the (isothermal) pretreatment phase. However, some hemicellulose hydrolysis may occur during the last part of the heating phase, named the "active heating", while in the cooling phase the temperature drops quickly such that hydrolysis during this phase is considered negligible compared to the other two phases. Assuming an active heating from 160 °C up to the pretreatment temperature, within this range the pilot temperature was on average 3.8 °C higher than the laboratory temperature. In case of Figure 3.1, the duration of this active heating was 30 min, which was 4 times lower than the total pretreatment time. In other cases, the pretreatment time was shorter. Consequently, the experiments performed with 30 min pretreatment time may be slightly "overestimated" for the pilot plant. However, for the rest of the pretreatment times used, both profiles are considered analogous, facilitating the comparison of scales.

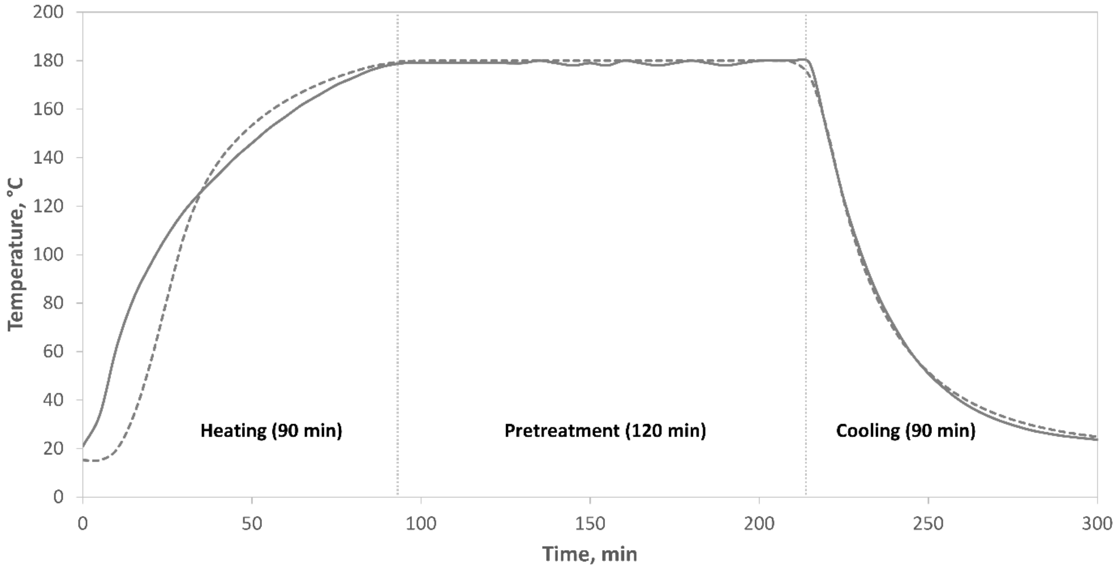

Figure 3.1. *Temperature profiles at laboratory (—) and pilot plant (- - -) scale during liquid hot water pretreatment of poplar at 180 °C and 120 min.*

Nevertheless, it was observed that the temperature profiles for the "higher temperature" experiments (188 °C pretreatment temperature) from lab to pilot did not fully match (see supplementary material). It took longer to heat the reactor to the set temperature at pilot scale (~120 min) than at lab scale (~60 min). This happened probably because there was a shorter temperature difference between the inside of the reactor and the temperature of the heating source (thermal oil) at the pilot plant, limited to a maximum of 200 °C, while in the lab, the heating source reached higher values (~260 °C). Consequently, despite keeping the same pretreatment times at both scales, the doubled heating time at pilot scale probably increased the extent of reaction during the active heating.

It is important to point out that heating is equipment dependent, and therefore heating profiles depend on scale (Negro et al. 2003) unless they are controlled at both scales like in this paper.

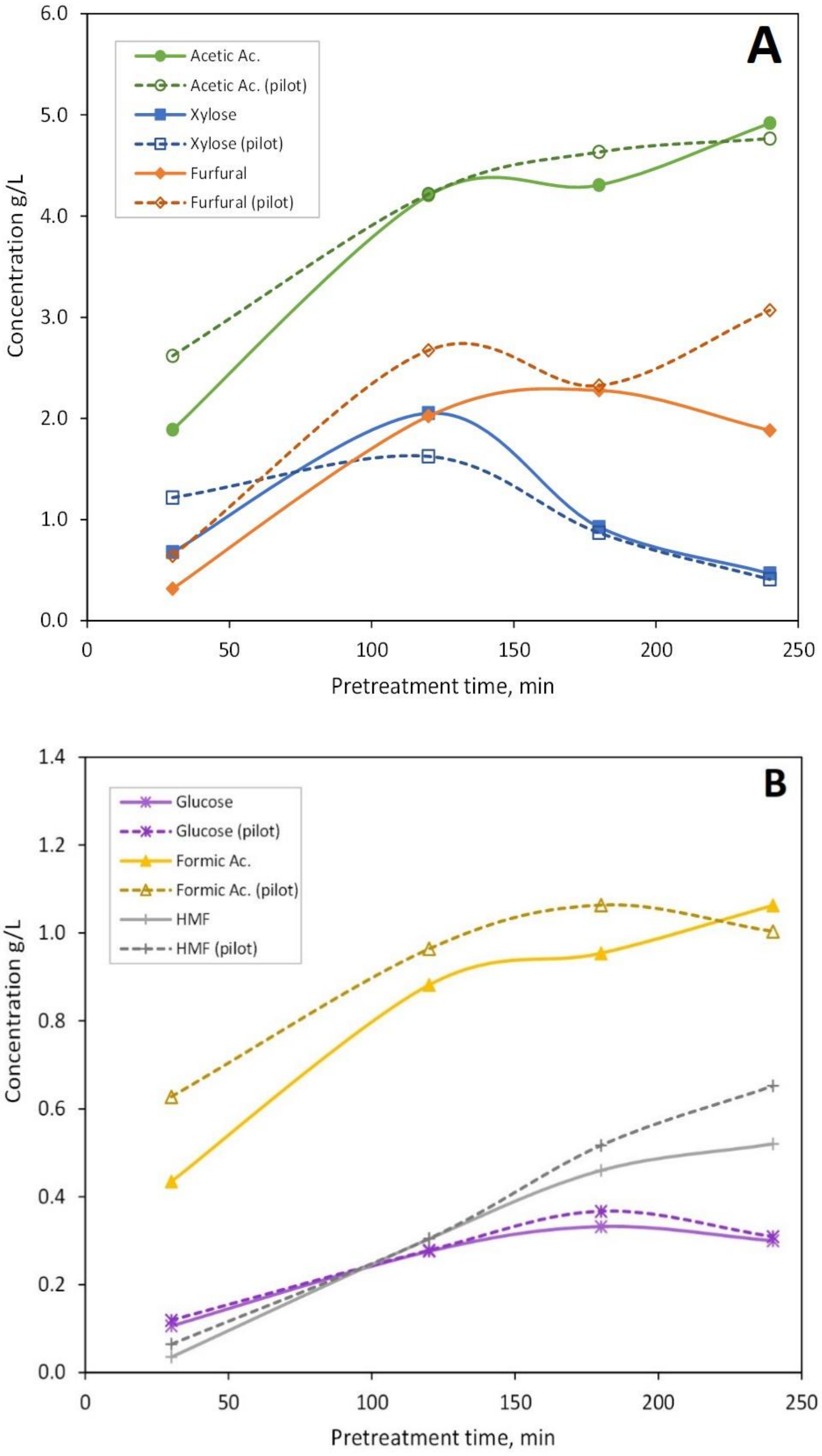

Figure 3.2. *Concentration of compounds released by LHW pretreatment at lab scale (closed markers) and pilot scale (open markers) at 180 °C. The lines describe the trend. A: Acetic acid, xylose and furfural. B: Glucose, formic acid and HMF.*

3.2. *Effect of time*

Results from analysis of the liquid fraction samples after LHW pretreatment at 180 °C and different pretreatment times at laboratory and pilot scale are comparable, as shown in Figure 3.2A, B. Slightly higher concentrations of carbohydrate degradation products (HMF, furfural) are obtained at the pilot scale (from 45-50% more at 30 min pretreatment time, to 20-40% at 240 min, respectively). In addition, formic acid concentrations were generally higher at pilot scale. However, increased carbohydrate degradation is not reflected by lower xylose and glucose concentrations. The concentrations of xylose and glucose reached a maximum point from which their degradation overtook their release by hemicellulose hydrolysis. Such maximum points are generally seen in literature, also when conditions differ somewhat (Hongdan et al. 2013, Aguilar et al. 2018).

Figure 3.3A, B represent the glucan and xylan content of the solids remaining from the same experiments, considering glucan as cellulose representative and xylan as hemicellulose representative. Corrected to the anhydrous form of glucose and xylose respectively, these values are calculated taking into accounts the mass loss due to handling/processing (Table 3.S3, supplementary material) and the raw/pretreated biomass ratio in dry basis, with a moisture content of 10.8% for the raw material. The fraction of glucan in the pretreated solids seems to increase slightly, indicating that most of the cellulose remained intact in the solid fraction, whereas the hemicellulose was progressively dissolved, which is in line with literature (Li et al. 2017) and with the data obtained from the analysis of the liquid.

In general, the LHW pretreatment might seem to be slightly faster at pilot scale as compared to lab scale. However, given the analytical error threshold and the relative small difference in the values from both scales, it would be more realistic to claim that the concentration of the compounds analyzed followed similar trends with increasing the pretreatment times, and therefore concluding that LHW pretreatment of poplar at the used laboratory and pilot scale behaved in the same way. Besides experimental error, small gradients inside the pilot vessel and small differences between lab and pilot scale procedures may explain the minor differences in concentrations between pilot and lab. In line with this, also the pH values measured from the liquid fraction after pretreatment were in a similar range for both scales (see Figure 3.S1, supplementary material); additionally, no significant difference at the surface of the pretreated material were observed at the microscope (see Figure 3.S3, supplementary material), supporting analogous performance at lab and pilot scales. Besides the compounds analyzed, there are numerous other compounds produced, such as humins and pseudo-lignin. We assume that reaction conditions that do not influence the concentrations of analyzed compounds also do not influence the concentrations of non-analyzed compounds, because of constraints imposed by mass balances reaction kinetics. Therefore, concentrations of non-analyzed compounds are assumed to be similar at lab and pilot scale, although deviations cannot be excluded.

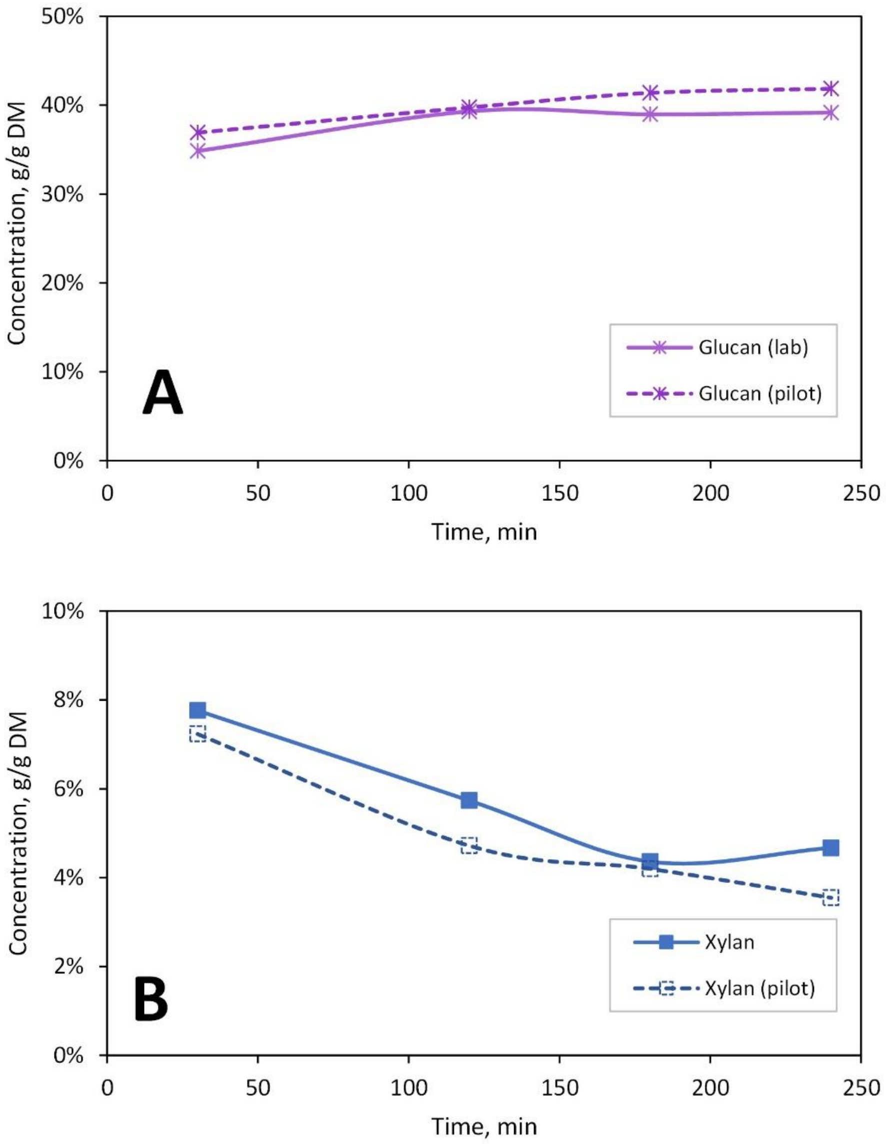

Figure 3.3. *Concentration of glucan (A) and xylan (B) in the solid fraction analyzed after LHW pretreatment at labs scale (closed markers) and pilot scale (open markers lines) at 180 °C, performed at different pretreatment times.*

The absence of scale effects suggests that mass and heat transfer are not limiting at the conditions and scales used. Hence, lab tests can be used for proper optimization of LHW pretreatment, before switching to the used pilot scale. For even larger scales, mass and heat limitations might still occur and lead to deviations from lab scale results.

3.3. *Temperature effect*

When pretreating at higher temperature, in general more hydrolysis of biomass is expected. Concentrations of different compounds of the liquid fraction after LHW pretreatment of poplar at two different temperatures are shown in Table 3.1. More glucose was obtained at 188 °C than at 180 °C, as more cellulose hydrolysis occurs at higher temperatures (Thomsen et al. 2006). Additionally, a larger quantity of HMF was found, though still in very dilute amounts. Processing at higher temperatures is generally known to lead to a higher HMF concentration (Weil et al. 1997). Regarding hemicelluloses, a considerable difference in the dissolved xylose concentration was observed when comparing these two temperatures. The LHW process at 188 °C reduced the xylose concentration, at first sight due to formation of degradation compounds such as furfural. However, the amount of furfural produced is not proportional to the lower xylose concentration (on basis of a 1:1 stoichiometry in the degradation reaction) (Mayr et al. 2015) but lower, in case of both lab and pilot scale. This phenomenon can be explained by thermal degradation of furfural, which is also enhanced at higher temperatures (Danon et al. 2013), and contributes to the formation of other degradation compounds, such as formic acid. Nevertheless, the concentration of formic acid just slightly increased at higher temperatures, following the trend observed previously (Jimenez-Gutierrez et al. 2020), suggesting that most furfural degradation products were not analyzed in this work.

The absolute value of the difference between lab and pilot scale is larger for the concentrations in the case of pretreating at 188 °C (19%) than at 180 °C (13%). This may be explained by the larger difference at 188 °C between lab and pilot experiments with respect to the heating profiles, as described in section 3.1.

The relatively severe heating in the pilot pretreatment at 188 °C led to more degradation (xylose being 30% lower, HMF 21% higher, and furfural 20% higher than in the lab). Others (Nascimento et al. 2017) found less degradation at pilot than at lab scale when pretreating at 190 °C. This could be explained by their heating profiles, which were very different from lab to pilot, being 90 min longer at lab scale. Values of the solid fraction composition at 188 °C pretreating temperature are in line with those found in literature (Menezes et al. 2016) when using a 350 L batch reactor to obtain Enzymatic Hydrolysis Residual Lignin from sugarcane bagasse at 190 °C during a shorter time.

***Table 3.1**. Concentration of different compounds in the liquid fraction after LHW pretreatment of poplar at two temperatures, both at lab and pilot scale, with 120 min pretreatment time and 10% initial biomass fraction.*

Scale	Temperature (°C)	Glucose (g/L)	Xylose (g/L)	Formic acid (g/L)	Acetic acid (g/L)	HMF (g/L)	Furfural (g/L)
Lab	180	0.28	2.05	0.88	4.20	0.30	2.02
	188	0.33	0.56	0.97	4.40	0.54	1.80
Pilot	180	0.28	1.62	0.96	4.22	0.31	2.67
	188	0.31	0.39	1.06	4.67	0.65	2.15

3.4. *Biomass/water ratio effect*

Since different amounts of solid loading and water were used, the results were expressed as yield in "milligrams of compound per gram of biomass" (Table 3.2), estimated by multiplying measured concentrations by the liquid volume initially added and dividing by the biomass loaded. When increasing the biomass fraction up to 15-16%, results indicated again that LHW pretreatment worked correspondingly at both scales. For instance, the yield of acetic acid remained similarly at either scale when adding more solids. A few inconsistencies can be observed, however. For example, the amount of xylose obtained was significantly lower (26%) at pilot scale than at the lab in case of 10% biomass fraction. This is mirrored by a furfural increase. Yet, at 15-16% biomass no such difference between lab and pilot scale was seen, such that no correlation between scale and conversion remains.

Table 3.2. *Yield of different compounds on initial dry biomass for experiments involving 120 min pretreatment time at 180 °C.*

Scale	Initial biomass fraction	Glucose mg/g	Xylose mg/g	Formic acid mg/g	Acetic acid mg/g	HMF mg/g	Furfural mg/g
Lab	10%	2.48	18.46	7.94	37.84	2.74	18.19
	15%	2.75	9.55	8.83	36.46	2.92	16.17
Pilot	10%	2.50	14.62	8.68	37.96	2.74	24.05
	16%	2.98	9.91	9.71	37.63	3.28	16.85

Irrespective of scale, a minor correlation can be observed between the initial biomass fraction and the yields. Increasing biomass fractions may lead to higher severity, as observed from a decrease in xylose yield and a slight increase in glucose and HMF yield. The higher severity could be explained by the release of more carboxylic acid (from more biomass) in less aqueous liquid. Thus, although the amounts of acetic acid obtained per gram of biomass were almost equal at higher biomass fraction and at both scales, a higher aqueous concentration of acetic acid was observed at larger initial biomass loading (see Table 3.S2, supplementary material).

Consequently, when expressing the results as "concentration in g/L" the about 50% increase in the initial biomass fraction led to proportional increments in the concentrations of most of the compounds analyzed (see Table 3.S2, supplementary material).

3.5. Complete de-acetylation

During pretreatment of lignocellulosic biomass, acetic acid is released as a result of the hydrolysis of the acetyl groups located in the hemicellulose structure. The selected biomass for this study comprises a high acetyl content. Acetic acid is considered an inhibitor for several microorganisms (Casey et al. 2010), thus its presence would not be desired for the subsequent enzymatic hydrolysis and fermentation steps after pretreatment (Gurram et al. 2011). According to results in Figure 3.2A, acetic acid release showed the expected increase with pretreatment time before reaching a plateau, as observed in previous laboratory experiments (Jimenez-Gutierrez et al. 2020), which suggested a complete hydrolysis of acetyl groups from hemicelluloses. Similarly, results from the higher biomass/water ratio experiments (Table 3.2) indicate full acetic acid release regardless the initial biomass fraction loaded. Data obtained from the two-step hydrolysis LAP applied to the solid fraction, as well as the analysis of washing water after pretreatment and filtration (Table 3.S4 in supplementary material) confirmed the de-acetylation of the poplar biomass by LHW after 120 min at 180 °C at both scales according to the HPLC detection limit (0.01% acetyl content on dry basis as the minimum value for the analytical calibration in HPLC).

4. Conclusions

LHW pretreatment of poplar biomass has been compared at laboratory and pilot scale. At either scale there was a considerable extent of hemicellulose hydrolysis and formation of degradation compounds, such as formic acid and furfural. In general, at similar conditions, including a similar temperature profile, LHW pretreatment behaved in a similar way at laboratory and pilot plant scale, allowing optimization of the process at lab scale before moving to larger scale. Complete de-acetylation of poplar wood was achieved at both scales applying 180 °C for 120 min as pretreatment condition. At both scales, higher pretreatment temperature led to larger glucose and acetic acid release, but simultaneously to more formation of sugar degradation compounds. Increasing the biomass/water ratio resulted also in similar behavior at both scales, and no significant correlation between scale and conversion per amount of initial biomass loaded was observed.

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Supplementary material

Conditions and pH values

***Table 3.S1**. Conditions and pH values for LHW pretreatment of poplar biomass at lab and pilot scales.*

Sample	Biomass fraction	Temperature (°C)				Pressure (atm)				Time (min)			pH
		T.set	**T.init**	**T.pret**	**T.final**	**P.set**	**P.init**	**P.pret**	**P.final**	**t.heat**	**t.pret**	**t.cool**	
Lab													
P19N2	10%	180	22.2	180	20.8	0.1	0.1	0.98	0.15	80	120	52	3.64
P33N	10%	180	22.0	180	24.0	0.1	0.1	0.96	0.13	70	6	30	4.10
P34N	10%	180	21.7	180	21.7	0.1	0.1	0.98	0.16	62	240	418	3.60
P35N	10%	180	21.8	180	20.1	0.1	0.1	0.98	0.16	55	180	40	3.60
P36N	10%	180	21.7	180	21.8	0.1	0.1	0.97	0.13	56	30	177	4.30
P37N	10%	188	21.5	188	13.0	0.1	0.1	1.17	0.15	57	120	93	3.66
P38N	15%	180	21.6	180	22.4	0.1	0.1	1.00	0.14	60	120	135	3.60
P39N	11%	180	20.3	180	21.7	0.1	0.1	0.98	0.15	55	120	150	3.75
P40N	10%	180	18.1	180	13.3	0.1	0.1	0.98	0.14	56	120	54	3.67
P34N2	10%	180	25.5	180	20.0	0.1	0.1	0.97	0.16	94	240	26	3.61
Pilot													
71	10%	180	18.9	180	20.1	0.1	0.1	0.98	n.a.	105	33	95	3.92
73	10%	180	17.0	180	24.0	0.1	0.1	0.88	n.a.	80	30	93	4.12
75	10%	180	16.9	180	28.2	0.1	0.1	1.02	n.a.	80	180	71	3.71
77	10%	180	20.3	180	28.3	0.1	0.1	0.90	n.a.	105	243	78	3.68
79	10%	180	20.9	180	19.8	0.1	0.1	1.03	n.a.	74	122	127	3.74
82	10%	180	20.0	180	29.0	0.1	0.1	0.96	n.a.	81	33	76	4.01
83	10%	188	16.4	187	24.7	0.1	0.1	1.20	n.a.	125	120	95	3.68
85	10%	180	19.1	180	27.4	0.1	0.1	0.96	n.a.	79	245	78	3.69
87	16%	180	17.3	180	24.1	0.1	0.1	1.01	n.a.	70	121	69	3.68
89	10%	180	15.3	180	21.1	0.1	0.1	1.01	n.a.	81	123	128	3.75

Temperature profiles at pilot plant

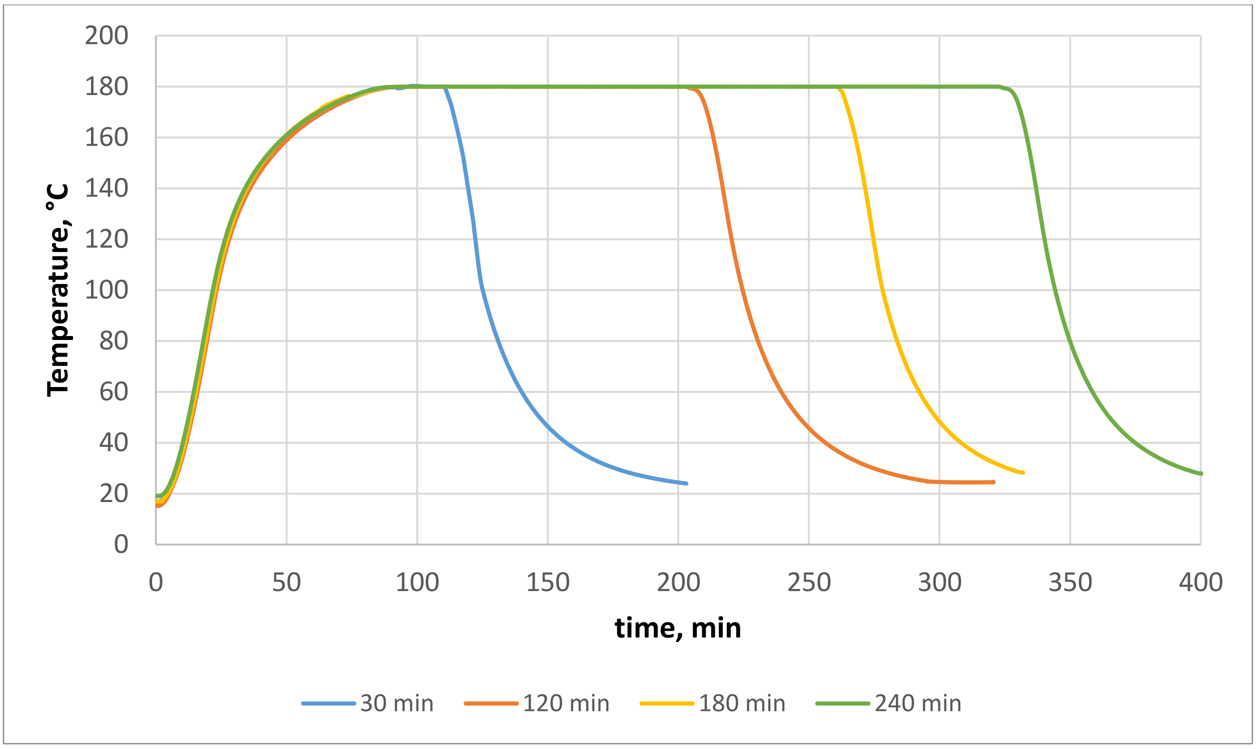

Figure 3.S1. *Temperature profiles during LHW pretreatment of poplar biomass at pilot plant at different pretreatment times.*

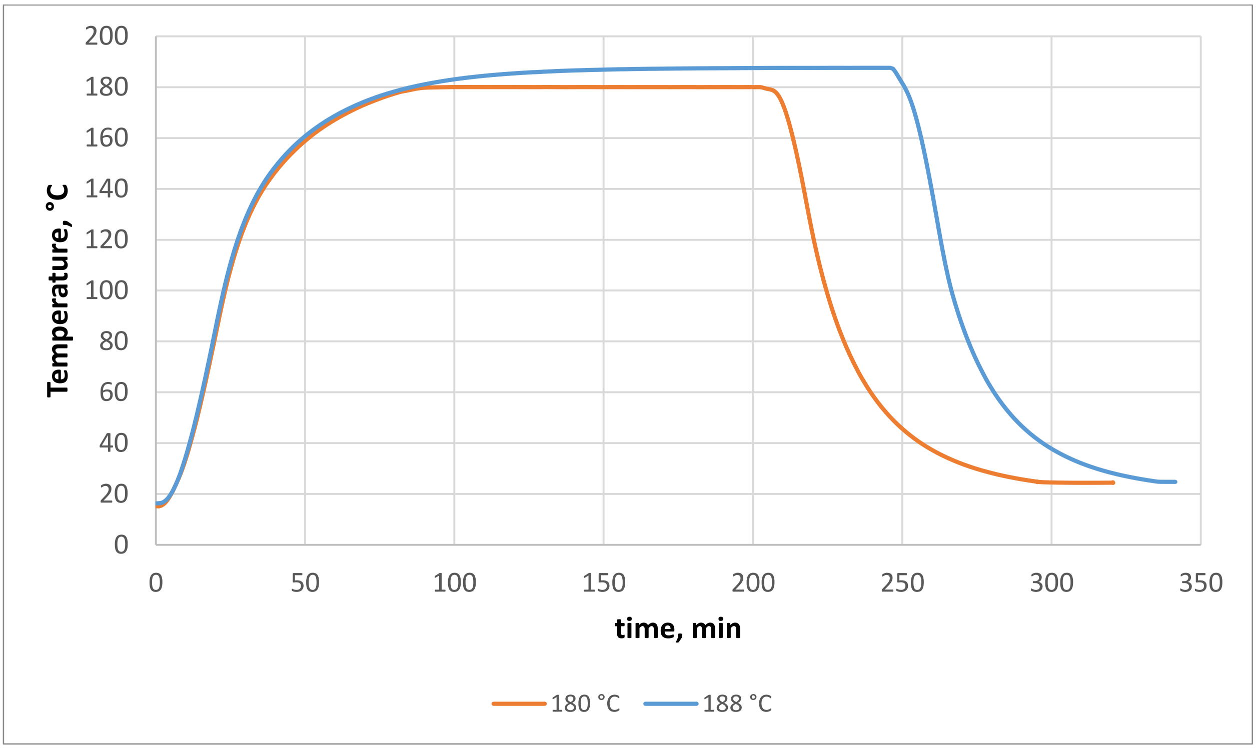

Figure 3.S2. *Temperature profiles during LHW pretreatment of poplar biomass at pilot plant at two different pretreatment temperatures, for 120 min pretreatment time and 10% initial biomass fraction.*

Visual analysis of pretreated biomass

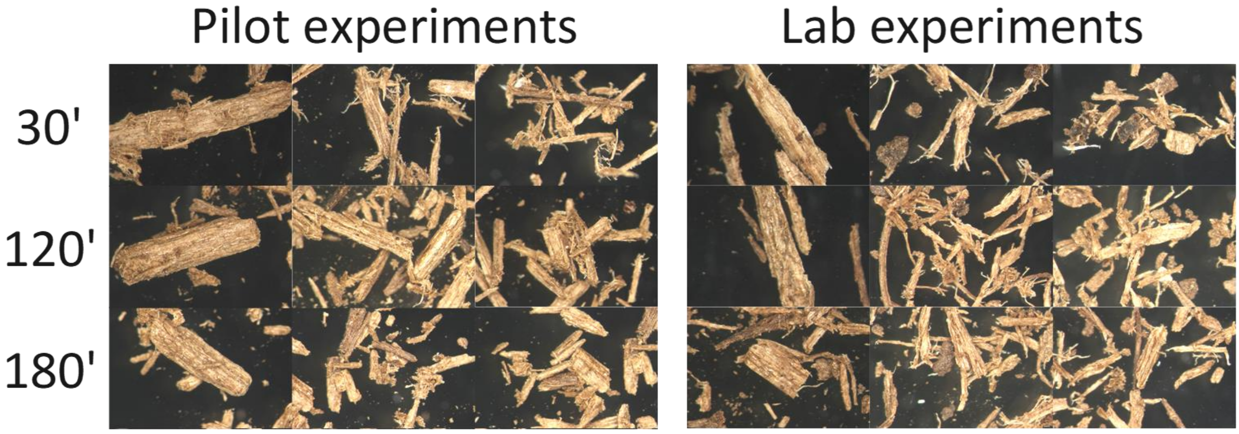

Figure 3.S3*. Visual analysis at the microscope of the surface of the LHW pretreated material at different pretreatment times at lab and pilot scales.*

Results from higher biomass loading fraction, in g/L

Table 3.S2*. Concentration of different compounds for LHW experiments for 120 min pretreatment time at 180 °C.*

Scale	Initial biomass fraction	Glucose g/L	Xylose g/L	Formic ac. g/L	Acetic ac. g/L	HMF g/L	Furfural g/L
Lab	10 %	0.28	2.05	0.88	4.20	0.30	2.02
	15 %	0.49	1.69	1.56	6.44	0.51	2.85
Pilot	10 %	0.28	1.62	0.96	4.22	0.31	2.67
	16 %	0.568	1.888	1.849	7.168	0.625	3.210

In the pilot plant trials, increasing 50% the dry mass content loading provided proportional increments in the concentrations in g/L of most of the compounds analysed, with the exception of xylose and furfural (see Table 3.S2). Whereas glucose, formic acid and HMF experienced an increase in double concentration, acetic acid was 70% higher and a 20% growth was observed for furfural. At the lab trials, except for xylose and furfural, all the species show an average increment of approximately 70% in concentrations in g/L. On the other hand, xylose showed only a decrease of 18% at the lab experiments. By increasing the biomass/water ratio for LHW pretreatment, the concentration of glucose, carboxylic acids and furans increased mostly proportionally due to the lower liquid fraction used.

Overall mass balance

***Table 3.S3**. Overall mass balance for all LHW pretreatment experiments.*

Sample	Biomass	Water	Total mass	Liquid frac.	Solid frac.	% mass loss	% water adsorbed	Feature
Lab	**g**	**g**	**g**	**g**	**g**			
P34N	5	45	50	27.50	18.40	8.2 %	29.8 %	240 min
P35N	5	45	50	25.23	20.26	9.0 %	33.9 %	180 min
P36N	5	45	50	23.50	23.15	6.7 %	40.3 %	30 min
P37N	5	45	50	24.36	17.83	15.6 %	28.5 %	188 °C
P38N	5.25	29.75	35	16.24	13.24	15.8 %	26.9 %	15 %DM
P39N	5	40	45	23.33	14.49	16.0 %	23.7 %	11 %DM
P40N	5	45	50	25.18	20.08	9.5 %	33.5 %	120 min
P34N2	5	45	50	27.58	12.58	19.7 %	16.9 %	240 min
Pilot	**kg**	**kg**	**kg**	**kg**	**kg**			
71	2.5	22.5	25	14.88	9.54	2.3 %	31.3 %	30 min
73	2.5	22.5	25	13.56	10.18	5.0 %	34.1 %	30 min
75	2.5	22.5	25	15.40	7.14	9.8 %	20.6 %	180 min
77	2.5	22.5	25	17.26	7.30	1.8 %	21.3 %	240 min
79	2.5	22.5	25	15.72	8.00	5.1 %	24.4 %	120 min
82	2.5	22.5	25	15.30	9.28	1.7 %	30.1 %	30 min
83	2.5	22.5	25	16.06	7.02	7.7 %	20.1 %	188 °C
85	2.5	22.5	25	16.46	6.82	6.9 %	19.2 %	240 min
87	2.0	10.5	12.5	4.90	6.30	10.4 %	41.0 %	16 %DM
89	2.5	22.5	25	16.70	7.40	3.6 %	21.8 %	120 min

Acetic acid in washing steps

Table 3.S4. *Amount of acetic acid in the recovered liquid fraction and in the washing water, compared with the total acetic acid per amount of biomass, estimated from the initial liquid added (including mass loss).*

Sample	in liq. fraction†, mg	in W1*, mg	in W2*, mg	Total recovered, mg/g biomass	Total estimated‡, mg/g biomass	Feature
Lab						
P35N	108.7	46.4	7.7	32.53	38.76	180 min
P36N	44.4	37.9	8.1	18.08	16.99	30 min
P37N	107.1	73.9	9.6	38.13	39.57	188 °C
P38N	104.5	64.9	14.2	36.72	38.29	15 %DM
P39N	101.2	70.4	26.1	37.67	33.06	11 %DM
P40N	107.1	55.1	6.0	33.65	38.27	120 min
P34N2	146.5	37.4	16.7	40.12	47.81	240 min
Pilot						
72	171.2	86.2	24.6	22.54	20.21	30 min
74	91.9	52.5	10.5	14.97	14.00	30 min
76	449.3	163.1	53.1	44.23	39.33	180 min
78	423.5	138.8	38.5	46.75	42.20	240 min
80	372.4	137.6	38.8	36.71	33.83	120 min
81	179.8	74.7	19.3	20.14	19.13	30 min
84	555.4	185.1	52.7	45.61	41.30	188 °C
86	609.6	178.7	61.4	48.93	44.68	240 min
88	286.8	272.4	84.6	37.40	31.98	16 %DM
90	367.3	130.6	35.5	41.05	36.71	120 min

*W1 regards the first washing steps, and w2 the second washing steps, both cases with 100ml demi-water.

†Liquid fraction at pilot plant estimated from wet solids sampled and the proportion liquid/solid fractions obtained (Table 3.S3).

‡Values estimated by multiplying the concentration of the liquid fraction by the total initial water added and divided by the initial biomass (both initial liquid and biomass minus the proportional mass loss (Table 3.S3)). In case of pilot plant values, the initial water added was estimated by the proportion liquid fraction†/initial water added. Similarly, the initial biomass was also estimated by multiplying the wet solids sampled by the average raw/wet biomass ratio obtained at the lab (0.335 $g_{\text{raw.biomass}}/g_{\text{pretreated.biomass}}$).

Chapter 4

Modelling of acetic acid release from lignocellulosic biomass during liquid hot water pretreatment process

Abstract

Biomass pretreatment is one of the most crucial biorefinery stages, needed for the disruption of the recalcitrant structure of lignocellulosic biomass. Liquid hot water pretreatment is considered a very promising technology, because it only requires mild operational conditions and no addition of extra chemicals. This hydrothermal process dissolves most of the hemicellulose in the biomass, including its fraction of acetyl groups. Extensive studies have attempted to model the complex matrix of reactions and compounds produced during this pretreatment method. In this work, a simplified model to describe the liquid hot water pretreatment is developed, using acetyl hydrolysis reaction as the progress indicator. Heating, cooling, thermal expansion and pH of the reaction mixture are included in the model, as the reaction will be temperature-dependent and catalysed by released acetic acid. The model fits well to experimental data for acetic release, but does not fit the experimental pH closely. A more detailed picture is required of the buffer capacity of the biomass.

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Nomenclature

A_0^{HL}	Frequency factor for glucuronic acid degradation reaction	1/s
A_0^{LH}	Frequency factor for glucuronolactone degradation reaction	1/s
A_1	Frequency factor for acetyl hydrolysis reaction	mol/(L·s)
A_{HT}	Available area for heat transfer	m^2
c	Combined constant	J/(s m^3 K)
C_i	Concentration of species "i"	mol/m^3
C_p	Specific heat capacity	J/(kg K)
E_{a1}	Activation energy for acetyl hydrolysis reaction	J/mol
E_a^{HL}	Activation energy for glucuronic acid degradation reaction	J/mol
E_a^{LH}	Activation energy for glucuronolactone degradation reaction	J/mol
h	Heat transfer coefficient	W/(m^2 K)
$K_{eq,HAc}$	Equilibrium constants for acetic acid dissociation	mol/m^3
$K_{eq,HGlucA}$	Equilibrium constants for glucuronic acid dissociation	mol/m^3
$K_{eq,HGlucL}$	Equilibrium constants for glucuronolactone dissociation	mol/m^3
k_{HL}	Reaction constant for glucuronic acid degradation	1/s
k_{LH}	Reaction constant for glucuronolactone degradation	1/s
K_w	Equilibrium constants for water dissociation	
R	Universal gas constant	J/(mol K)
r_1	Volume-specific production rate of acetic acid	mol/(s m^3)
$r_{eq,H2O}$	Volume-specific reaction rate of water dissociation	mol/(m^3 s)
$r_{eq,HAc}$	Volume-specific reaction rate of acetic acid dissociation	mol/(m^3 s)
$r_{eq,HGlucA}$	Volume-specific reaction rate of glucuronic acid dissociation	mol/(m^3 s)
$r_{eq,HGlucL}$	Volume-specific reaction rate of glucuronolactone dissociation	mol/(m^3 s)
T	Internal temperature	K
t	Time	s
V	Volume of the reaction mixture	m^3
w_r	Mass of the reaction mixture	kg
ρ	Density	kg/m^3

1. Introduction

The development of biobased economy has led to a special interest in using lignocellulosic biomass as main feedstock for biorefinery processes (Fonseca et al. 2020). The recalcitrance of lignocellulosic biomass implies a pretreatment step needed prior the enzymatic hydrolysis and fermentation, in order to disrupt the microstructure and facilitate the accessibility and digestibility of the sugars present in the complex matrix (Meng et al. 2016) (Sun et al. 2021).

Four pretreatment categories can be distinguished: physical, chemical, physico-chemical and biological, with various sub-categories (Haghighi Mood et al. 2013). Among all the possible methodologies, liquid hot water (LHW) pretreatment is considered a very promising method because there is no addition of extra chemicals and it operates at relatively mild conditions (Li et al. 2017). Typical conditions for LHW pretreatment include batch operation mode, a temperature range of 150-230°C (leading to increased pressure), residence time from minutes to hours (Ruiz et al. 2020) and around 1:10 solid/liquid ratio for a stirred tank reactor, since the upper limitation of solid loading in a batch reactor is determined by the viscosity of the reaction mixture, which can hinder the stirring of the reactor vessel (Cara et al. 2007).

This pretreatment technique has been proven to be an effective method to dissolve hemicelluloses in lignocellulosic biomass, while most of the lignin fraction remains in the solid biomass structure (Mosier et al. 2005) (Xiao et al. 2011). The elevated temperature applied enhances the formation of hydronium ions, which act as catalyst for the depolymerisation reactions of the lignocellulosic biomass. Besides, the release of acetic acid from the cleavage of the acetyl groups in hemicelluloses contributes also to lowering the pH, enhancing the hydrolysis of the lignocellulosic material (Carvalheiro et al. 2016).

During LHW pretreatment, hemicelluloses and (to a much smaller extent) celluloses are cleaved into pentose and hexose oligomers and monomers. These C-5 and C-6 sugars partly degrade to furfural and hydroxymethylfurfural (HMF), respectively (Yan et al. 2016). Moreover, under high severity conditions, these sugar degradation compounds degrade even further to levulinic acid and formic acid (Negro et al. 2003), for example.

Most of these degradation compounds produced during LHW pretreatment are inhibitors for the consecutive enzymatic hydrolysis and fermentation steps within the biorefinery (Jönsson and Martín 2016). Thus, it is important to take into account the formation of these side products, as the conditions applied during the LHW treatment determine directly the composition and properties of the product obtained (Kim 2018). Hence, special attention is required on the acetic acid release, because its concentration after pretreatment is higher than other inhibitors, meaning that its presence may certainly affect the following downstream processes (Lyu et al. 2019).

Removing those inhibitors after the pretreatment step could improve the whole biochemical process (Gurram et al. 2011). Apart from the benefit of eliminating the inhibitory effect for the microorganisms used in the fermentation step (Cho et al. 2010), recovering the acetic acid fraction from the biomass hydrolysate would increase the pH and therefore, reduce the amount of base required for pH adjustment before the enzymatic hydrolysis. Furthermore, other inhibitors could be also recovered, such as furfural or formic acid, adding consequently an extra value as potential side products (Scapini et al. 2021).

Some authors have used the acetyl content in the insoluble solid fraction obtained after LHW pretreatment of lignocellulosic biomass to assess pretreatment effectiveness (Pérez et al. 2007). In previous work, acetic acid release during LHW pretreatment was considered as indicator of hemicellulose hydrolysis, because it is originated exclusively from the cleavage of acetyl groups present in the hemicellulose structure, it is a stable compound and the main contributor to the acidity in LHW pretreatment (Jimenez-Gutierrez et al. 2020).

To obtain a better understanding of the mechanisms involved during this hydrothermal pretreatment and to be able to optimize these biochemical processes (Pérez et al. 2008), different models have been previously proposed with diverse degrees of complexity, in order to evaluate the reactions kinetics, as well as the influence of the conditions, such as temperature or pH.

Regarding LHW pretreatment, the core of these kinetic models is the depolymerisation of hemicelluloses, including the degradation of xylan (Garrote et al. 2001). Different reactor configurations have been used for modelling this hydrothermal treatment, such as batch, continuous-flow (Rogalinski et al. 2008) or semi-continuous fixed-bed (Ingram et al. 2009).

Concerning solubilisation of xylan, some authors included two sources of xylose formation during LHW pretreatment of lignocellulosic biomass: directly from xylan and via xylooligomers (Mittal et al. 2009). Alternatively, a kinetic model for hemicellulose hydrolysis previously proposed distinguished two fractions: fast and slow reacting hemicelluloses, which were assumed to decompose into xylooligosaccharides, and consecutively into xylose, furfural and degradation products (Parajó et al. 2004) (Shen and Wyman 2011) (Borrega et al. 2011). Shao and Lynd (2013) extended their model by adding the biomass bed shrinking during pretreatment of corn stover and triticale straw.

A multiscale model has been also developed, taking into account simultaneously chemical reaction (microscale), diffusion (mesoscale), mixing and bulk diffusion (macroscale), including four different xylooligomers in their kinetic model (Hosseini and Shah 2009).

Other authors have modelled a pilot-scale tubular reactor as a series of continuous stirred tank reactors (CSTR) for hydrothermal pretreatment of wheat straw. In their kinetic model, apart from slow and fast reactive hemicellulose fractions, they included sugar degradation reactions (furfural and HMF from xylose and glucose respectively) as well as acetic acid formation (González-Figueredo et al. 2015).

Some authors extended the amount of species used in their kinetic model, considering the cellulosic and hemicellulosic fractions (dos Santos Rocha et al. 2017). Therefore, cellobiose, glucose, formic acid and hydroxymethylfurfural (from cellulosic fraction) and xylose, arabinose, acetic acid, glucuronic acid and furfural (from hemicellulosic fraction) were taken into account in their semi-mechanistic model for hydrothermal degradation of sugarcane straw in a batch reactor.

Most recently, a dynamic model was proposed for xylan depolymerisation in a pilot-scale pretreatment continuous tubular reactor for three different feedstocks (Rodríguez et al. 2019).

However, in several aforementioned cases, inconsistencies were found between the simulation and the experimental data, due to the large amount of factors and dependencies comprised in those complex models. Hence, a simplified model capturing the essence of hydrothermal pretreatment of lignocellulosic biomass using acetic acid release as indicator of and catalyst for hemicellulose hydrolysis would be useful but has not been proposed yet.

In this work, we present a model specifically design for acetic acid release during LHW pretreatment of lignocellulosic biomass, with the purpose of understanding the effects of temperature, pH, and reaction time on the conversion rate of the acetyl hydrolysis. Based on another recent model of a similar system (Ahmad et al. 2018), our system considers much less transported species. We focus on acetyl groups, acetic acid, and other compounds that will determine pH and hence the rate of all reactions. We assume that proper prediction of acetic acid concentration and pH will allow extension of the model to predict the concentration of all other dissolved species. The advantage of acetic acid is that it does not degrade further under LHW conditions.

A wide variety of feedstocks have been considered for the study of hydrothermal pretreatment of lignocellulosic biomass, including agricultural wastes, hardwoods, as well as energy crops (Nitsos et al. 2013). In general, hardwoods contain larger amount of acetyl groups than other lignocellulosic materials. Poplar wood was chosen in this work as lignocellulosic biomass source, due to its availability and composition.

The model is using a semi-mechanistic approach to describe essentially the hydrolysis of acetyl groups during LHW pretreatment of lignocellulosic biomass. A preliminary model, based on a previous work for a similar system (Ahmad et al. 2018), was proposed and evaluated in order to determine the most significant factors involved in the acetic acid release from hemicelluloses.

2. Materials and Methods

2.1. System description

The system consists of ≈10% w/w solid wood particles in aqueous suspension, placed in a closed, insulated stirred tank reactor. The vapour phase present was assumed to not to influence the LHW pretreatment. The LHW pretreatment is performed in batch mode and controlled by external heating. Assuming mass transfer resistance to be negligible, the suspension was simplified to a homogeneous aqueous phase, reducing therefore the complexity of the system. Focusing on the acetyl hydrolysis, the relevant species involved in this work are described in Table 4.1. The model takes into account the reaction vessel as a 0D space where the basic reactions required for acetyl hydrolysis take place. A heating model is included too, which controls the temperature profile of the simulation, as well as the temperature dependency of certain parameters.

Table 4.1. *Transported species used in the numerical model*

Transport species	**Abbreviation**
Acetyl groups	RAc
Acetic acid	HAc
Acetate	Ac^-
Protons	H^+
Hydroxide	OH^-
Glucuronic Acid	HGlucA
Glucuronate	$GlucA^-$
Glucuronolactone	GlucL

2.1.1. Temperature

The heating period of the process has been modelled by equation (1), derived from an energy balance of the reaction mixture with a heat flux driven by temperature difference between the mixture and the heating source (Ahmad et al. 2016) (Kapu et al. 2016). Other heat effects were assumed to be negligible. The energy balance could be rewritten as equation (2):

$$w_r \cdot C_p \cdot \frac{dT}{dt} = h \cdot A_{HT} \cdot \Delta T \tag{1}$$

$$\frac{dT}{dt} = \frac{h \cdot A_{HT}}{w_r \cdot C_p} \cdot \Delta T \tag{2}$$

In these equations, w_r is the mass of the reaction mixture in kg, C_p is the specific heat capacity of the reaction mixture (J/(kg K)), h is the heat transfer coefficient (W/(m^2 K)), A_{HT} is the available area for heat transfer (m^2), and ΔT is the difference between internal (mixture) and external set temperatures (T and T_{set} respectively).

In equation (1), the constants of heat transfer coefficient h and heat transfer area A_{HT} were grouped together and divided by the volume of the reaction mixture V (m^3), introducing then a combined constant named c (J/s m^3 K). This was used to obtain a simplified energy balance:

$$c = \frac{h \cdot A_{HT}}{V} \tag{3}$$

$$\frac{dT}{dt} = \frac{c}{\rho \cdot C_p} \cdot (T_{set} - T) \tag{4}$$

Where ρ is the density of the mixture (kg/m^3). Due to the high water content in the reaction mixture, ρ and C_p were assumed to be the same as for pure water and temperature dependent. Formulas for temperature dependencies used in the model were taken from Gmehling et al. (2019) and are shown in the supplementary material. The heat transfer coefficient and area were assumed to be temperature independent. By integrating equation (4) with $T = T_0$ and $t = t_0$ as initial conditions, an expression for temperature over time was obtained, represented in equation (5):

$$T(t) = T_{set} - (T_{set} - T_0) \cdot e^{\frac{-c}{\rho \cdot C_p} \cdot (t - t_0)} \tag{5}$$

The function in equation (5) was compared with experimental temperature profile data (Jimenez-Gutierrez et al. 2021) for a LHW pretreatment process of 120 min at 180°C, in order to estimate the value of the constant c. Instead of including the temperature dependency of ρ and C_p in the energy balance, their values were calculated individually for each time step using the measured temperature in supplementary material. This resulted in a sufficiently accurate data fit for the determination of the value of c. Consequently, the Sum of Squared Residuals (SSR) between the simulated and the experimental value of internal temperature T was calculated and minimized by adjusting the value of c to the most optimal fit.

2.1.2.Liquid Volume calculation

According to representation in Figure 4.S1 (supplementary material), at temperatures above 160 °C, the density of pure water decreases around 10 % from room conditions. Since our reactor is a closed insulated vessel containing a liquid and a vapour phase, the pressure increases with temperature and the liquid phase is kept in liquid state, though a liquid volume expansion occurs proportionally due to the increase in temperature. Assuming that reaction rates depend on concentrations, the liquid volume of the system should not be considered constant along the pretreatment process and therefore, the reaction volume expansion needs to be predicted. These changes in volume are included in the mass balances for each species as follows:

$$\frac{d(V \cdot C_i)}{dt} = V\frac{dC_i}{dt} + C_i\frac{dV}{dt} \tag{6}$$

Where C_i is the concentration of species *"i"* in mol/m^3. Hence, changes in volume over time could be expressed as the variation of volume with temperature times changes in temperature over time:

$$\frac{dV}{dt} = \frac{dV}{dT} \cdot \frac{dT}{dt} \tag{7}$$

The total mass of the reaction mixture w_r does not change with temperature. Therefore, using equation (8), an expression was obtained that represents the changes in volume with temperature as function of changes in density over temperature (equation (9)).

$$\frac{dw_r}{dT} = 0 = \frac{d(V \cdot \rho)}{dT} = \rho \frac{dV}{dT} + V \frac{d\rho}{dT} \tag{8}$$

$$\frac{dV}{dT} = -\frac{V}{\rho} \frac{d\rho}{dT} \tag{9}$$

Assuming the density of the mixture as for pure water, the expression for the dependency of density with temperature was taken from Gmehling et al. (2019) and the derivative of this equation (10), shown as equation (11), is the term required to complete the mass balances.

$$\rho = 18.015 \cdot (A_0 + A_1 T + A_2 T^2 + A_3 T^3 + A_4 T^4 + A_5 T^5) \tag{10}$$

$$\frac{d\rho}{dT} = 18.015 \cdot (A_1 + 2A_2 T + 3A_3 T^2 + 4A_4 T^5 + 5A_5 T^4) \tag{11}$$

Thus, changes in volume due to thermal expansion are included in the mass balances as combination of equations (6), (7) and (9), with the term *dT/dt* from equation (4) and *dρ/dT* in equation (11).

$$\frac{d(V \cdot C_i)}{dt} = V \frac{dC_i}{dt} - C_i \frac{dT}{dt} \cdot \frac{V}{\rho} \frac{d\rho}{dT} \tag{12}$$

$$\frac{d(V \cdot C_i)}{dt} = V \frac{dC_i}{dt} - C_i \frac{V \cdot C}{\rho^2 \cdot C_p} \cdot (T_{set} - T) \frac{d\rho}{dT} \tag{13}$$

2.1.3.Reactions

The only irreversible reaction considered in our model is the acid hydrolysis of acetyl groups to dissolved acetic acid:

$$RAc + H_2O \overset{H^+}{\longrightarrow} ROH + HAc \tag{14}$$

The volume-specific production rate (mol/(s m^3)) of this conversion is represented by an Arrhenius-type expression in equation (15):

$$r_1 = C_{RAc} \cdot C_{H^+} \cdot A_1 \cdot e^{\frac{-E_{A1}}{RT}} \tag{15}$$

Equilibrium reactions implemented in the model included dissociation of acetic acid and water. The equilibrium constants for these reactions are expressed in equations (16) and (17):

$$K_{eq,HAc} = \frac{C_{H^+} \cdot C_{Ac^-}}{C_{HAc}} \tag{16}$$

$$K_w = C_{H^+} \cdot C_{OH^-} \tag{17}$$

These equilibrium constants are temperature dependent, which is described in the next section. The reaction rate of the equilibrium reactions is calculated using the unbalance in the equilibrium, meaning the difference between the current reactant concentration and the reactant concentration at equilibrium, as long as this difference is not zero. Once the equilibrium is balanced, both terms are equal and the reaction rate becomes zero. Reaction rates for these equilibrium reactions are represented in equations (18) and (19) respectively, with the parameter k determining the volume-specific rate of the equilibrium reaction:

$$r_{eq,HAc} = k \cdot \left(C_{HAc} - \frac{C_{H^+} \cdot C_{Ac^-}}{K_{eq,HAc}} \right) \tag{18}$$

$$r_{eq,H_2O} = k \cdot \left(1 - \frac{C_{H^+} \cdot C_{OH^-}}{K_w} \right) \tag{19}$$

2.1.4. *Temperature dependencies of equilibrium constants*

The temperature dependency for acetic acid dissociation is well known, and an expression for its dissociation in pure water was taken from Fisher and Barnes (1972), shown in equation (20):

$$RTln\left(K_{eq,HAc}\right) = \Delta S^0_{e,r} \cdot \left[T_r - T - 218 \cdot \left(1 - exp\left[exp(-12.741 + 0.01875 \cdot T) - 7.48 \cdot 10^{-4} + \frac{(T-T_r)}{219}\right]\right)\right] - \Delta H^0_r + T \cdot \Delta S^0_r + T \cdot a \cdot \left[ln\left(\frac{T}{T_r}\right) - 1 + \frac{T_r}{T}\right] + \frac{b}{2} \cdot (T - T_r)^2 \tag{20}$$

The parameters used in this expression can be found in Fisher and Barnes (1972) and are reproduced in Table 4.2. A graphical representation of this temperature dependency could be seen in Figure 4.S2 (supplementary material). This expression was used to estimate species concentrations related to the acetic acid dissociation during the heating of the reactor.

Table 4.2. Parameters used to calculate the temperature dependency of the equilibrium constant for acetic acid dissociation in water (Source: Fisher and Barnes (1972)).

Parameter	Value	Units
$\Delta S^0_{e,r}$	-7.42856	cal/mol K
ΔS^0_r	-22.1280	cal/mol K
ΔH^0_r	-108.273	cal/mol
Tr	298.15	K
a	-59.9424	-
b	0.112254	-

The temperature dependency for the equilibrium constant regarding the water dissociation reaction is described by equation (21) (Rumpf et al. 1998) and its graphical representation can be found in the supplementary material.

$$ln(K_w) = \frac{-13445.9}{T} - 22.4773 \cdot ln(T) + 140.932 \tag{21}$$

2.2. *pH and buffer component*

The selection of the buffer component is crucial, as it would affect the reaction kinetics of the simulation. The amount of buffer and its pK_a may influence considerably the initial pH of the simulation and the pH profile during the pretreatment process. A strong buffer will push the initial pH above 7, which is not in line with experimental values (Jimenez-Gutierrez et al. 2021). On the other hand, a weaker buffer with a similar pK_a to acetic acid would take up H^+ from the system without affecting significantly the initial pH. Glucuronic acid, which dissociates to glucuronate, is a potential buffer component for this system. It is an uronic acid present in the lignocellulosic biomass (Ahmad et al. 2018) and it is consequently released during the LHW pretreatment process. Additionally, previous studies proposed that removing glucuronic acid from the hemicellulose structure may improve the saccharification of lignocellulosic biomass (Lyczakowski et al. 2017).

The structure of hemicelluloses in poplar biomass includes branches of α-D-glucuronic acid (HGlucA) and 4-*O*-methyl glucuronic acid (4mGlucA), being the former less frequent than the latter (Sakamoto et al. 2015). Content of glucuronic acid in hardwoods (including 4-*O*-methyl glucuronic acid) is generally expressed as molar ratio of xylose to glucuronic acid, and it may vary from 4:1 to 25:1 (Zhou et al. 2017) (Bajpai 2018). In this work, both species are considered combined as glucuronic acid (HGlucA) and as unique buffer component, with a molar ratio xylose/HGlucA of 1:10 (Sjöholm et al. 2000) (Wyman et al. 2005) (Ebringerová 2005).

2.2.1. Glucuronic acid as single buffer

Glucuronic acid is a weak acid that forms an acid/base equilibrium with glucuronate:

$$HGlucA \leftrightarrow GlucA^{-} + H^{+} \tag{22}$$

The equilibrium constant for this dissociation is represented in equation (23):

$$K_{eq,HGlucA} = \frac{C_{H^+} \cdot C_{GlucA^-}}{C_{HGlucA}} \tag{23}$$

The reaction rate for this equilibrium reaction was expressed in equation (24), in a similar way that for the acetic acid and water dissociation reactions:

$$r_{eq,HGlucA} = k \cdot \left(C_{HGlucA} - \frac{C_{H^+} \cdot C_{GlucA^-}}{K_{eq,HGlucA}} \right) \tag{24}$$

Due to difficulties finding the temperature dependency of this equilibrium glucuronic acid/glucuronate, an approximation was made similarly that done in previous work (Ahmad et al. 2016), by using the same thermodynamic parameters for the temperature dependency of $K_{eq,HAc}$ (section 2.1.4).

This equilibrium has been included in the model to reflect the higher values of experimental pH measured after the LHW pretreatment, by assuming that a certain amount of glucuronic acid present in the biomass was released during the pretreatment process, acting as a buffering component (Ahmad et al. 2016).

2.2.2. Release method

Glucuronic acid (as well as 4-*O*-methyl glucuronic acid) is linked to the xylan backbone by α-(1→2) glycosidic bonds (Scheller and Ulvskov 2010) (Bajpai 2018). Though rather than leaving the carboxylic acid group free, this substituted glucuronic acid appears to be covalently cross-linked to lignin via ester bonds (Terrett and Dupree 2019). Therefore, a progressive availability of glucuronic acid as buffer along the pretreatment process seems to be a good estimation. A gradual release of glucuronic acid was the selected approach to describe this mechanism. This gradual release links the activation rate of the buffer to the reaction rate of acetyl hydrolysis, preventing excessive buffering during the first period of the simulation. Therefore, it was assumed that this buffering component in the lignocellulosic biomass is released at the same rate as the hydrolysis of acetyl groups occurs during the LHW pretreatment process. To implement this gradual release in the model, another irreversible reaction was included, represented by equation (25):

$$r_{release} = \frac{C_{base}}{C_{RAc}} \cdot r_1 \tag{25}$$

The buffer was added initially as an inactive compound with concentration $C_{base,0}$ and then it was converted to the active buffer at the same rate as acetyl groups are hydrolysed by reaction rate r_1 in equation (15). Subsequently, C_{base} was converted into a mixture of C_{HGlucA} and C_{GlucA-} according to the acid/base ratio in equation (27).

In order to have an initial pH of 7, the required ratio between acid and base was calculated using the equilibrium equation (26), knowing that the value for the equilibrium constant $K_{eq,HGlucA}$ at room temperature is of $10^{-3.14}$ (Ahmad et al. 2018).

$$K_{eq,HGlucA} = \frac{C_{H^+} \cdot C_{Base}}{C_{Acid}} \tag{26}$$

$$\frac{C_{Acid}}{C_{Base}} = \frac{C_{H^+}}{K_{eq,HGlucA}} = \frac{10^{-7}}{10^{-3.14}} = 10^{-3.86} \tag{27}$$

2.2.3. *Glucuronolactone formation reaction*

Under LHW conditions, glucuronic acid is converted into glucuronolactone in an equilibrium reaction (Wang et al. 2010). This reversible reaction removes glucuronic acid from the system, altering the buffering equilibrium and increasing the pH value. Arrhenius-type equations (28) and (29) can be used to calculate the forward and reverse constants involved in the equilibrium, used to calculate the equilibrium constant in equation (30), as described by (Wang et al. 2010):

$$k_{HL} = A_0^{HL} \cdot e^{\frac{-E_a^{HL}}{R \cdot T}} \tag{28}$$

$$k_{LH} = A_0^{LH} \cdot e^{\frac{-E_a^{LH}}{R \cdot T}} \tag{29}$$

$$K_{eq,GlucL} = \frac{k_{HL}}{k_{LH}} \tag{30}$$

The values of the activation energy E_a and frequency factor A_0 used in the Arrhenius expressions are shown in Table 4.3 (Wang et al. 2010). Although this equilibrium may not produce significant increase of pH at room temperature, the effect of glucuronolactone might become more prominent at higher temperatures, due to the Arrhenius plot of the equilibrium reaction (Wang et al. 2010).

***Table 4.3**. Kinetic parameters of degradation reaction of glucuronic acid to glucuronolactone (from Wang et al. (2010)).*

Parameter	E_a^{HL}**, kJ/mol**	A_0^{HL}**, 1/s**	E_a^{LH}**, kJ/mol**	A_0^{LH}**, 1/s**
Value	88.5	1.86 x 10^8	63.2	4.43 x 10^4

The pH measurements are generally conducted at room temperature, as well as equilibrium calculations use pK_a values at room temperature. The glucuronic acid dissociation has a conversion rate quick enough to be considered instantaneous (Dutton 2012). However, at room temperature the reaction rate of the glucuronolactone equilibrium has a k_{LH} in the order of 10^{-7} (s^{-1}), meaning that the built up of glucuronolactone will take multiple weeks to revert back to glucuronic acid via equilibrium, at room temperature. Therefore, it was assumed that the glucuronolactone will not convert back as the reaction mixture is cooled down to room temperature and pH is measured. To represent this, the equilibrium constant $K_{eq,GlucL}$ was considered constant at the pretreatment temperature. The glucuronolactone reaction rate was represented using a similar expression as for glucuronic acid in equation (24):

$$r_{eq,GlucL} = k \cdot \left(C_{HGlucA} - \frac{C_{GlucL}}{K_{eq,GlucL}} \right) \tag{31}$$

2.3. Model Implementation

The model was solved using the numeric computing program MATLAB, specifically by the ordinary differential equation solver ODE15s. A general mass balance for a compound *"i"* in a batch reactor would be:

$$\frac{d(V \cdot C_i)}{dt} = \sum r_i \cdot V \tag{32}$$

Where r_i represents the volume-specific reaction rate for all the reactions in which species *"i"* is involved. As described in section 2.1.2, the volume of the mixture should not be considered constant and the changes due to thermal expansion need to be accounted. Hence, combining equation (13) with (32) and re-arranging the expression, it is possible to obtain the following equation for a general compound *"i"*:

$$\frac{dC_i}{dt} = \sum r_i + C_i \frac{c}{\rho^2 \cdot C_p} \cdot (T_{set} - T) \frac{d\rho}{dT} \tag{33}$$

The differential equations then were expressed to calculate the change over time in the concentration of the species and the temperature. This set of differential equations has contributions from irreversible and equilibrium reactions, as well as the energy balance and the term for the thermal volume expansion. They were implemented in the software as follows:

$$\frac{dC_{RAc}}{dt} = -r_1 + C_{RAc} \cdot \frac{c}{\rho^2 \cdot C_p} (T_{set} - T) \frac{d\rho}{dT} \tag{34}$$

$$\frac{dC_{HAc}}{dt} = r_1 - r_{eq,HAc} + C_{HAc} \cdot \frac{c}{\rho^2 \cdot C_p} (T_{set} - T) \frac{d\rho}{dT} \tag{35}$$

$$\frac{dC_{Ac^-}}{dt} = r_{eq,HAc} + C_{Ac^-} \cdot \frac{c}{\rho^2 \cdot C_p} (T_{set} - T) \frac{d\rho}{dT} \tag{36}$$

$$\frac{dC_{H^+}}{dt} = r_{eq,HAc} + r_{eq,H_2O} + r_{eq,HGlucA} + C_{H^+} \cdot \frac{c}{\rho^2 \cdot C_p} (T_{set} - T) \frac{d\rho}{dT} \tag{37}$$

$$\frac{dC_{OH^-}}{dt} = r_{eq,H_2O} + C_{OH^-} \cdot \frac{c}{\rho^2 \cdot C_p}(T_{set} - T)\frac{d\rho}{dT} \tag{38}$$

$$\frac{dC_{HGlucA}}{dt} = r_{release} \cdot 10^{-(7-pK_{eq,HGlucA})} - r_{eq,HGlucA} - r_{eq,GlucL} + C_{HGlucA} \cdot \frac{c}{\rho^2 \cdot C_p}(T_{set} - T)\frac{d\rho}{dT} \tag{39}$$

$$\frac{dC_{GlucA^-}}{dt} = r_{release} \cdot \left(1 - 10^{-(7-pK_{eq,HGlucA})}\right) + r_{eq,HGlucA} + C_{GlucA^-} \cdot \frac{c}{\rho^2 \cdot C_p}(T_{set} - T)\frac{d\rho}{dT} \tag{40}$$

$$\frac{dC_{GlucL}}{dt} = r_{eq,GlucL} + C_{GlucL} \cdot \frac{c}{\rho^2 \cdot C_p}(T_{set} - T)\frac{d\rho}{dT} \tag{41}$$

$$\frac{dC_{base}}{dt} = -r_{release} + C_{base} \cdot \frac{c}{\rho^2 \cdot C_p}(T_{set} - T)\frac{d\rho}{dT} \tag{42}$$

$$\frac{dT}{dt} = \frac{c}{\rho(T) \cdot C_p(T)} \cdot (T_{set} - T) \tag{43}$$

The initial concentration of acetyl groups in the supplied biomass $C_{RAc,0}$ was equal to 97.856 mol/m^3, while the initial concentration of buffer in the biomass $C_{base,0}$ was 13.666 mol/m^3, based on this 10:1 xylose/GlucA ratio (both parameters are calculated in supplementary material). The rest of the species has a zero concentration in the mixture at initial time t_0. Initial temperature T_0 used was room temperature, assumed to be 20 °C.

2.3.1. Selection of data set

The experimental setup of the process, as well as the analytical procedures, have been described in Jimenez-Gutierrez et al. (2020) and (Jimenez-Gutierrez et al. 2021), where LHW pretreatment was applied at different conditions to poplar biomass at laboratory and pilot scale, up to almost complete hydrolysis of hemicellulose. The experimental data used in this work consists of values for the heating time, temperature and pretreatment time of each experiment, as well as measurements of pH and acetic acid concentration of the liquid fraction obtained after pretreating the lignocellulosic biomass. Additionally, for the temperature profile, experimental measurements of temperature over time at laboratory scale were used from Jimenez-Gutierrez et al. (2021).

2.3.2. Parameter fitting

To determine the kinetic parameters for the acetyl hydrolysis E_{A1} and A_1 in equation (15), the simulation model was compared with the experimental data and optimized for these parameters. During the simulation in MATLAB, each experiment in the dataset generates a value for the total acetic acid concentration (the sum of acetic acid and acetate ions at pretreatment temperature) as well as a value for the final pH after the processing time (heating plus pretreatment times). Experimental values of pH and C_{HAc} from Jimenez-Gutierrez et al. (2020) were measured at room temperature, while the simulated values of these two parameters were calculated at the pretreatment temperature. Thus, to make the values comparable, a conversion factor was applied, which consisted of the ratio between densities at room and pretreatment temperature.

Simulated (sim) and converted experimental (exp) values of pH and acetic acid concentrations for each experiment were compared by calculating the sum of (relative) squared estimate of errors with the following expression, in which the overbar indicates the average value:

$$SSE = \sum \left(\frac{C_{HAc.sim}^{end} - C_{HAc.exp}^{end}}{\bar{C}_{HAc,exp}} \right)^2 + \sum \left(\frac{pH_{sim}^{end} - pH_{exp}^{end}}{\overline{pH}_{exp}} \right)^2 \qquad (44)$$

The value of SSE obtained from equation (44) is then used as input for the optimization of the model, which determines the optimal values of E_{A1} and A_1 for a given set of experimental data by finding those for which the SSE value is minimum.

2.4. Characterization of biomass

Moisture content of the lignocellulosic biomass was determined by placing samples of milled biomass in the oven at 115 °C overnight. Difference in weight show a moisture mass fraction of 10.8%.

Composition of dried solid biomass was determined following a slightly modified methodology based on the NREL (National Renewable Energy Laboratory) laboratory analytical procedure (LAP) for determination of structural carbohydrates in biomass (Sluiter et al. 2008). A two-step acid hydrolysis process was carried out. Firstly, 1.5 g of the dried, milled and sieved solids (180 µm mesh size) were incubated with a 72% w/w solution of H_2SO_4 during 60 min at 30 °C and then, after dilution to 4% w/w, the mixture was autoclaved for 1 h at 121 °C. Once room temperature was reached, calcium carbonate was added to an aliquot of the filtered liquid (Whatman 0.2 µm) to neutralize the acid until pH reached 6-7. The neutralized liquid samples were filtered again and analysed by HPLC for determination of carbohydrates, whereas the acetyl content of biomass was determined by the acetic acid concentration of the non-neutralized liquid samples. Liquid samples were analysed on a Waters HPLC system with a Bio-Rad HPX-87H column (300 x 7.8 mm) at 60 °C. Phosphoric acid (1.5 mmol/L at 0.6 mL/min) was used as eluent. Glucose, xylose and acetic acid were detected by refraction index (Waters 2414). Quantification was done using external standards. Volumes of injection of standards and samples were 10 µL at 15 °C. All samples were analysed in triplicate. Lignin and ash content were not determined in this work.

2.5. Materials

Acetic acid (≥ 99.7%) and xylose (≥99%) were from Sigma-Aldrich, while D-Glucose (≥99.5%) was from Merck. The lignocellulosic material (poplar wood with stems and bark) was provided by Woodoo GmbH & Co. KG, through the Bioprocess Pilot Facility B.V, Delft, the Netherlands. Samples were pre-milled in a bench mill and sieved to a mesh size of ~1 cm.

3. Results and Discussion

In the system, two phases were initially considered: a wood chip phase and a bulk phase, representing respectively the lignocellulosic biomass and the liquid in the reactor. For simplicity, the solid woodchip phase was replaced by a liquid phase that represents the water in between the fibres of the woodchips, meaning that the corresponding chemicals reactions would take place in in this fibre liquid phase instead. After assessing this preliminary model, it was decided to consider only a single liquid phase.

To calculate the initial concentration of acetyl groups and glucuronic acid as single buffer, the composition of the selected biomass was required, and it is represented in Table 4.4. Our values are comparable to the values of Mante et al. (2014).

***Table 4.4**. Composition of poplar biomass. Values expressed in mass percentage, on dry basis (g/100 g DM).*

	This work	**Literature***
Glucan	41.34	42.73
Xylan	18.22	16.20
Acetyl group	4.25	4.25
Lignin	Not determined	26.14

*Source: (Mante et al. 2014)

3.1. Simulated Temperature Profile

Arrhenius-type equations were used to determine the conversion kinetics of the reactions. Thus, temperature inside the reactor is one of the key factors for the reaction rates. For the simulation, it was important to mimic the temperature profile similarly to the one observed in the laboratory experiments, as the relevance of heating period in this LHW pretreatment has been previously highlighted.

The experimental temperature profile from Jimenez-Gutierrez et al. (2021) was used as an average profile for the heating and cooling periods during the LHW pretreatment processes at 180 °C. The heating and cooling phases were fitted using equation (5) as temperature over time, by minimizing the difference between experimental and simulation temperature inside the reactor, obtaining a value of 2123 J/(m^3 K s) for the constant *c* for the heating, and 3120 J/(m^3 K s) for the cooling.

When comparing the experimental data of temperature over time for the LHW pretreatment at 180 °C for 120 min (Jimenez-Gutierrez et al. 2021) with the simulated temperature profile represented by equation (5), it was observed a slight difference at the end of the heating period (see Figure 4.S3, supplementary material). This may affect the conversion rates, as the acetyl hydrolysis reaction rate increases with temperature. Thus, an alternative simulation of the heating profile was also performed by minimizing the SSR between the simulated and experimental values of time, using the following expression derived from equation (5):

$$t = -\frac{(\rho \cdot C_p)}{C} \cdot ln\left(\frac{T_{set} - T}{T_{set} - T_0}\right) + t_0 \qquad (45)$$

Hence, the temperature dependencies of ρ and C_p were implemented within the integrated expression. In this case, values for the constant *c* differ slightly, obtaining 2528 J/(m^3 K s) for the heating phase and 3177 J/(m^3 K s) for the cooling part. The comparison between experimental and simulated temperature profile for this case is represented in Figure 4.1.

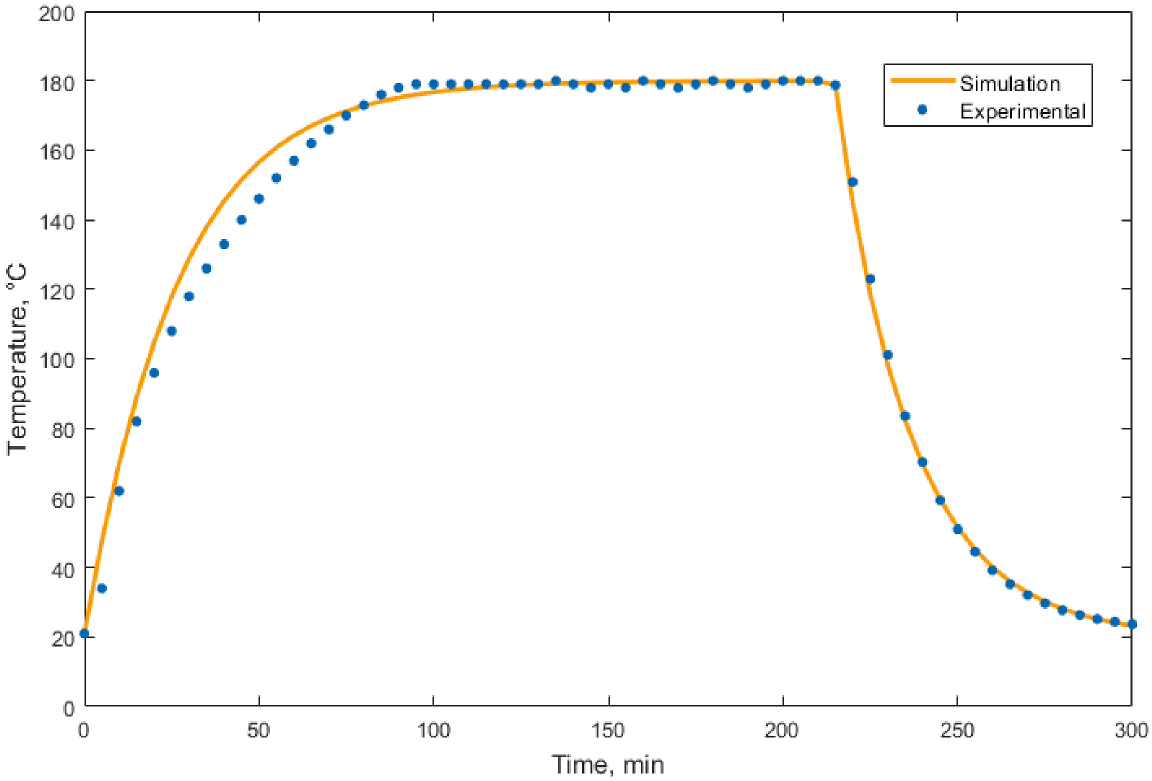

Figure 4.1. *Temperature profile from experimental and simulated LHW pretreatment process of poplar at 180 °C for 120 min, using equation (45).*

During the cooling period, temperature dropped sharply, decreasing gradually while reaching room temperature. Consequently, these mostly low reaction temperatures involve low reaction rates, due to the Arrhenius-type kinetics, and therefore the cooling period was assumed to be negligible for the acetyl hydrolysis simulation.

The heat capacity of the experimental setup did change slightly between experiments, due to small differences in application of the heating elements (placing the heating tape and the insulation fabric). Consequently, the experimental data used to fit the kinetic parameters have different heating times and temperature set values for each experiment. Then, temperature curves were adjusted just by setting the corresponding T_{set} value in equation (5), as it is assumed that the heat capacity of the system does not change. The slight differences in the heating time for experiments with the same T_{set} were balanced by using the average heat capacity of the experimental setup, determined by a 90 min heating period.

3.2. *pH and buffer effect*

Previous studies showed that experimental values of pH after LHW pretreatment of lignocellulosic biomass were higher than expected on basis of the concentrations of acetic acid obtained, suggesting the presence of buffering compounds (Jimenez-Gutierrez et al. 2020). Thus, a buffering species is required and has been used to adjust the pH during the simulation. In this work, the buffering capacity of the pretreated mixture was evaluated by considering glucuronic acid as a single buffer component, gradually release from the biomass with similar ratio as the acetyl hydrolysis, as a good estimation.

However, it would be expected that the presence of salts (Du et al. 2014) and carbohydrates, as well as other uronic acids and degradation compounds, such as formic, levulinic or lactic acid (Burtscher et al. 1987) (Gao et al. 2016), may affect substantially the final pH of the pretreated biomass. Even the untreated biomass presents a high buffer capacity due to its ash content (Pascoli et al. 2021). In any case, to keep the model simplified, the single buffering component estimation was implemented, which in turn might be used with comparable species.

Glucuronic acid content in poplar biomass was not measured experimentally in this work. Instead, the initial concentration of buffer was calculated based on a 1:10 xylose/HGlucA molar ratio (Sjöholm et al. 2000) (Wyman et al. 2005). Additionally, glucuronolactone was also introduced as the degradation product of the buffering component due to high severity of the process. These two species might not seem enough to represent accurately the reality, as the pretreated biomass contains other compounds that may contribute to the buffering effect on the pH during pretreatment. In fact, this complex matrix of compounds might be the explanation of some discrepancies found when comparing the experimental values of pH with those obtained from the simulation of our system at similar conditions.

Furthermore, originally the glucuronic acid branches (including the 4-*O*-methyl glucuronic acid) are not attached by ester bonds to the xylose backbone. This leaves the carboxylic groups partly free, which may contribute to certain initial acidity of the system (Bajpai 2018), although as it was previously discussed, most of them would be esterified to cross-link lignin (Terrett and Dupree 2019).

Additionally, the model considers the degradation of glucuronic acid to glucuronolactone due to the elevated temperatures used. However, knowing that the molar ratio of 4-*O*-methyl glucuronic acid to glucuronic acid in poplar biomass is around 1:20 (based on the analysis performed by Sakamoto et al. (2015)), the single buffer component considered in our model might not be fully degraded, if this 4-*O*-methylated fraction is accounted as non-degradable.

Despite that these and many other mechanisms should be taken into account in order to evaluate accurately the buffer capacity of this complex system, using the glucuronic acid and the 4-*O*-methyl glucuronic acid together, in combination with the glucuronolactone, in the simulation seem to work fine as “ideal” buffer, as one could see in the next section.

3.3. *Kinetic fitting*

The model described in this work was fitted to experimental values of pretreatment temperature and time, as well as measurements of pH and acetic acid concentration of the biomass hydrolysate after LHW pretreatment, to obtain the kinetic parameters for the acetyl hydrolysis. In order to evaluate more accurately the acetyl hydrolysis of the poplar biomass, the dataset described in section 2.3.2 was reduced to experiments performed at 160 °C or higher. After the fitting, the values for the kinetic parameters of the acetyl hydrolysis were 92.2 kJ/mol for the activation energy E_{A1}, and $1.95 \cdot 10^{11}$ mol/(L·s) for the frequency factor A_1. Figure 4.2A and 2B compare simulated and experimental values of acetic acid and pH for the LHW pretreatment at 180 °C, showing a good correspondence. Similar results were noticed for pretreatment at 160 °C and 200 °C (not shown). It can be observed that the gradual release of the buffer caused a sharp drop of in pH, resulting in an earlier conversion in the simulation at lower temperature. Besides, it also shows that the simulated pH remains stable during the reaction, since the buffer was released at the same rate as the acetic acid. However, while the correlation for the acetic acid concentration in the reactor was well fitted, the experimental pH values were not that close to the simulation line, suggesting that an alternative releasing mechanism of the single buffer component may improve the fitting with the experimental pH values. Perhaps taking into account the formic or levulinic acid formation (Jimenez-Gutierrez et al. 2020) would decrease the simulated pH too, which could be implemented by indicating a fraction of these degradation compounds as a percentage relative to acetic acid.

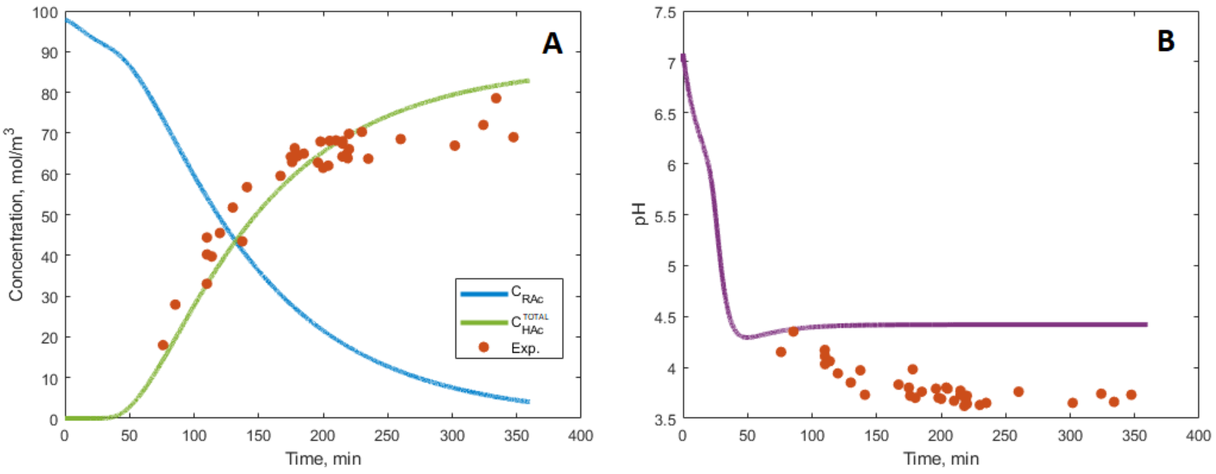

Figure 4.2. *Experimental and simulation values of acetic acid (A) and pH (B) over time, obtained after liquid hot water pretreatment of poplar biomass at 180 °C.*

On the other hand, in spite of the simulation describe quite well the acetic acid release during LHW pretreatment, the simulated values of acetic acid concentration during this pretreatment process, compared to the experimental ones, seem to be slightly underestimated at shorter pretreatment times while oppositely overestimated at longer pretreatment times, as can be observed in Figure 4.3A. From a mathematical viewpoint, the relation between acetic acid concentration and pH are proportionally inverse in the optimization function, meaning that optimizing the kinetic parameters using these two variables results in a compensation of both. Therefore, by decreasing the frequency factor of the acetyl hydrolysis, the simulated values of pH get closer to the experimental ones, at the expenses of increasing the difference from simulation to experimental in acetic acid concentration. Consequently, with our current model structure, it is not possible to find a perfect fitting for the acetic acid concentration and the pH simultaneously. Although the optimization function used in the computational simulation seems to balance both proportionally, results are strongly dependent on the initial given values of the kinetic parameters for the iterations. Several pairs of values were tested and the final selection was done based on the fitting for the acetic acid concentration, getting as expected a not so good correlation for the pH (Figure 4.2A, B).

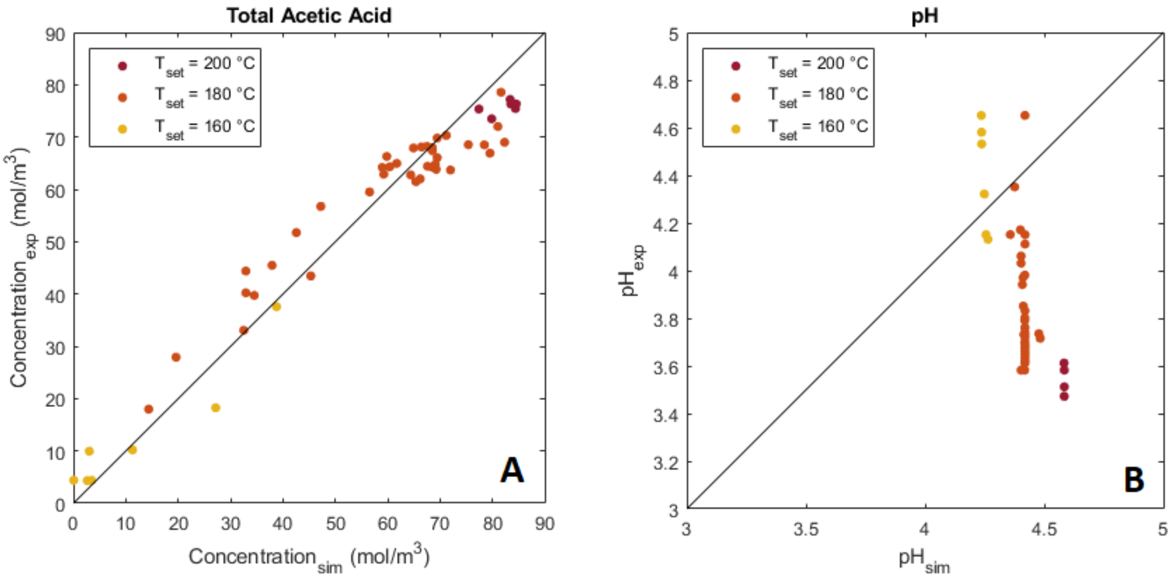

Figure 4.3. *Parity plot comparing experimental and simulation values of acetic acid concentration (A) and pH (B) in the biomass hydrolysate after LHW pretreatment process at different temperatures.*

Since there were these discrepancies when fitting the pH and acetic acid concentration, during the optimization the system converges into a very similar point, due to the considerable differences between experimental and simulated values (especially for the pH fitting). The glucuronic acid releasing estimation may not be enough to describe the buffering capacity of the complex mixture and a modification of the releasing mechanisms might compensate these differences. Besides, the tolerance selected for the optimization function may also influence the final results, regarding accuracy and running time for each simulation test. Moreover, results from the optimization are also strongly dependent on the buffer concentration, or by extension, on the buffering mechanism. Thus, different alternative cases described below were also tested to see if better fitting was achievable.

The model considers both glucuronic acid and 4-*O*-methyl glucuronic acid species combined as a single buffer component. As it was previously discussed in section 3.2, the methyl ether form of the uronic acid is assumed to be responsible for certain initial acidity for the untreated biomass (Bajpai 2018). Previous experiments of soaking poplar biomass with water for certain amount time (Jimenez-Gutierrez et al. 2020) confirmed a slightly acidic pH (around 6.8) prior the pretreatment. This initial value was also included in the simulation as initial concentration of hydronium ions, but there was not much difference in the outcome.

Alternatively, the amount of glucuronic acid released during the pretreatment in the model may not be fully susceptible of degradation to glucuronolactone, as it has been already indicated that the presence of 4-*O*-methyl glucuronic acid is 20 to 1 (molar based) regarding the glucuronic acid content in poplar (Sakamoto et al. 2015). This case was also tested in the software by reducing proportionally the amount of glucuronic acid degraded to glucuronolactone, but again the results did not improve much.

Another case considered was slowing down the release of the glucuronic acid, which after the optimization showed a better fitting for the pH between simulated and experimental, but the acetic acid concentration curve deviated considerably from the experimental points.

In a further attempt, the initial buffer concentration was introduced as an extra optimizable variable in the simulation. Results from this case showed a better fitting for both the acetic acid concentration and the pH (supplementary material). Concerning the kinetic parameters, the value for the activation energy was in the same order but halved, while the frequency factor was 10^5 times lower. Nevertheless, the initial buffer concentration in biomass was 10 times lower than calculated from literature.

After all these alternative possibilities, and keeping the simplify model with one unique single buffer, for the purpose of this work it was finally decided to focus on optimizing the acetic acid concentration (at the expenses of the pH fitting).

3.4. Implications: dilute acid hydrolysis

Since the hydronium ions catalyse the acetyl hydrolysis, lowering the pH by initial addition of acid should speed up the pretreatment process. This methodology similar to LHW is known as acid pretreatment and it is the most employed technique to disrupt the lignocellulosic biomass (Solarte-Toro et al. 2019). While extensive research has been done based on dilute sulfuric acid pretreatment (Mosier et al. 2005), some other authors have studied the use of acetic acid to successfully solubilize a high percentage of the hemicellulose in corncobs (Zhang et al. 2017) and poplar sawdust (Huang et al. 2018). In this section, dilute acetic acid pretreatment was explored as an alternative methodology to increase the acetyl conversion rate, and therefore to lower the processing time as well as consequently the equipment and operating costs.

Thus, the model described in this work was use to predict the behaviour of the system for a dilute acetic acid pretreatment. The amount of acid may vary depending on the conditions applied (Esteghlalian et al. 1997). In this simulation, 1% w/w of acetic acid was selected for the initial concentration, which was quickly converted into H^+ via the acetic acid equilibrium, and corresponded to an initial pH of 2.76. Results from the dilute acetic acid simulation predicted a decrease of 53% in time for the reaction to reach 90% acetyl conversion ($t_{90\%}$).

Previous studies indicated 90% conversion rate after 30 min for dilute acid of poplar wood using 4% sulphuric acid compared to 90 min for LHW pretreatment (Li et al. 2014). Those results show that a sharp decrease in the initial pH can contribute significantly to the hydrolysis rate during pretreatment, and therefore suggest a potential reduction in operational costs. Accordingly, further simulations might be used for optimizing the cost of the acid or a possible recycling from the downstream processing. However, our model may lack in accuracy for prediction on dilute acetic acid pretreatment, because the dataset used for the fitting contained pH 3.3 as lowest value, while dilute acid pretreatments generally are initiated below pH 2.7. A similar model fitted with a larger range of data might be useful to validate the current model for such optimisation routines.

3.5. Temperature effect

The acetyl hydrolysis reaction rate is highly dependent on the temperature in the reactor. The sensitivity of the system to the pretreatment temperature was assessed by comparing results after simulation at three different temperatures (160 °C, 180 °C and 200 °C). Results from the concentration of acetic acid confirmed indeed that changes in temperature had a substantial effect on the acetyl hydrolysis rate, as higher values of temperature increased the acetyl conversion (Figure 4.3A). Regarding the pH, one could expect a similar effect, which was perceived during the heating period of the reaction by a quicker drop in the pH curve at higher temperatures. However, after this heating stage, it was observed that an increase in the temperature resulted in a higher average pH curve, due to the temperature dependencies of the equilibrium reactions for the acetic acid and water dissociation. Consequently, this highlights the importance of the temperature dependencies for modelling the pH.

The acetyl conversion rate was investigated by determining the time for 90% conversion ($t_{90\%}$) for 160 °C and 200 °C reaction temperatures, and compared them to the 180 °C case, obtaining an increase in 91% and a decrease in 38% respectively. Similar results in acetyl hydrolysis rate were seen in literature (dos Santos Rocha et al. 2017) (Vallejos et al. 2015). Therefore, the relevance of the temperature fitting during the heating phase is reasserted.

3.5.1.Heating phase

Frequently LHW pretreatment processes involve relatively short heating times compared to the total reaction time (Ahmad et al. 2016), and consequently the conversion during this heating phase is often neglected. On the other hand, when the heating period is significantly larger (Carvalheiro et al. 2005) (Jimenez-Gutierrez et al. 2021), relevant conversion may occur prior to reaching the pretreatment temperature.

As described in section 3.1, the heating phase of the LHW pretreatment was simulated, obtaining an average temperature profile (Figure 4.1). Results from the simulation using the fitted kinetic parameters indicate that the acetyl conversion begins after 35-40 min approximately. At his point, the reactor is about to reach 140 °C, suggesting it as the starting temperature for relevant acetyl hydrolysis, which is determined by the activation energy of the kinetic constants. A similar result was found by Jimenez-Gutierrez et al. (2020) using the same dataset, confirming the fitting of this model.

Based on the results obtained from the simulation, the conversion of acetyl groups during the heating period was a substantial amount of the total acetyl content in the system, with almost 33% converted during the heating period. Hence, this cannot be disregarded, as long as the heating phase involves a long timeframe between 140 °C and the reaction temperature, which in our case was for around one hour. Nevertheless, it is important to mention that for the simplification of the fitting, a total "processing temperature" was used as the addition of the heating and pretreating times, regardless the duration of the heating, for each experiment in the dataset. By doing this, the experimental heating time was integrated within the processing time, and therefore taken into account for the conversion.

3.5.2. Cooling phase

Similar to the heating, the cooling phase involves a certain period between 140 °C and the pretreatment temperature, though shorter. Acetyl conversion during the cooling phase was calculated by running the model for 300 min processing time (heating and pretreating), then changing the set point to room temperature and simultaneously switching the heating constant c to its cooling value (see section 3.1). Some conversion of acetyl groups was detected during the first part of the cooling phase, but it decreased rapidly as the temperature dropped steeply below 140 °C. The amount of conversion during the cooling period was around 1.5% of the total acetyl groups in the biomass, which could be safely neglected.

During the cooling phase a small drop in the pH curve was also observed, due to the temperature dependencies of the equilibrium constants. This small dip of pH increased slightly the conversion just after the temperature went below 140 °C, as the pH became low enough to facilitate some acetyl hydrolysis. However, the temperature quickly dropped further, and the conversion stopped.

4. Conclusions

The model proposed in this work seems adequate to satisfactorily predict the acetic acid production through LHW pretreatment. The single buffer component approach is an acceptable approximation, although the releasing method involved should be improved to suit the complexity of the buffering capacity for this system, as this model is strongly dependent on the buffering conditions. Some discrepancies were observed when optimizing the fitting of simulated to experimental values of concentration of acetic acid and pH, which indicate multiple factors affecting the buffering capacity. Temperature is one of the most relevant parameters for the design and optimization of LHW pretreatment processes. While the cooling phase might be negligible, significant acetyl hydrolysis occurs at the end of the heating period.

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Supplementary Material

Temperature dependency of specific heat capacity and density of pure water

Based on Gmehling et al. (2019), the specific heat capacity of water (in J/(mol K)) can be correlated with temperature by the polynomial formula in equation (S1):

$$C_p = R\left(\frac{A}{\tau} + B + C\tau + D\tau^2 + E\tau^3 + F\tau^4\right), \quad \tau = 1 - \frac{T}{T_c} \tag{S1}$$

Where T_c is the critical temperature (647.096 K), and R is the universal gas constant (8.134471 J/(mol K)). This expression is valid for temperatures between 273 K and 586 K. Values for the parameters are represented in **Error! Reference source not found.**.

Table 4.S1. *Values of parameters for specific heat capacity correlation.*

	A	**B**	**C**	**D**	**E**	**F**
Water	0.255980	12.545950	-31.408960	97.766500	-145.423600	87.018500

Density of liquid water (in kg/m^3) is described as function of temperature by Gmehling et al. (2019) in equation (S2), with T in Kelvin and values for the parameters in Table 4.S2:

$$\rho = 18.015 \cdot \sum_{i=1}^{5} A_i(T)^i \tag{S2}$$

Table 4.S2. *Values of parameters for water density correlation.*

A0	**A1**	**A2**	**A3**	**A4**	**A5**
-13.418392	0.6884103	$-2.44970115 \cdot 10^{-3}$	$3.7060667 \cdot 10^{-6}$	$-2.11062995 \cdot 10^{-9}$	$-1.12273895 \cdot 10^{-13}$

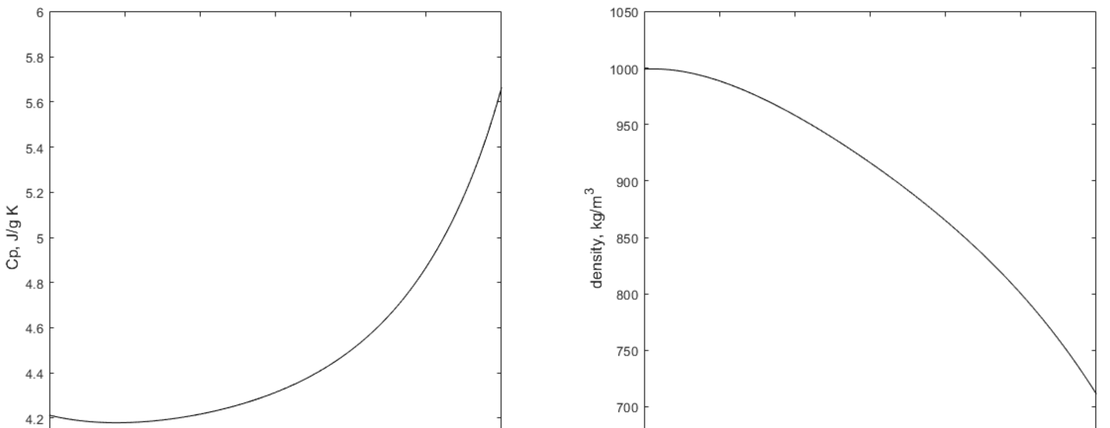

Figure 4.S1. *Temperature dependency of density and specific heat capacity of pure water (calculated from Gmehling et al. (2019)).*

Calculating the value of the combined constant *c*

To simulate the same temperature gradient as the experimental data, the c constant needs to be determined, using equation (S3).

$$\frac{dT}{dt} = \frac{c}{\rho \cdot C_p} \cdot (T_{set} - T_{int}) \tag{S3}$$

Because ρ and C_p are temperature dependent, both values were determined for every time step using experimental data. It was required an equation for temperature over time, which was calculated by integrating the energy balance in equation (S3):

$$\int_{T_0}^{T} \frac{dT}{(T_{set} - T)} = \frac{c}{\rho \cdot C_p} \cdot \int_{t_0}^{t} dt \tag{S4}$$

$$-ln\left(\frac{T_{set} - T}{T_{set} - T_0}\right) = \frac{c}{\rho \cdot C_p} \cdot (t - t_0) \tag{S5}$$

$$T_{set} - T = (T_{set} - T_0) \cdot e^{\frac{-c}{\rho \cdot C_p} \cdot (t - t_0)} \tag{S6}$$

$$T(t) = T_{set} - (T_{set} - T_0) \cdot e^{\frac{-c}{\rho \cdot C_p} \cdot (t - t_0)} \tag{S7}$$

Experimental data for the temperature profile was used, consisting of measurements of the internal temperature of the reactor with a time step of 5 minutes. Instead of including the temperature dependency of ρ and Cp in the energy balance (S7), their values were calculated individually for each time step using the measured temperature in equations (S1) and (S2). This resulted in a sufficiently accurate data fit for the determination of the value of c.

Initial concentration of acetyl groups and glucuronic acid in biomass

The initial concentration of acetyl groups $C_{RAc,0}$ was determined using the molar mass of acetyl groups and the mass fraction water/poplar wood. Therefore, the composition of the selected biomass was required, represented in Table 4.S3.

Table 4.S3. *Composition of poplar biomass. Values expressed in mass percentage, on dry basis (g/ 100 g DM).*

	This work	Literature*
Glucan	41.34	42.73
Xylan	18.22	16.20
Acetyl group	4.25	4.25
Lignin	Not determined	26.14

*Source: (Mante et al. 2014)

The rest of the calculation is described as follow:

$$M_{Ac} = 43.045\ \frac{g}{mol} \qquad X_{water}^{wood} = 0.108\ \frac{kg\ water}{kg\ wood}$$

$$Poplar\ Biomass = 0.0425\ \frac{kg\ acetyl\ groups}{kg\ dry\ wood}$$

$$Acetyl\ content = \frac{0.0425\ \cdot 1000}{43.045} = 0.9873 \frac{mol}{kg\ dry\ wood} = 0.8807 \frac{mol}{kg\ wood}$$

Regarding the initial concentration of glucuronic acid as single buffer component $C_{base,0}$, the molar ratio of xylose/glucuronic acid used was 10:1 as average from values on literature (Sjöholm et al. 2000) (Zhou et al. 2017). From the xylan content to xylose concentration, the correction factor was 0.88. Therefore, from Table S3, the xylose content on dry basis in biomass would be 20.70 % w/w.

The rest of the calculation is described as follow:

$$M_{Xylose} = 150.13\ \frac{g}{mol} \qquad X_{HGlucA}^{Xylose} = 10 \frac{mol\ Xylose}{mol\ HGlucA}$$

$$Poplar\ Biomass = 0.207\ \frac{kg\ Xylose}{kg\ dry\ wood}$$

$$Glucuronic\ acid\ content = \frac{0.207\ \cdot 1000}{150.13} \cdot \frac{1}{10} = 0.138 \frac{mol}{kg\ dry\ wood} = 0.123 \frac{mol}{kg\ wood}$$

The reaction mixture has a 1:9 biomass/water mass ratio. Therefore, using the density of pure water at room temperature, the initial concentration of acetyl groups $C_{OAC,0}$ and buffer $C_{base,0}$ is calculated as:

$$C_{RAc,0} = \frac{0.8807 \frac{mol}{kg\ wood}}{0.009 \frac{kg\ wood}{m^3\ water}} = \mathbf{97.856}\ \frac{\mathbf{mol}}{\mathbf{m^3}} \qquad C_{base,0} = \frac{0.123 \frac{mol}{kg\ wood}}{0.009 \frac{kg\ wood}{m^3\ water}} = \mathbf{13.666}\ \frac{\mathbf{mol}}{\mathbf{m^3}}$$

Graphical representation of temperature dependency of equilibrium constants

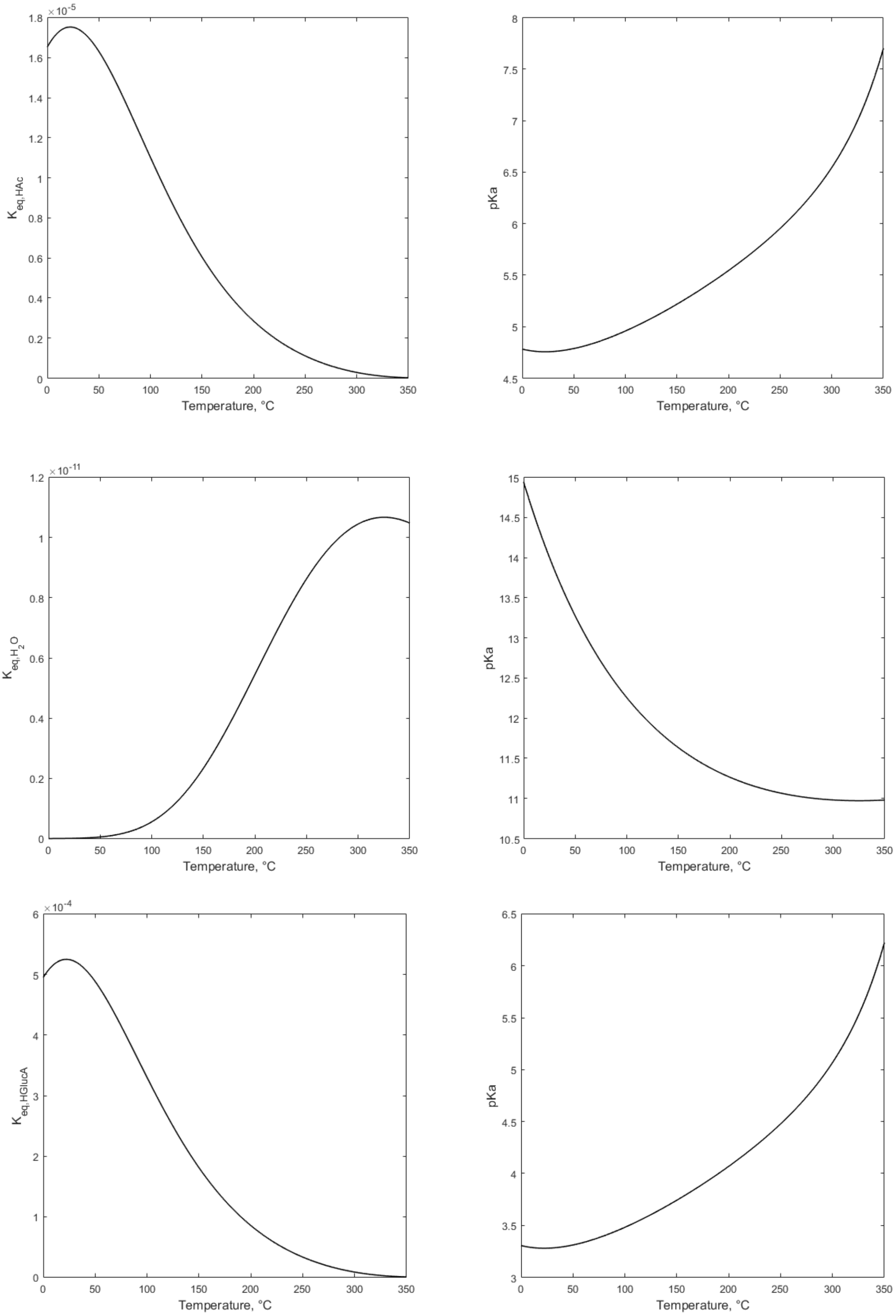

Figure 4.S2. *Temperature dependency of acetic acid, water and glucuronic acid dissociation reactions.*

Alternative temperature profile fitting

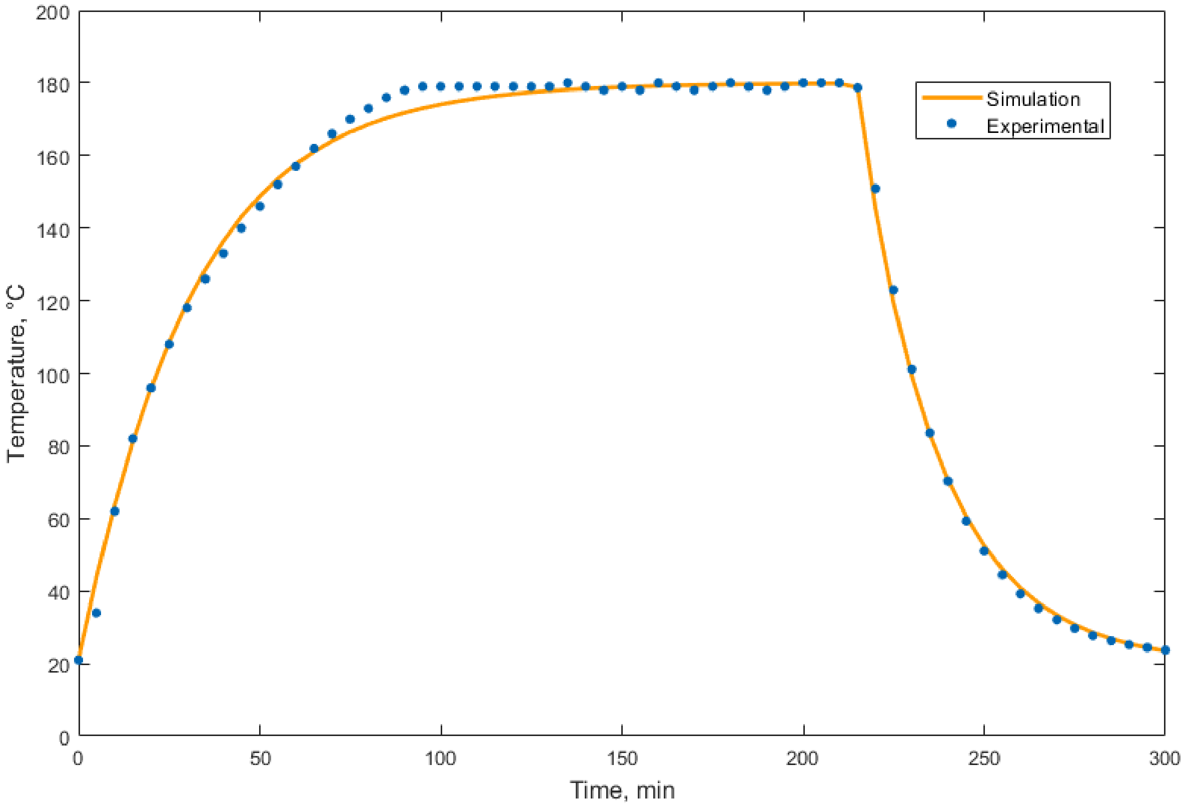

Figure 4.S3. *Temperature profile from experimental and simulated LHW pretreatment process of poplar at 180 °C for 120 min, using expression in equation S7.*

Results from optimization of kinetic parameters and initial buffer concentration

When optimizing the kinetic parameters for the acetyl hydrolysis simultaneously with the initial buffer concentration, the values obtained were 54.13 kJ/mol for the activation energy E_{A1} and $1.39 \cdot 10^{6}$ mol/L·s for the frequency factor A_1, while the optimized initial buffer concentration of glucuronic acid was $1.78 \cdot 10^{-3}$ mol/L. These values for the kinetic parameters are in a similar range from previous research on acetyl hydrolysis of poplar (Zhang and Runge 2012). It can be observed in Figure 4.S4 and Figure 4.S5 that the fitting with experimental values is quite good for both the acetic acid concentration and the pH, noticing that the simulation for the former was slightly underestimated. On the other hand, experiments with a shorter pretreatment time were not fully well represented, as the experimental pH was higher than the simulation.

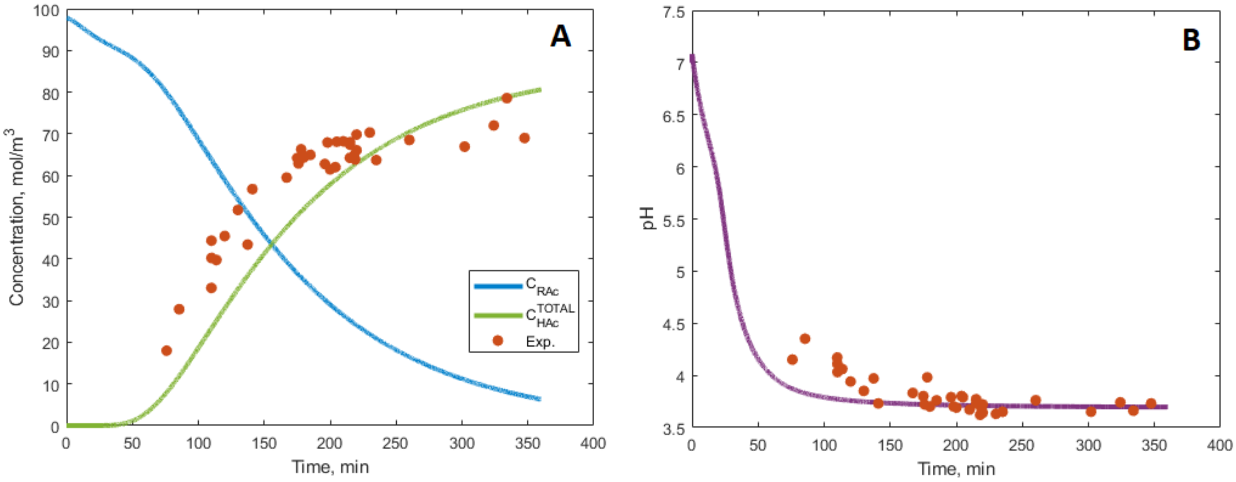

Figure 4.S4. Experimental and simulation values of acetic acid (A) and pH (B) over time, obtained after LHW pretreatment of poplar biomass at 180 °C (when optimizing kinetic parameters and initial buffer concentration).

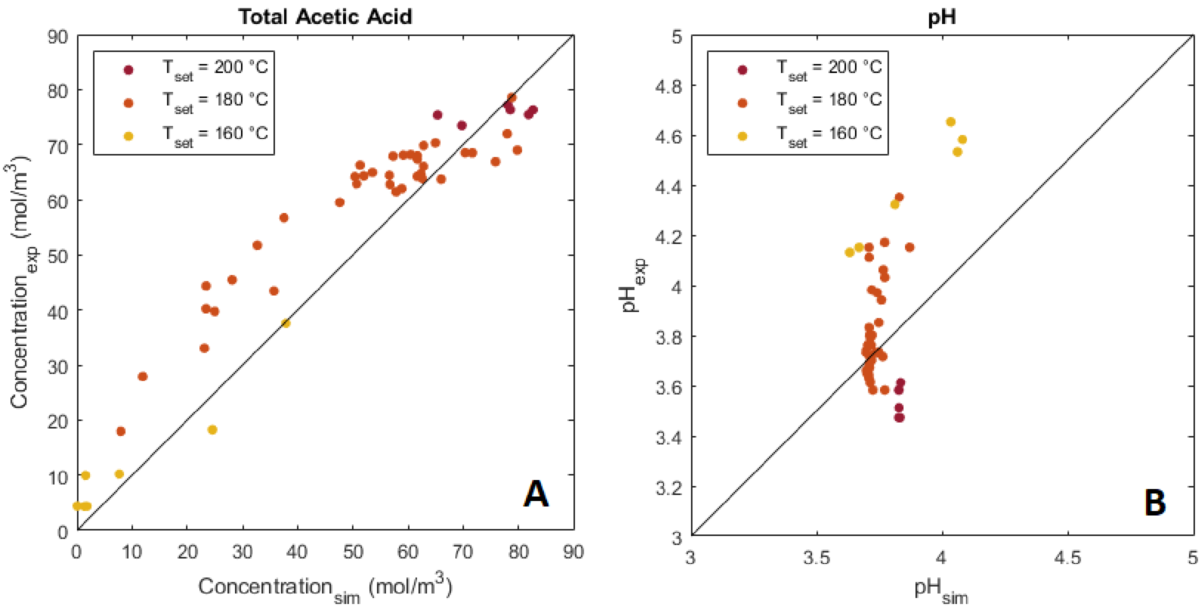

Figure 4.S5. Parity plot comparing experimental and simulation values of acetic acid concentration (A) and pH (B) in the biomass hydrolysate after LHW pretreatment process at different temperatures (when optimizing kinetic parameters and initial buffer concentration).

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Chapter 5

Dilute acetic acid pretreatment of poplar biomass

Abstract

Diluted acid hydrolysis is an alternative methodology to liquid hot water pretreatment of lignocellulosic biomass, typically with the addition of a strong acid such as sulfuric acid. Adding a weak acid instead, such as acetic acid, might be an alternative option, because it might provide the low pH required. In this work, dilute acetic acid was compared with liquid hot water pretreatment at similar conditions. Both methods have analogous results for longer processing times, and no much difference was found regarding the acetic acid release. However, shorter pretreatment times for the dilute acetic acid showed an enhanced hydrolysis with higher glucose and xylose concentrations, as well as their degradation compounds. Since acetic acid is obtained from the hydrolysis of acetyl groups in hemicelluloses during pretreatment, the recirculation of the liquid fraction after the liquid hot water pretreatment was also explored for longer processing times, performing three cycles and obtaining a linear release of acetic acid.

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1. Introduction

The path towards circular economy is not exempt from challenges but opportunities too, where renewable resources are used to obtain valuable products (Hassan et al. 2019). This concept of biorefinery involves the fractionation and production of different compounds from a sustainable perspective, with great potential allocated to the second generation feedstocks as starting point (FitzPatrick et al. 2010). Lignocellulosic biomass (LB) is usually perceived as the most promising source for biochemical activities, due to their abundance, worldwide availability and price, despite the drawbacks, such as the recalcitrance of its structure or the extensive range of variability in composition.

Targeting the disruption of the LB complex matrix and releasing oligosaccharides to enhance accessibility for a further enzymatic hydrolysis into fermentable sugars, the pretreatment has been frequently indicated as one of the crucial steps within the biorefinery process (Moreno et al. 2019). In the past decades, several pretreatment techniques have been extensively researched and classified based on the type of the process involved (Kumar and Sharma 2017) (Baruah et al. 2018).

Previous studies have highlighted the potential of hydrothermal pretreatment methods, due to the mild conditions applied and the lack of addition of extra chemicals, in combination with their green, eco-friendly, low-cost and low corrosion to equipment features (Yue et al. 2022). With the major effect on hemicellulosic fraction depolymerisation, the mechanism involved is considered a combination of the auto-ionization of water at high temperatures and pressures, with the release of carboxylic acids from the LB. Both effects cause the reduction of pH required for an acid hydrolysis (Yue et al. 2022).

Acetic acid (HAc), which could be considered responsible for the major contribution to that low pH during the hydrothermal pretreatment, is generated by hydrolysis of the acetyl groups present in the hemicellulose structure (Jimenez-Gutierrez et al. 2021) (Negro et al. 2003). The presence of these acetyl groups within the polysaccharides in LB is directly related to their properties and resistance (chemical, physical and biological) (Gille and Pauly 2012) (Huang et al. 2017). Depending on the conditions applied during the pretreatment process, there might be a significant hemicellulose depolymerisation, although at the expenses of side reactions, which produced important amounts of degradation compounds, such as furfural or 5-hydroxymethyilfurfural (HMF) (Pienkos and Zhang 2009). Besides, lignin from delignification and re-polymerisation after pretreatment (Yoo et al. 2020), and humins as condensation products of HMF with sugars (Steinbach et al. 2017), can be also found in the solid residues (Kang et al. 2018). All these compounds generally are inhibitors for the subsequent enzymatic hydrolysis and fermentation steps in the biorefinery process (Ko et al. 2015). Thus, reducing their presence from the pretreated fraction is commonly desired and needed in order to achieve a reasonable saccharification and fermentation (Klinke et al. 2004).

1.1. *Dilute acid pretreatment*

Acid pretreatment methodologies use the similar hydronium-based hydrolysis reaction as hydrothermal methods (Ruiz et al. 2020). Therefore, both pretreatment processes are analogous, regarding relative high temperatures, though with the main difference of the addition of an acid pretreatment time can be shortened or pretreatment temperature can be decreased. This catalyst might be a mineral acid (sulfuric, hydrochloric, nitric, phosphoric) or an organic one (formic, acetic, propionic, maleic) (Behera et al. 2014). Depending on its concentration, the process may be considered concentrated or dilute acid pretreatment (Jung and Kim 2015). While concentrated acid pretreatment uses lower operational costs than dilute acid, in terms of lower temperature, process equipment must be resistant to corrosion, which involves extra costs (Jung and Kim 2015). In both cases, inhibitory compounds are formed, although to less extent during dilute acid pretreatment. On the other hand, concentrated acid pretreatment reduces cellulose crystallinity while dilute acid removes only the hemicellulosic fraction of LB, with the consequent requirement of an enzymatic saccharification step to complete the cellulose hydrolysis (Solarte-Toro et al. 2019).

From an economic and environmental perspective, dilute acid pretreatment presents more advantages than the concentrated acid pretreatment. Dilute acid pretreatment technologies have been extensively studied and currently applied, giving important results in dissolving hemicelluloses, and consequently in xylan recovery (Allen et al. 2001) (Cao et al. 2012). Moreover, almost full deacetylation and hemicellulose removal have been reported when using dilute sulfuric acid pretreatment on poplar biomass (Kumar et al. 2009). Hence, due to its simplicity and effectiveness, dilute acid pretreatment is considered the technology closest to commercialization (Jung and Kim 2015), together with hydrothermal techniques (Chandel et al. 2021).

Typical operating conditions comprise relative high temperatures (120 – 215 °C) and pressures (0.2 – 1 MPa), low acid concentrations (0% w/w – 5% w/w), and a wide range of pretreatment times (from 10 to 120 min) (Solarte-Toro et al. 2019). Compared to hydrothermal methods, such as liquid hot water (LHW), dilute acid pretreatment implicates less energy consumption to produce the disruption and modification of the lignin structure and almost completely hydrolysis of the hemicellulose fraction, with the additional benefit of partial cellulose solubilisation (Jung and Kim 2015) (Wen et al. 2019). On the other hand, LHW pretreatment without addition of an acid releases less inhibitors (Dimitrellos et al. 2020).

Some authors have compared LHW with dilute sulfuric acid pretreatment (Allen et al. 2001), obtaining similar results for both cases, though with most favourable conditions for the latter, meaning lower processing temperature. Additionally, longer pretreatment time was observed as most favourable for the dilute acid than for the LHW (Allen et al. 2001), which was unexpected as, in general, the addition of an acid to the pretreatment aims the reduction in processing time.

1.2. Acetic acid as catalyst

Previous studies have showed faster hemicellulose hydrolysis when using dilute sulfuric acid, although at the expense of producing more inhibitory compounds (Qin et al. 2012) (Jackson de Moraes Rocha et al. 2011). The use of weak organic acids, such as acetic acid, seems to be a potential alternative to avoid this extensive side product formation.

Concentrated acetic acid pretreatment has been previously studied (Rattanaporn et al. 2017). Some authors used concentrated HAc for xylan hydrolysis, recovering mostly xylooligosaccharides (XOS) and traces of xylose and furfural (low sugar degradation), with the reaction temperature as the most significant factor for the XOS yield (Zhang et al. 2017).

In other studies (Zhang et al. 2017), HAc provided the highest XOS yield compared to sulfuric and hydrochloric acid at similar conditions (temperature 150 °C, pH 2.7 and 30 min reaction time), as well as formation of other products such as xylose, formic acid, furfural and HMF.

Other authors pretreated poplar sawdust at 170 °C for 30 min using HAc at 5% v/v, removing more than 70% of the xylan in the biomass and obtaining mainly XOS, xylose and furfural, which enhance the further enzymatic hydrolysis of cellulose (Huang et al. 2018). The solid pretreated fraction was washed prior to saccharification in order to eliminate the inhibitory effect of HAc, as well as the other degradation compounds.

In any case, a synergistic effect was observed for acetic acid pretreatment in combination with an enzymatic hydrolysis step upon co-production of XOS and fermentable sugars (Zhang et al. 2017) (Huang et al. 2018).

A limited removal effect of lignin during acetic acid pretreatment has been reported before too (Zhang et al. 2016), as well as cellulose acetylation (Espinoza-Acosta et al. 2014). Furthermore, some authors found degradation, acetylation and condensation of a significant fraction of lignin after acetic acid pretreatment of poplar (Wang et al. 2021) (Pan et al. 2006). Thus, the inhibitory effect of the residual lignin to cellulose digestibility was enhanced by using acetic acid as pretreatment catalyst.

A possible solution might be proceeding with a delignification after the acetic acid pretreatment, proposed by adding an alkali treatment, which in fact improved the cellulose accessibility and enzymatic hydrolysis (Lai et al. 2019).

Peng et al. (2019) reported significant changes in lignin structure, as well as improvement in biomethane yield when pretreating rice straw with dilute HAc at low temperature (80°C) but longer processing times (24-48 h).

In combination with hydrogen peroxide, acetic acid pretreatment has been also applied to lignocellulosic biomass, aiming the selective removal of lignin to enhance enzymatic hydrolysis (Tan et al. 2010). A synergistic effect was reported, regarding production of XOS and fermentable sugars, when proceeding with a first HAc pretreatment of poplar and a consequent delignification step using hydrogen peroxide-HAc (Wen et al. 2019).

Alternatively, addition of sulfuric acid to the hydrogen peroxide-HAc pretreatment resulted more suitable for a two-step enzymatic hydrolysis of poplar to produce XOs and glucose (Hao et al. 2020).

As abovementioned, HAc is usually one of the most abundant inhibitors obtained during hydrothermal pretreatment processes. Using dilute HAc pretreatment instead of hydrothermal methods, such as LHW, might be interesting for several reasons. Apart from providing the mild-low pH necessary for the pretreatment, HAc is already produced *in situ*, unlike other organic acids, thus no new compounds are added, and the amount of pre-catalyst can be reduced (Zhao et al. 2014). Since HAc is released during degradation of hemicelluloses, its content increase from the initial concentration along with the pretreatment process, leading to higher HAc concentrations after the pretreatment (Qin et al. 2012). Furthermore, an interesting option might be aiming two simultaneous objectives: improve the digestibility of the lignocellulosic material and at the same time, produce, separate and refine the acetic acid, which could be considered generated from a green source.

Alternatively, recycling the liquid fraction obtained after LHW pretreatment might be considered as a dilute acetic acid pretreatment, because it contains certain amount of HAc, and therefore its concentration, as well as the other side compounds, may accumulate along the pretreatment process, increasing the possible added value once separated. On the other hand, to recover these side products, and hence embrace that potential revenue associated to them, a relative high concentration might be required, in addition to a concentration and a separation steps within the downstream process (Jönsson and Martín 2016).

In previous work (Jimenez-Gutierrez et al. 2021), results suggested full deacetylation of poplar after certain set of conditions. Concentrations of HAc were up to 5 g/L after LHW processing at 200°C and 90 min. In this work we evaluate the effect of dilute HAc addition on LHW pretreatment of LB in order to achieve higher HAc concentrations. Additionally, comparable to the dilute acid pretreatment, the liquid fraction obtained from previous pretreatment steps is used several times, emulating a recycling in the process design. Poplar wood was selected as LB source due to its relative high acetyl content.

2. Materials and Methods

2.1. Materials

Acetic acid (≥99.7%), furfural (≥99%), 5-hydroxymethylfurfural (HMF) (≥98%) and xylose (≥99%) were from Sigma-Aldrich. D-Glucose (≥99.5%) was from Merck. Formic acid (≥98%) was from Honeywell Fluka. The lignocellulosic material (poplar wood with stems and bark) was provided by Woodoo GmbH & Co., KG, Grunow-Dammendorf, Germany, through the Bioprocess Pilot Facility B.V, Delft, the Netherlands. Samples were pre-milled in a bench mill and sieved to a mesh size of ~1 cm (moisture mass fraction of 10.8%).

2.2. *Pretreatment*

A laboratory pressurized reactor was used as described in Jimenez-Gutierrez et al. (2020), which consists of a stainless steel 100 mL vessel with an electrical heating and an agitation system. The reactor was connected to independent inlet lines for N_2 and CO_2 and to two outlet lines for gas, one of them with a safety relief valve set at 2.2 MPa.

A sample of 5 g of biomass, including its 10.8% moisture content, was suspended with the liquid (being water, different acetic acid solutions, or the liquid fraction from previous pretreatment cycles, depending on the conditions applied from the design of experiments), at the same solids loading (1:9 w/w), and then the mixture was loaded into the reactor vessel. Insulation fabric was then placed around the vessel. Before starting the experiments, all pipes and valves were shortly flushed with N_2 (or CO_2), assuming negligible evaporation of water. Then, the agitation and the heating system were switched on, increasing the temperature inside the vessel up to 180 °C, which increased the pressure up to the "pretreatment pressure" value. The reactor was kept at the set temperature by the controller during the pretreatment time. Subsequently, the heating system was switched off and the insulating fabric was removed from the reactor, allowing a fan to speed up the cooling of the vessel to room temperature. When ambient conditions were reached, the reaction suspension was filtered (Whatman 0.2 μm), and the pH of the liquid fraction was measured. Several liquid samples were then taken and stored at −20 °C until further analysis by HPLC. The solids were washed twice with demi-water, dried overnight in an oven at 105 °C and weighed. Samples of the dried solids were stored for further analysis.

2.3. *Analysis of samples*

Solid samples were analyzed following a slightly modified methodology based on the NREL (National Renewable Energy Laboratory) laboratory analytical procedure (LAP) for determination of structural carbohydrates in biomass (Sluiter et al. 2008). A two-step acid hydrolysis process was carried out. Firstly, 1.5 g of the dried, milled and sieved solids (180 μm mesh size) were incubated with a 72% w/w solution of H_2SO_4 during 60 min at 30 °C and then, after dilution to 4% w/w, the mixture was autoclaved for 1 h at 121 °C. Once room temperature was reached, calcium carbonate was added to an aliquot of the filtered liquid (Whatman 0.2 μm) to neutralize the acid until pH reached 6–7. The neutralized liquid samples were filtered again and analyzed by HPLC as described below. Lignin and ash content were not determined in this work.

Liquid samples were analyzed on a Waters HPLC system with a Bio-Rad HPX-87H column (300 × 7.8 mm) at 60 °C. Phosphoric acid (1.5 mmol/L at 0.6 mL/min) was used as eluent. Glucose, xylose, acetic acid, furfural and HMF were detected by refraction index (Waters 2414), while formic acid was detected using UV (Waters 2489) at 210 nm. Quantification was performed using external standards. Volumes of injection of standards and samples were 10 μL at 15 °C. All samples were analyzed in quadruplicate. After statistical analysis of these data, in most of the cases, the values of each analysis showed less than 5% of relative standard deviation, and in many cases lower than 1%.

The small error bars have not been displayed in the graphical representation of the results to facilitate readability.

2.4. Calculations

In previous studies (Chapter 4 of this thesis), it was observed that after LHW pretreatment at 180°C, the concentration of HAc in the liquid increased in time to certain point, when it seemed to level off to reach a sort of "steady state" for longer processing times. This simulation indicated that full deacetylation of hemicelluloses was not 100% possible to achieve, due to the equilibrium reactions. In practice, the acetyl esterification of hemicelluloses by presence of acetic acid may play a significant role. Hence, and for simplicity, the previously considered irreversible acetyl hydrolysis reaction would be then switched to reversible in this work, including therefore the opposite of deacetylation of hemicelluloses. Despite the aim of lowering the pH is to enhance the hydrolysis of acetyl ester bonds (reaction not considered an equilibrium *per se*), the inclusion of initial acetic acid in the process may produce then an opposite unwanted esterification reaction.

$$RAc + H_2O \overset{H^+}{\leftrightarrow} ROH + HAc \tag{1}$$

From a kinetic perspective, at the beginning of the pretreatment the concentration gradient as driven force for the hydrolysis of acetyl groups is larger for the LHW than for the dilute acetic acid, and the species are distributed between phases along the process based on equilibrium reactions (Chapter 4 of this thesis). On the other hand, the lower pH for the dilute acetic acid case might also speed up the hydrolysis reactions. Thus, in principle it is not fully clear which pretreatment conditions will be more favourable for the acetic acid released.

A total mass balance could be described as follow:

$$m_0^L + m_0^S = m^L + m^S \tag{2}$$

With m_0 as the initial mass of the liquid (L) or solids (S), and m as the mass of liquid or solids after the pretreatment. Note that the moisture content of the initial biomass is included as liquid in $m_0{}^L$, and therefore not accounted in the initial solid mass $m_0{}^S$. An acetic acid mass balance would have the following expression, which includes also the acetic acid added initially in the liquid (if done so):

$$m_{HAc,0}^S + m_{HAc,0}^L = m_{HAc}^L + m_{HAc}^S \tag{3}$$

$$m_0^S \cdot X_{HAc,0}^S + m_0^L \cdot X_{HAc,0}^L = m^L \cdot X_{HAc}^L + m^S \cdot X_{HAc}^S \tag{4}$$

In equation (4), $X_{HAc}{}^S$ (g/g) is the mass fraction (on dry basis) of acetic acid in the solid phase, $X_{HAc}{}^L$ (g/g) is the acetic acid mass fraction in the liquid, $X_{HAc,0}{}^L$ is the mass fraction of acetic acid added initially to the liquid, and $X_{HAc,0}{}^{S}$ is the mass fraction of acetic acid equivalent in the biomass on dry basis, which is calculated from the actual acetyl group content on dry basis in the biomass (0.036 g/g, obtained from analysis of solid raw biomass), and using a correction factor of 0.72 to compensate the differences in molecular weight between acetyl groups and acetic acid (Jimenez-Gutierrez et al. 2021). From the analysis of the liquid samples, values for the acetic acid concentration C_{HAc} are obtained in g/L. Consequently, the mass of HAc in the liquid phase $m_{HAc}{}^{L}$ equals:

$$m_{HAc}^{L} = m^{L} \cdot X_{HAc}^{L} = V^{L} \cdot C_{HAc}^{L} \qquad (5)$$

V^{L} is the total volume of the liquid, calculated from the sum of the initial mass of liquid, assuming no evaporation during the pretreatment, and the moisture content of the raw biomass. Therefore, knowing $m_{HAc,0}{}^{S}$, $m_{HAc}{}^{L}$ and $m_{HAc,0}{}^{L}$, it is possible to obtain the mass of HAc remaining in the solid phase after pretreatment $m_{HAc}{}^{S}$ with Equation (3). Values of $m_{HAc}{}^{L}$ can be taken relative to the total amount of acetic acid added initially in the mixture, including the acetyl groups in the biomass ($m_{HAc,0}{}^{S}$ + $m_{HAc,0}{}^{L}$). Similarly, values of and $m_{HAc}{}^{S}$ can be represented as relative to the initial mass of acetic acid equivalents in the raw biomass, obtained from the acetyl content using the correction factor ($m_{HAc,0}{}^{S}$).

During the pretreatment process, part of the solid biomass is dissolved into the liquid, as well as some fraction of the liquid is adsorbed to the solids after the filtration step. Additionally, there are some mass losses due to handling, which are negligible as it is assumed to be proportional to both liquid and solid phases (and therefore their compositions are also proportional).

3. Results and Discussion

3.1. Effect of initial HAc concentration on LHW pretreatment

The first set of experiments was performed at common conditions of 180 °C and 120 min of pretreatment temperature and time respectively. Initial HAc concentrations were 0.5% and 1% w/w. Experiments were carried out in presence of CO_2 and N_2. Since no effect was observed of carbon dioxide on LHW pretreatment in a previous study (Jimenez-Gutierrez et al. 2020), the results shown in Table 5.1 for these different gases can be considered to be duplicates.

***Table 5.1**. Concentration in g/L of compounds after LHW and with initial acetic acid, in presence of CO_2 or N_2.*

HAc (initially)	Glucose		Xylose		Acetic acid		Formic acid		Furfural		HMF	
g/L	N_2	CO_2	N_2	CO_2	N_2	CO_2	N_2	CO_2	N_2	CO_2	N_2	CO_2
0	0.462	0.501	2.179	2.607	4.378	4.467	1.145	1.058	2.353	2.077	0.269	0.272
5	0.294	0.327	1.462	1.791	8.488	8.775	0.969	0.952	2.813	2.666	0.384	0.413
10	0.348	0.369	1.133	1.230	13.419	13.397	1.052	1.062	2.794	2.682	0.502	0.530

In previous work (Jimenez-Gutierrez et al. 2020) it was suggested that the low pH required for the hydrolysis of the biomass during LHW was mostly originated from the HAc produced *"in situ"*, not from the pressurized CO_2. When adding initially HAc, it is even more obvious that HAc causes a low pH.

Table 5.1 shows that, for different initial concentrations of HAc, no significant difference in concentrations of formic acid was observed (less than 10% relative standard deviation). The increase in HAc concentration of the liquid fraction after pretreatment follows a linear correlation with the increase in initial HAc, although not proportionally. While less xylose was recovered when adding higher initial acidity, concentrations of furfural increased, indicating more degradation of xylose induced by the initial low pH. When using HAc at 0.5% w/w, glucose and HMF concentrations were reduced and increased respectively than when using 1% w/w HAc. This suggested that the presence of very diluted HAc at the beginning of the process enhanced the hydrolysis and the degradation of glucose to HMF, but when increasing the concentration of initial HAc, this effect is cancelled. Additionally, those trends are in line with data from previous studies (Jimenez-Gutierrez et al. 2021), and therefore comparable to pretreating with LHW at longer processing times.

Analysis of raw LB provided values of 45.9%, 18.8% and 3.6% for the glucan, xylan and acetyl content respectively (lignin content was not determined in this work).

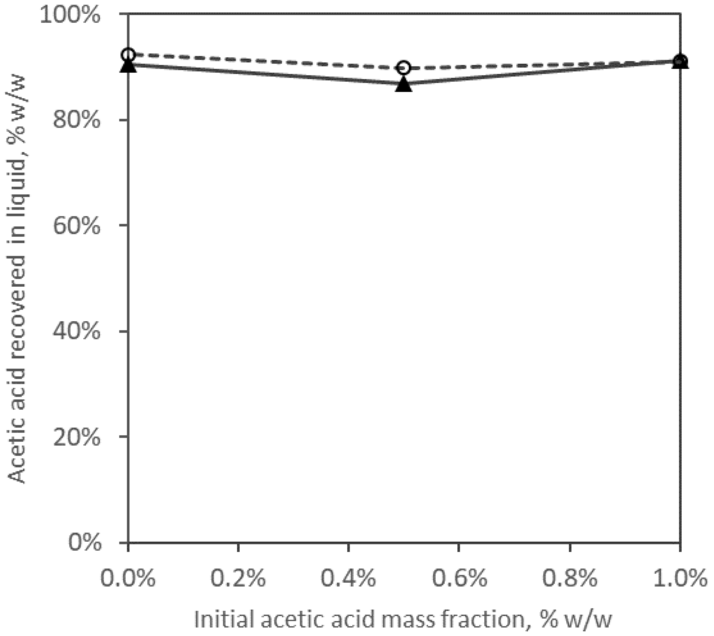

***Figure 5.1**. As function of the acetic acid mass fraction of the initial liquid (% w/w), the lines represent the relative amount of acetic acid recovered in the liquid phase (g/g), as per total of acetic acid added initially, after pretreatment at 180°C for 120 min in presence of CO_2 (▲) or N_2 (○).*

The relative amount of acetic acid obtained in the liquid phase after pretreatment is depicted in Figure 5.1. It can be observed that by adding initial acidity to the mixture there is no improvement regarding the release of HAc. Results indicate that approximately 90% of the initial acetic acid added (including the acetyl groups in the biomass) is recovered in the liquid after pretreating at 180°C for 120 min, regardless the initial concentration of acetic acid. The extra acidity appears to not have a big effect on the hydrolysis mechanisms, while the initial presence of acetic acid seems to contribute to the acetyl esterification of the LB, following the conceptual equilibrium described in Equation (1).

However, these results correspond to longer pretreatment times (120 min), suggesting that the major fraction of acetyl groups are hydrolysed. Therefore, for the next set of experiments, intermediate conditions with shorter pretreatment times were tested.

3.2. *Time effect*

Aiming to lower the severity of the process by reducing the processing time, four different pretreatment times were tested (5, 10, 15 and 30 min), each of them in absence and initial presence of 1% w/w HAc. Only N_2 was used for all experiments, at 180°C pretreatment temperature. Results from these experiments can be found in Table 5.2.

As general trend, the concentration of all the species increased with the pretreatment time, which is more noticeable for the compounds related to hemicellulose hydrolysis. It can be observed that initial addition of 1% w/w HAc during pretreatment improved the release of xylose, and consequently proportional the furfural production. On the other hand, no significant effect was observed on the formic acid obtained, and a slight increased on glucose concentration when treating the biomass for 30 min, compared to the plain LHW process. However, similar improvement than furfural could be seen for HMF when adding initial HAc, despite it did not correspond with the glucose trend.

Table 5.2. *Concentration (g/L) of compounds after LHW and dilute acetic acid (1% w/w) at different pretreatment times.*

Time	**Glucose**		**Xylose**		**Acetic acid**		**Formic acid**		**Furfural**		**HMF**	
min	**LHW**	**HAc 1%**	**LHW**	**HAc 1%**	**LHW**	**HAc 1%**	**LHW**	**HAc 1%**	**LHW**	**HAc 1%**	**LHW**	**HAc 1%**
5	0.089	0.094	0.240	0.596	1.071	10.339	0.246	0.152	0.056	0.213	0.013	0.023
10	0.102	0.097	0.267	1.061	1.287	10.592	0.349	0.263	0.061	0.355	0.017	0.046
15	0.099	0.126	0.349	1.259	1.362	11.116	0.311	0.335	0.115	0.475	0.020	0.055
30	0.109	0.160	0.649	2.187	1.897	12.058	0.413	0.420	0.237	1.195	0.038	0.133

Overall, these values indicated clear effect of low pH due to initial addition of HAc at short pretreatment times, so the mechanisms of hydrolysis of the lignocellulosic polymers, and consequent sugar degradation compounds were acting from the beginning of the process, with no need to wait for hydrolysis of acetyl groups.

Figure 5.2 represents the relative amount of acetic acid in the liquid phase (calculated as described in section 2.4) after pretreatment at different times, in absence and presence of initial HAc 1% w/w. Increasing the processing time proportionally enhances the acetyl hydrolysis, although values in Figure 5.2 go only up to 30 min pretreatment. Thus, it would be expected to have lower hydrolysis than longer experiments, and therefore proportionally less acetyl esterification of biomass, in case HAc is added initially. It can be observed that from 20% to 40% of the acetyl groups in the biomass is recovered without initially adding HAc. For the dilute acetic acid process, much higher recovery values are obtained (70% - 80%), mostly due to the HAc that was already present in the mixture at the beginning. Therefore, in order to have comparable results, it becomes interesting to calculate the acetic acid remaining in the solid phase.

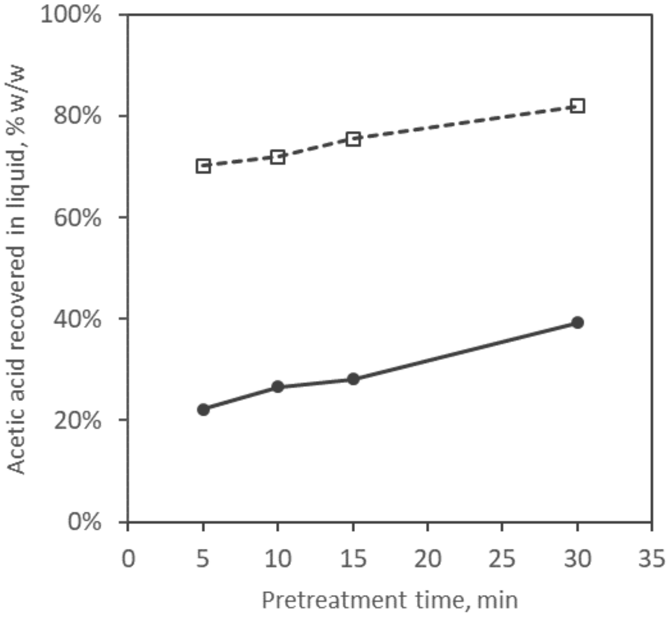

Figure 5.2. *Recovery of acetic acid in the liquid phase (g/g), as percentage of acetic acid added initially (including the acetyl groups in the biomass), after LHW (●) and dilute acetic acid (□) pretreatment (with initial HAc 1% w/w) at 180°C.*

At different pretreatment times, Figure 5.3 represents the relative amount of acetic acid remaining in the solid phase (g/g), expressed as the mass of HAc in the solids after pretreatment over the initial acetic acid equivalent in the biomass. At the beginning of the process (5-10 min), deacetylation of the biomass seems to be less when initially HAc is introduced in the system. This might be explained by the big initial difference in dissolved acetic acid concentration, which counteracts hydrolysis equilibrium. From certain point (≈15 min), the release of acetic acid from the hemicellulose seems to overtake the acetylation phenomena and match the values of acetyl hydrolysis for the LHW. Therefore, these results indicate that the dilute acetic acid pretreatment have similar performance as the LHW process, which small differences at the beginning, regarding the acetic acid release. To confirm this, the next set of experiments was designed to test several concentrations of initial HAc for a 30 min pretreatment, keeping the rest of the conditions the same.

Comparing these results to those from the previous subsection, it is possible to see that indeed longer pretreatment times produced more degradation compounds and more release of acetic acid, though not proportionally. When processing LB by LHW pretreatment at 180°C for 120 min, the pH of the liquid fraction obtained was around 3.6, with a concentration of 4.3 g/L of HAc, while only for 30 min pretreatment, the acetic acid was 1.9 g/L with a pH of 4.0. Therefore, to double the concentration of HAc, a 4 times longer pretreatment was required. Regarding the dilute acetic acid process, an initial 1% w/w solution corresponds to 10 g/L of HAc. After 120 min of acid pretreatment, 13.4 g/L of HAc was obtained, compared to 12.1 g/L of HAc obtained for a 30 min process (pH values 3.4 and 3.5 respectively). Thus, aiming the acetyl hydrolysis, in both cases it seemed more efficient to proceed with several short stages in series for the pretreatment processes than a very long treatment. This may cause then accumulation of HAc, among the other compounds, and consequently increasing their concentration, without degrading in excess the sugars. The challenge would be to find the optimum conditions for this pretreatment in series.

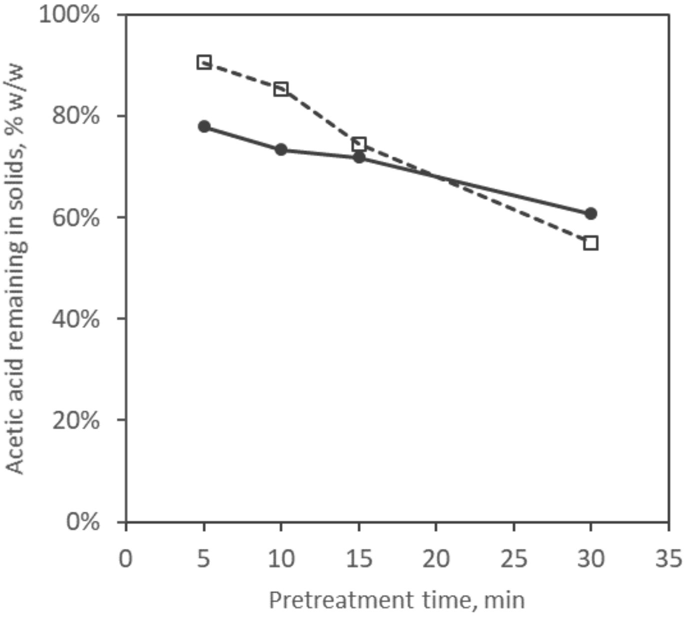

__Figure 5.3__. Amount of acetic acid in the solid phase (g/g), relative to the initial acetic acid equivalents in the biomass, after LHW (●) and dilute acetic acid (□) pretreatment (with initial HAc 1% w/w) at 180°C.

3.3. *Lower initial HAc concentrations at mid-short pretreatment time*

With the idea of a possible recirculation of the liquid fraction from LHW and using it as media for the diluted acid hydrolysis, this set of experiments aimed to test the effect of using lower concentrations of initial HAc. After 30 min and 120 min LHW pretreatment at 180°C, acetic acid concentrations were 1.9 g/L and 4.4 g/L, respectively. To cover that range of concentrations, the values for the mass fraction of the initial HAc selected were 0.25%, 0.5% and 1% w/w. Results can be observed in Table 5.3.

It could be observed that higher initial HAc concentrations enhanced the hemicellulose hydrolysis, even for that short period of pretreatment, as more xylose, and proportionally furfural is obtained. Glucose and HMF have similar trend, though in a less noticeable amount, while formic acid production is not much affected. Results indicate that during the 30 min dilute acid pretreatment, the hydrolysis of hemicelluloses is more significant than the sugar degradation reactions.

***Table 5.3**. Concentration in g/L of compounds after LHW and dilute acetic acid pretreatment, at different initial %HAc, for 30 min at 180°C.*

	% HAc w/w	Time, min	Glucose	Xylose	Acetic Acid	Formic Acid	Furfural	HMF
LHW	0.00	30	0.109	0.649	1.897	0.413	0.237	0.038
Dilute Ac.	0.25	30	0.106	1.185	4.445	0.371	0.523	0.062
	0.50	30	0.116	1.181	6.821	0.399	0.621	0.071
	1.00	30	0.160	2.187	12.058	0.420	1.195	0.133

Regarding the acetic acid release, values for the relative amount of HAc in the liquid and the solid phases are depicted in Figure 5.4. It could be observed that the relative HAc recovered in the liquid slightly increases with the acetic acid mass fraction of the initial mixture until a point when it seems to level off. This trend may be expected with an increase in the initial concentration of HAc, as if the difference with the initial acetyl content in the solid is too large, the contribution from of the acetyl hydrolysis to the final HAc concentration might be negligible compared to the amount of HAc added initially.

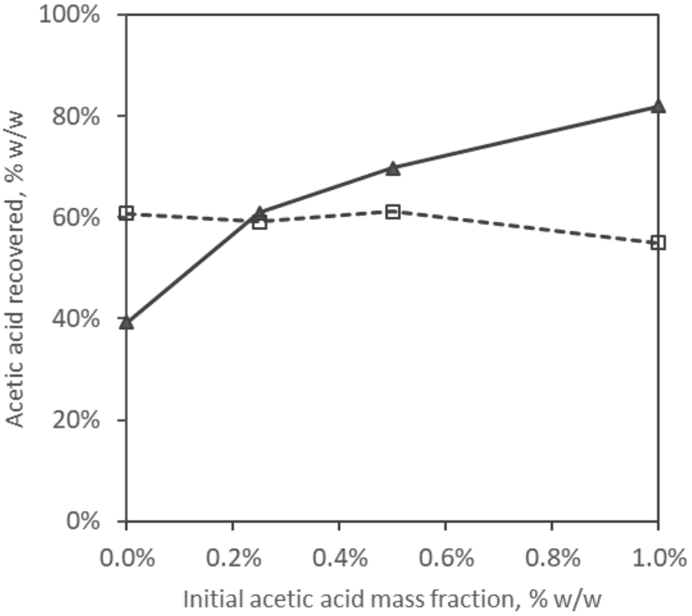

***Figure 5.4**. (▲) Recovery of acetic acid in the liquid phase, defined as the HAc mass in the liquid after pretreatment over the total mass of initial HAc (in the liquid and solid raw biomass); (□) Percentage of acetyl equivalents remaining in the dry biomass. Pretreatment conditions were 180°C during 30 min in presence of* N_2.

On the other hand, the relative amount of acetic acid in the solids after the 30 min pretreatment remain constant around a value of 60%, confirming that after the first few minutes of the process (10-15 min), the initial acidity has not much substantial effect on the acetic acid release form the LB, and therefore, at the conditions applied, LHW and dilute acetic acid pretreatment produce similar results.

The last set of experiments was designed with two simultaneous purposes: confirm the influence of initial presence of HAc and investigate the effect of recirculating the liquid fraction after the pretreatment several times, observing the accumulation (and possible interactions) of not only the acetic acid but the rest of the compounds.

3.4. Recirculation of liquid fraction

In this last series of experiments, four rounds of LHW pretreatment process were performed, all at 180°C and for 120 min, and all with the same solid/liquid ratio (1:9 w/w). In the first round plain demi-water was used as liquid added to the biomass, while in the second, third and fourth, the liquid obtained from the previous round was used instead. An overview of this recirculating procedure is depicted in Figure 5.5.

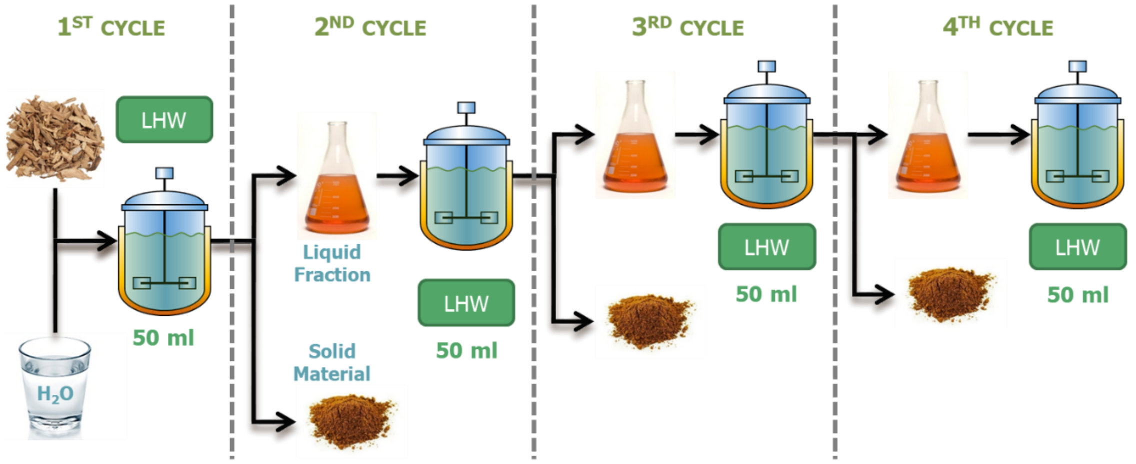

***Figure 5.5**. Graphical description of the recirculating procedure, using the liquid fraction from previous cycle.*

Recirculation of the liquid fraction after LHW has been explored before (Wu et al. 2017), although for other types of LB and at different conditions, such as shorter pretreatment times with 1:20 solid-liquid ratio (Lu et al. 2016). By recycling the liquid fraction from a previous pretreatment to a pretreatment with fresh biomass at the same conditions, accumulation of dissolved compounds was expected (Lü et al. 2017). The composition of the liquid fraction in subsequent cycles is depicted in Figure 5.6. The concentration of acetic acid in the liquid increases proportionally with the number of cycles, which indicates that at the conditions applied, the amount of acetyl groups that is hydrolysed per cycle is not increased due to the initial presence of acetic acid or other hydrolysis products. A similar trend can be seen for formic acid, which like acetic acid, seems to be not be further degraded at the conditions applied, and therefore simply accumulates due to recirculation of the liquid.

The monosaccharides and furans are much less stable. The concentration of glucose seems to increase until reaching a maximum at the second cycle, and then decreases slightly to level off. The same trend can be observed in the values of furfural, though at higher concentrations, while HMF increases and remains steady from the third cycle on. This indicates that before the fourth cycle, the production and degradation rates become equal for glucose and per furan. Xylose concentrations decrease after the first cycle, suggesting that a similar sort of "steady state" might be reached after the 4th cycle. Despite the use of different feedstock and pretreatment conditions, these trends are in line with previous literature (Lu et al. 2016).

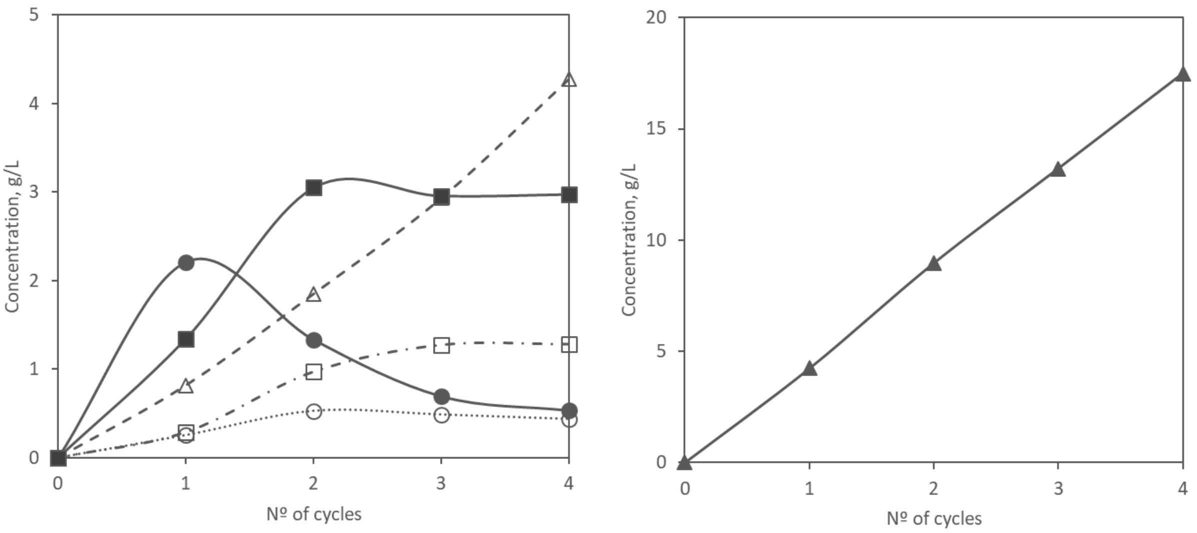

Figure 5.6. Dissolved concentrations as function of the number of cycles (pretreatment at 180°C during 120 min in presence of N_2, using the liquid fraction from the previous cycle); The symbols/lines represent the concentration (g/L) in the liquid phase of different compounds: Glucose (○); Xylose (●); Acetic acid (▲); Formic acid (Δ); Furfural (■); HMF (□).

The calculated recovery of acetic acid in the liquid phase follows a similar trend as represented in Figure 5.1, though in this case around 95% ± 5% for all the cycles. Correspondingly, the calculated relative amount of acetic acid remaining in the solids has a similar value for all the cycles, moving around 8% ± 5%. On the other hand, experimental values of the composition of the solid phase indicate no HAc remained after the pretreatment in any cycle, which is not in line with the unachievable 100% deacetylation proposed. This mismatch revealed that with the analytical method used, the small amounts of acetic acid in the pretreated biomass were very diluted and therefore, below the detection limit.

Figure 5.7 represent the glucan and xylan content in the pretreated biomass after the cycles. Despite not in a strongly marked way, there is some slight linear decrease in both glucan and xylan content in the biomass with the recirculation of the liquid fraction. While the former goes from a 35.8 to 28.7 g/g DM, the later drops from 4.4 to 2.7 g/g DM, from cycle 1 to cycle 4. Thus, these results indicate a clear but restrained effect of the initial HAc presence in the hydrolysis of LB.

Values from the 2nd cycle were compared with the analogous dilute acetic acid experiment, which would be the pretreatment with 0.5% w/w initial HAc from section 3.1. It could be observed that the acetic acid release follows similar trend for both cases, although for the recirculation experiment, most of the concentrations of the other compounds analysed were higher. While the formic acid doubled because its accumulation, glucose did it mostly due to enhanced biomass hydrolysis. This is confirmed by the values of HMF, which more than doubled for the recirculation experiment. Moreover, similar concentrations of xylose were obtained in both experiments, indicating that rather than accumulating from previous cycles, its degradation is enhanced instead when recirculating the liquid fraction.

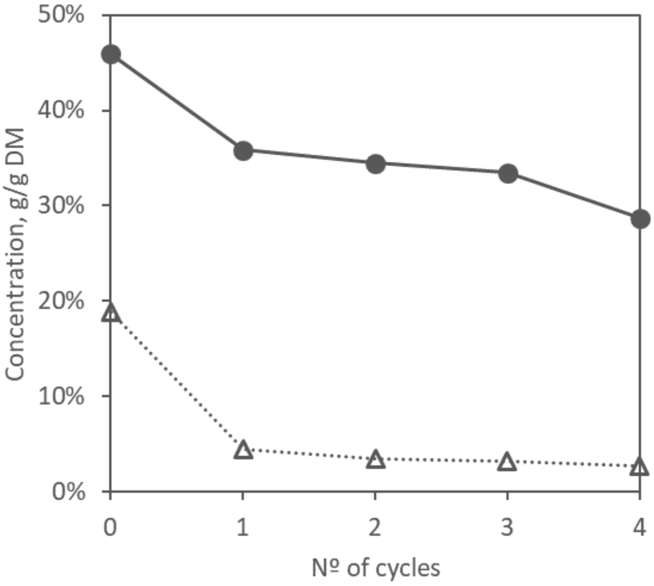

***Figure 5.7**. Glucan (●) and Xylan (Δ) content in the pretreated biomass as function of cycles (pretreatment at 180°C for 120 min in presence of N_2, using the liquid fraction from the previous cycle). Composition of the solid fraction in dry basis (g/g DM).*

Prior the subsequent enzymatic hydrolysis and fermentation steps following the pretreatment, higher concentrations are preferred for consecutive separation and purification of side-products, such as furfural, formic acid and especially acetic acid. Consequently, the recirculation of the liquid fraction would be more favourable than the dilute acetic acid pretreatment, as larger concentrations of these compounds could be achieved than in a single dilute acetic acid process. On the other hand, the reduction in xylose content after several cycles may also play a significant role, as its degradation is enhanced over its production. However, the steady furfural and glucose concentrations after 3 cycles indicate that the recirculation might be optimized between 2 and 4 cycles, depending on the target: more cycles for higher carboxylic acid production (formic, acetic…) whereas less cycles for larger xylose content at the expenses of lower concentration of acids.

3.5. pH

The initial solution of 1% w/w HAc used gave a measured pH of 2.6, while dilutions of 0.5% and 0.25% w/w provided values of pH of 2.9 and 3.1 respectively. After none of the LHW and dilute acetic acid pretreatment experiments, the pH was below 3.4, probably due to the presence of compounds that acted as buffer, as suggested in previous work (Jimenez-Gutierrez et al. 2020). Recirculation of the liquid fraction in several cycles did not have any impact on the final pH value, in line with literature (Wu et al. 2017).

4. Conclusions

Dilute acetic acid has a significant effect on the hydrolysis of LB compared to LHW pretreatment. Results suggested that the release of glucose and xylose, and consequently the degradation to HMF and furfural respectively, are enhanced by the initial presence of dilute HAc, especially at the beginning of the pretreatment. The low pH due to initial addition of HAc may initiate the hydrolysis of the lignocellulosic polymers, and the consequent degradation reactions although to a lower extent, from the beginning of the process, with no need to wait for the hydrolysis of acetyl groups.

However, the acetyl esterification of biomass seems to have more relevance than the acetyl hydrolysis at the first 10 min of the process for the dilute acetic acid pretreatment. After the first 15 min, the release of acetic acid from hemicelluloses has similar trends for LHW and dilute acetic acid pretreatment.

Results showed that four times longer processing was required to double the concentration of HAc, indicating that it would be more efficient to proceed with a pretreatment in several short stages in series, in case the acetyl hydrolysis would be the target.

Nevertheless, by recirculating the liquid fraction for longer times, the concentration of acetic acid is linearly increased, as well as the formic acid, with each cycle. This would be interesting as a minimum concentration of these potential side products (such as HAc, formic acid, or even furfural) would be essential for a subsequent separation and purification steps.

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Chapter 6

Outlook

As discussed in previous chapters, the pretreatment step is crucial in the biochemical conversion of lignocellulosic biomass, as a primary way to tackle its recalcitrance. Hydrothermal methods, as well as acid pretreatments, lead mostly to hydrolysis of hemicelluloses, producing therefore its derivatives, including acetic acid from the cleavage of acetyl groups present in hemicelluloses. The acetyl content of lignocellulosic biomass is a potentially interesting source of acetic acid, as its fraction in dry basis may reach up to more than 5% w/w in hardwoods, such as poplar. Hence, liquid hot water (LHW) pretreatment of lignocellulosic biomass was investigated in this work in order to improve the process, focusing on the release the acetic acid fraction as value-added side-product.

In this thesis, the potential of LHW as a pretreatment technique was explored to achieve specific degrees of hemicellulose depolymerisation in a green way, as no extra chemicals are added. Using subcritical CO_2 as a prospective catalyst to lower system pH did not lead to extra improvement during LHW pretreatment of poplar biomass (**chapter 2**). Comparable performance at lab and pilot scales were obtained for LHW pretreatment of poplar biomass with equivalent temperature profiles (**chapter 3**). Moreover, results indicated that it is possible to achieve complete de-acetylation of poplar wood using LHW. Regarding the acetic acid release, a simulated model of the LHW pretreatment, including the acetyl hydrolysis reactions, described the acetic acid production accurately (**chapter 4**). LHW was compared to dilute acetic acid pretreatment, obtaining similar release of acetic acid in both methods for longer processing times. However, the presence of initial acetic acid enhanced the hemicellulose hydrolysis at the beginning of the pretreatment, as well as promoted the production of degradation compounds initially (**chapter 5**). Furthermore, the recirculation of the liquid fraction obtained after LHW pretreatment is a method to increase the acetic acid concentration in the biomass hydrolysate, as well as other degradation inhibitors, such as formic acid, 5-hydroxymethylfurfural (HMF) and furfural.

1. Dealing with inhibitors

Formation of inhibitors during pretreatment is a limiting factor in production of valuable chemicals from biomass (Kumar et al. 2020). Multiple degradation compounds are produced during LHW pretreatment of lignocellulosic biomass, and therefore special importance needs to be paid on their synergistic inhibiting effect. Carboxylic acids, (acetic, formic, levulinic), furans (furfural, HMF) and phenolic compounds are some of them (Steinbach et al. 2017). The presence of these side products requires a process design with an optimal strategy to avoid inhibition of the microbial and enzymatic biocatalysts (Jönsson and Martín 2016). Different strategies might be adopted to effectively reduce the inhibitory effect of various compounds on fermentation process by microbes (Kumar et al. 2020).

The production of side compounds depends on the pretreatment conditions. Adjusting the pretreatment process to keep the concentration of the inhibitors below the tolerance threshold for the enzymes and the microorganisms might be a possible solution. Using a biomass source with low acetyl content instead of hardwood may also result in lower concentration of acetic acid after the hydrothermal pretreatment (Du et al. 2010) (Michelin and Teixeira 2016). Otherwise, tolerance to acetic acid from the microorganisms might be improved by adaptation (Trcek et al. 2015) (González-Ramos et al. 2016) or by using evolutionary engineering, allowing higher concentrations of HAc in the substrate (Morales et al. 2017) (Ko et al. 2020). Furthermore, converting the acetic acid present in the biomass hydrolysate by reducing it to ethanol via metabolic engineering has been successfully studied too (Medina et al. 2010) (Papapetridis et al. 2016).

Alternatively, detoxification or conditioning is considered a powerful way to counteract the inhibitory effect (Nogueira et al. 2021). These methodologies generally involve the use of additives and chemicals, and commonly a separation step would be required within the process too (Jönsson and Martín 2016). However, the profitability of the whole process might be increased by generating side products plus the inhibitor-reduced sugar-rich stream for the saccharification and fermentation steps, apart from the additional costs saving due to lower chemicals use for the pH adjustment prior enzymatic hydrolysis, less residual solids (waste disposal cost), and higher productivity for the fermentation (Ranjan et al. 2009). In this case, a holistic perspective seems relevant in order to determine the positive and negative factors of fractionating lignocellulosic biomass, and consequently producing, separating and purifying different value-added bio-based compounds as side-products of the carbon source for the fermentation.

2. Recovery of acetic acid

Different technologies can be employed for the recovery of acetic acid. In most commercial processes, HAc is separated and concentrated from aqueous mixtures by distillation methods, providing initial concentration sufficiently high. However, since LHW pretreatment usually generates a biomass hydrolysate with dilute amounts of acetic acid, standard distillation may require a large number of plates and reflux ratio. Therefore, other techniques have been studied regarding separation and purification of acetic acid from pretreated lignocellulosic biomass (see Table 6.1).

***Table 6.1**. Detoxification methods studied for acetic acid removal from lignocellulosic pretreated biomass.*

Detoxification technique	**Literature**
Azeotropic distillation	(Galeotti et al. 2018) (Le Berre et al. 2014)
Extractive distillation	(Le Berre et al. 2014) (Ghose and Bhadra 1985)
Solvent extraction	(Roque et al. 2019) (Binder 2015) (Cebreiros et al. 2017)
Vacuum distillation	(Huang et al. 2019)
Steam stripping	(van Zyl et al. 1991)
Adsorption	(Sainio et al. 2011)
Ion exchange	(Wickramasinghe and Grzenia 2008) (Chen et al. 2017)
Membrane Techniques	
Membrane filtration	(Lakra et al. 2013) (Nguyen et al. 2016) (Zhou et al. 2013)
Membrane extraction	(Grzenia et al. 2008) (Grzenia et al. 2010)
Emulsion liquid membrane	(Lee 2017)
Electrodialysis	(Suwal et al. 2018)
Vacuum membrane distillation	(Zhang et al. 2015)

Most of these studies have been performed for lignocellulosic hydrolysates at laboratory scale. Operational factors such as long residence times, low recovery efficiencies or high capital/operating costs may lead to non-economically attractive HAc recovery processes at industrial scale. By combining different techniques, a gradual recovery and concentration of HAc from biomass hydrolysate may be achieved, allowing not only separation from the carbon source but also additional purification from the rest of inhibitors. Examples of this approach are the combination of vacuum membrane distillation with adsorption (Zhang et al. 2015), steam stripping with solvent extraction (Tolan et al. 2015), or vacuum evaporation with steam stripping (Zhu et al. 2009).

In a recent study in our research group (Garcia-Muñoz 2020), different alternatives to recover acetic acid from the mixture obtained after LHW pretreatment of lignocellulosic biomass were investigated. By making use of process design and simulation tools, it was possible to conceptually select, design and evaluate the best options. An overview of all the considered recovery processes was created by emulating the concept of superstructure in a simplified manner, being able to compare alternative processes simultaneously (Figure 6.1).

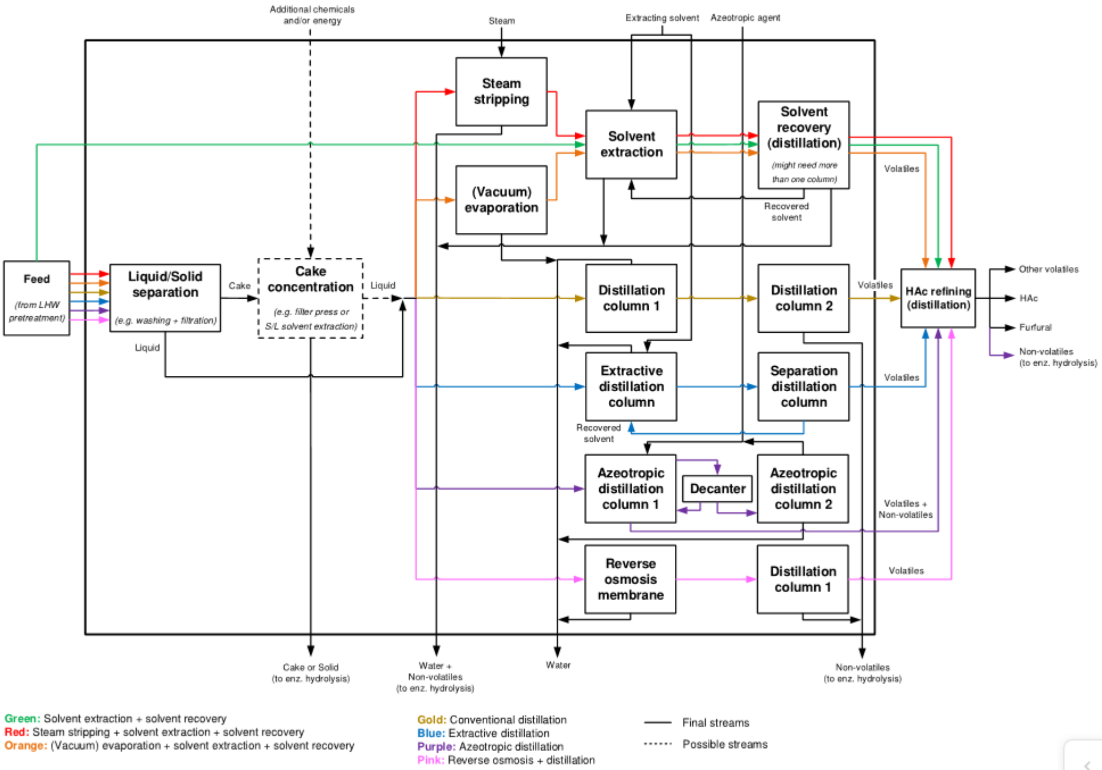

Figure 6.1. *Overview and summary of the possible HAc recovery process, combining the most relevant techniques.*

These processes were ranked by qualitative analysis, combining technical and economic factors, and finally selecting the two most promising alternatives: solvent extraction and conventional distillation. Both methods were studied in detail via simulation software, evaluating different scenarios and optimising several operational features and parameters, such as recycle streams, recovery ratios, energy savings and utilities consumption.

Additionally, the techno-economic analysis performed of these two methodologies highlighted the benefits of recovering furfural too (Cai et al. 2014). Produced as degradation product of xylose, its separation and purification from the lignocellulosic biomass hydrolysate seems reasonably viable, instead of fermenting it to produce ethanol (Luo et al. 2019).

The existence of multiple azeotropes due to the presence of acetic acid, formic acid and furfural in the system makes the separation and recovery process remarkably challenging (Gmehling 2004). Further studies of this complex system are required in order to obtain sufficiently high purities of these side products after an optimal recovery process.

3. Increasing concentration of acetic acid

It is recommended to obtain high concentrations of acetic acid and furfural after LHW pretreatment, as those species play a significant role in the multifactorial-dependent recovery process proposed above. Thus, full deacetylation of biomass and full conversion of xylose to furfural would be desired. In order to reach higher concentrations of acetic acid, recirculation of the liquid fraction obtained after LHW pretreatment was considered in chapter 5. Results showed that for long processing times, concentrations of acetic and formic acid increased linearly with the amount of cycles, while furfural seemed to increase and level off after the second cycle. On the other hand, the efficiency of proceeding with several short stages in series instead of longer pretreatment times was also discussed.

Reducing the amount of water used during the LHW pretreatment would be an alternative method to increase the concentration of acetic acid and the other inhibitors. However, using high solids loading lead to ineffective mixing, heat and mass transfer limitations and substrate viscosity, requiring excessive amounts of energy to operate (Modenbach and Nokes 2012). A 50% increase in the solid/liquid ratio was explored at pilot and laboratory scales in chapter 3, leading to proportional increments in concentration of most of the compounds analysed. While 15% w/w solid loading seems to be reaching the stirring limitation for a batch reactor setup, LHW pretreatment has been studied at different configurations, such as fixed-bed reactor, with the possibility to process lignocellulosic biomass at higher solid/liquid ratios (Ingram et al. 2009).

In this semi-continuous configuration, the hot water is pumped through the retained biomass in the reactor, solubilizing mainly the hemicellulose fraction while preventing excessive degradation product formation due to the shorter retention times (Ruiz et al. 2020). It has been claimed that this flow-through hydrothermal pretreatment technique may achieve near-complete hemicellulose recovery and high lignin removal with considerable release of sugars (Meng et al. 2022). This technique generally would require a large amount of water as well as high pressures to push the liquid through the biomass, leading to high-energy requirements and larger fermentation vessels (Yang et al. 2018). Therefore, recirculation of the liquid (similarly as proposed in chapter 5) would be then necessary to increase the concentration of acetic acid (up to complete deacetylation of biomass), and to reduce the water usage.

4. From wood to acetic acid

A general biorefinery process involves the consumption of pentoses (C5) and hexoses (C6) as main carbon source for the microbial fermentation, producing in most cases bioethanol (Aditiya et al. 2016). However, a different compound could be considered as the main target to be obtained from the conversion of these fermentable sugars (Song et al. 2013) (Sandström et al. 2014). That would be the case of acetic acid, which might be produced from poplar biomass by following a bioprocess of pretreatment, enzymatic hydrolysis, acetogen fermentation, and acid purification (Morales-Vera et al. 2020). The major expense of this process proposed was the separation of acetic acid from water, similarly to the recovery of acetic acid as side product exposed before.

On the other hand, conversion of ethanol to acetic acid is also carried out via oxidation by acetic acid bacteria in the production process of vinegar. Usually from fruit juices, such as grapes (wine) or apples (cider), vinegar is a solution of acetic acid produced by a two-step bioprocess: anaerobic fermentation of sugars to ethanol (by yeast), and oxidation of ethanol to acetic acid (by acetic acid bacteria) (Bhat et al. 2014). Nevertheless, other sources of sugar/ethanol could be used for the production of vinegar, such as beer, malt, rice and many more (Hailu et al. 2012), including kombucha (Akbarirad et al. 2017). Even fruit-based by-products are widely employed in the vinegar industry (Luzón-Quintana et al. 2021). Furthermore, other side streams have been already used for production of food-grade vinegar via subcritical water treatment (Yamato et al. 2021).

As one of the requirements for the production of vinegars is the bio-based green nature of the source used, a hypothetical "petrol-based vinegar" would not be accepted for human consumption. However, it could arguably be possible to consider the acetic acid solution obtained from the treatment of lignocellulosic biomass as "food-grade" (Baumann and Westermann 2016). Nevertheless, this may bring some confusion, because the term "wood vinegar" already exists, alluding to pyroligneous acid obtained from condensation and clarification of the liquid products generated during carbonization of lignocellulosic biomass (Wu et al. 2015) (Mathew and Zakaria 2015). This wood vinegar consists of a complex matrix of different compounds (Yang et al. 2016) (Liu et al. 2018) and it has several applications, including some in the food industry (Choi et al. 2009) (Li et al. 2018) (Wang et al. 2020).

5. Alternative hydrothermal pretreatment: Steam explosion

Instead of applying LHW, another possible hydrothermal pretreatment is the extensively employed steam explosion, which combines both mechanical forces and chemical effects. In this methodology, the biomass is heated for 10-30 min with high-pressure saturated steam, followed by a sudden decompression, producing an explosion and disruption of the biomass structure, and increasing then the surface area (Sarker et al. 2021). Typically used as precursor process for bioethanol production, this pretreatment could also lead to other conversion of the lignocellulosic biomass, such as densification or syngas production (Yu et al. 2022). Similar to LHW, the high temperature and pressure applied in this pretreatment enhance also the acetyl hydrolysis, the cleavage of glycosidic bonds and the solubilisation of hemicelluloses (Yu et al. 2022) (Duque et al. 2016). The slurry obtained after the steam explosion contains less water than the biomass hydrolysate from LHW, and therefore lower concentration of inhibitors. Although both hydrothermal pretreatment methodologies (LHW and steam explosion) could be carried out in batch or continuous operation mode, the last one brings several limitations due to the challenging scale-down to laboratory size (Ruiz et al. 2020).

Alternatively, the biomass is very often impregnated with a chosen catalyst before introduction in the reactor, such as sulphur dioxide or sulfuric acid, performing then an acid-catalysed steam explosion (Mackie et al. 1985). Analogous to dilute acid pretreatments, this modification has the advantages of improving the hydrolysis rate, promoting complete hemicellulose removal and allowing operational conditions, such as reduction in pretreatment temperatures and residence times (Duque et al. 2016).

This technique has been largely investigated and frequently proposed for an industrial scale pretreatment process (Silveira et al. 2018). However, it is important to highlight the difficulties and challenges to tackle for its further development and application industrially. These may include special equipment requirements due to operational conditions and resistance to corrosion.

6. Using CO_2 in LHW pretreatment

There are many different pretreatment methods and depending on several factors, such as the main target aimed or the type of biomass source, the selection of the most promising technique would be done accordingly. During hydrothermal pretreatment, pressurized carbon dioxide reacts with water producing carbonic acid *"in situ"*, decreasing consequently the pH of the system (Magalhães da Silva et al. 2014). This mechanism was the motivation for a possible improvement during LHW pretreatment of lignocellulosic biomass in chapter 2. The acetic acid release was used as a progress indicator to evaluate the hemicellulose depolymerisation during this CO_2-assisted hydrothermal pretreatment. No significant effect on the acetyl hydrolysis of poplar biomass was observed when using CO_2 instead of N_2. Moreover, results also indicated no relevant contribution to acidification due to the production of carbonic acid and therefore deacetylation of lignocellulosic biomass can be achieved regardless the pressure or gas type used during LHW pretreatment. This outcome was claimed at specific conditions applied, meaning temperatures from 120 to 200 °C, pretreatment times from 5 to 240 min and subcritical pressure values. On the contrary, the use of supercritical CO_2 has been also widely studied, with remarkable results regarding enhancement of enzymatic hydrolysis (Kim and Hong 2001) (Gao et al. 2010) (Relvas et al. 2015) (Badgujar et al. 2021) (de Carvalho Silvello et al. 2020).

Supercritical CO_2 is considered a eco-friendly nontoxic green solvent, that does not generate harmful chemicals and can be easily removed from products by depressurization (de Carvalho Silvello et al. 2020) (Sohni et al. 2020). This methodology is commonly applied in combination with another technique, such as ultrasound (Yin et al. 2014) (Zhang et al. 2019) ionic liquids (Gu et al. 2013), alkaline hydrogen peroxide (Phan and Tan 2014) or even with steam explosion (Alinia et al. 2010). Supercritical CO_2 pretreatment is often coupled with a CO_2 explosion step, in which a sudden decompression after the processing time produces a comparable physical disruption than steam explosion (Zhao et al. 2019).

Similar mechanism as LHW pretreatment with subcritical CO_2 is observed when using supercritical CO_2 too, regarding the production of carbonic acid due to its reaction with water, which increased the solubilisation of hemicelluloses (Narayanaswamy et al. 2011).

However, compared to the subcritical case (chapter 2), the temperature range for the supercritical CO_2 might be lower, although the pressure values are much higher. The disruption of the lignocellulosic biomass structure to enhance of the cellulose accessibility for the enzymatic hydrolysis step could be improved by longer processing times, even at lower pretreatment temperatures (50-80 °C), preventing consequent hemicellulose degradation (Zhao et al. 2019).

7. Final remarks

In a recent publication, a pretreatment technology is proposed that combines supercritical CO_2 with acetic acid and steam explosion, obtaining a reduced amounts of fermentation inhibitors and allowing the decrease or even removal of use of washing water, besides the enhancement of the reducing sugars yield (Zabihi et al. 2021). Therefore, it seems that rather than focusing in one unique pretreatment method, a combination of two or more techniques may lead to a synergistic effect with an optimal, efficient and selective solubilisation of lignocellulosic biomass, release of side products or limitation of inhibitors formation, depending on the specific target required.

The versatility and robustness of the pretreatment methodology applied within the biorefinery is considered one of the most important factors, especially because of the wide range of diverse feedstock that could be used, with many differences in composition and structure (Badgujar and Bhanage 2018). It is important to understand the properties and mechanism of different feedstock to adopt accordingly the suitable pretreatment method (Zheng et al. 2022). Apart from the duality between softwood or hardwood, or even blending them to obtain a synergistic effect on biomass bioconversion (Vera et al. 2015), it should be suggested to extend the options towards 3rd and 4th generation feedstock, which may not have the lignocellulosic biomass recalcitrance though they would bring associated challenges (Shahid et al. 2021). In any case, extensive investigation would be recommended, not only to increase the data obtained and to improve the contribution to the scientific community, but also to support the development of clean, eco-friendly and sustainable advanced biorefinery practices.

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List of publications

Journal articles

- Jimenez-Gutierrez, J. M., L. A. M. van der Wielen and A. J. J. Straathof (2020). "Subcritical CO_2 shows no effect on liquid hot water pretreatment of poplar wood." Bioresource Technology Reports **11**: 100442.

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Oral presentations

- “Lignocellulosic biomass pretreatment for acetic acid co-production”, Netherlands Biotechnology Conference (NBC) – Biobased Economy parallel session (online), 2020.

Poster presentations

- “High pressure CO_2 process for acetic acid recovery from biomass”, BE-Basic Symposium, Noordwijkerhout, 2017
- “Equilibria of ternary system acetic acid—water—CO_2 under subcritical conditions”, Netherlands Biotechnology Conference (NBC), Wageningen, 2017.
- “Equilibria of ternary system acetic acid—water—CO_2 under subcritical conditions” 10th World Congress of Chemical Engineering (WCCE), Barcelona, 2017

About the Author

Jose Maria Jimenez Gutierrez was born on the 28th of April 1982 in Granada (Spain). He grew up in the warm South of Spain and once finished high school, he decided to continue with higher education. He graduated in 2009 as Chemical Engineer at the University of Granada (UGR). During this period, he developed interests in bioprocess engineering and as a result, decided to follow a course in fundamentals of Biotechnology Engineering.

Always looking for new challenges, he moved to Edinburgh, Scotland (UK) in 2011, in order to learn English while working in hospitality. His passion about innovation and bioprocesses guided him to move later to The Netherlands and study Biotechnology, with specialization in Process Technology, at Wageningen University & Research (WUR). His MSc thesis *"Exergetic Analysis of Protein Extraction from Sugar Beet Leaves"* was performed in the Food Process Engineering department at WUR, under the supervision of Prof. dr. ir. A.J. van der Goot. His internship was done at Corbion (The Netherlands), under the supervision of Dr. ir. A. Morão and Dr. ir. P.M. Slegers. He worked on "*Sustainable Process Design of Bio-based Chemicals*", allowing him to improve his computer skills as he developed a tool to evaluate the sustainability of processes in their early stage of design. He graduated in 2015 and moved to Antwerp, Belgium.

It was right after following a MOOC in Biobased Economy when he finally joined Delft University of Technology (TUD), to pursue a PhD in Bioprocess Engineering. With Prof. dr. ir. L.A.M. van der Wielen and Dr. ir. A.J.J. Straathof as promotors, his project *"Pretreatment of lignocellulosic biomass for acetic acid co-valorization"* involved all the ingredients required for a stimulating and motivational investigation. The research, presented in this thesis, implied a combination of experimental and computational work at both laboratory and pilot scales, making use of chemical engineering concepts coupled with biotechnological applications.

After more than 10 years living in 3 different countries, he recently moved back to the south of Spain, starting a new professional stage.

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Dear reader, if you have reached this page, and you find your name below, it is for sure because our paths crossed at certain point during this doctoral stage. And even if your name is not here, most probably you helped me…and you do not know it. Believe me, just the simple gesture of having this book in your hands and reading these words, you are already collaborating. For any of these reasons, let me say: Thank you!

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