



# LUND UNIVERSITY

## Base-catalyzed depolymerization of kraft lignin for valuable chemical production

Li, Kena

2021

*Document Version:*

Publisher's PDF, also known as Version of record

[Link to publication](#)

*Citation for published version (APA):*

Li, K. (2021). *Base-catalyzed depolymerization of kraft lignin for valuable chemical production* (Media-Tryck ed.). [Doctoral Thesis (compilation), Lund University]. Chemical Engineering, Lund University.

*Total number of authors:*

1

### General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117  
221 00 Lund  
+46 46-222 00 00

# Base-catalyzed depolymerization of kraft lignin for valuable chemical production

KENA LI | CHEMICAL ENGINEERING | LUND UNIVERSITY





Her name is Kena Li, and she was born in Shanxi, China in 1991. She started her doctoral studies at the Department of Chemical Engineering, Lund University in 2016. When she started to study chemistry in middle school, she was laughed at by her classmates because her name was pronounced almost the same as sodium hydroxide, which is such a corrosive chemical. At that time, she would never have imagined that many years later, this corrosive chemical was to be used by her to degrade lignin, recovered from a paper and pulp waste stream, to produce value-added chemicals.

ISBN: 978-91-7422-800-7

Chemical Engineering  
Faculty of Engineering  
Lund University



LUND  
UNIVERSITY



# Base-catalyzed depolymerization of kraft lignin for valuable chemical production

Kena Li

Department of Chemical Engineering  
Lund University, Sweden  
2021



**LUND**  
UNIVERSITY

## DOCTORAL DISSERTATION

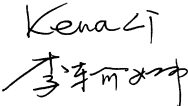
by due permission of the Faculty of Engineering, Lund University, Sweden.  
To be defended on 7<sup>th</sup> of May 2021 at 9.15 in lecture hall KC:B at  
Kemicentrum, Naturvetarvägen 14, Lund.

*Faculty opponent*

Dr. Hans Grundberg, Department of Process Engineering, MoRe Research,  
Domsjö, Sweden

<b>Organization</b> LUND UNIVERSITY Department of Chemical Engineering P.O. Box 124 SE-22100 Lund, Sweden		<b>Document name</b> DOCTORAL DISSERTATION	
		<b>Date of issue</b> 7 May 2021	
<b>Author(s)</b> Kena Li		<b>Sponsoring organization</b> Swedish Energy Agency Swedish Foundation for Strategic Research	
<b>Title and subtitle</b> Base-catalyzed depolymerization of kraft lignin for valuable chemical production			
<b>Abstract</b> <p>Lignin is one of the three predominant biopolymers that make up plants together with cellulose and hemicellulose, and it is the only plant component that is based on aromatic units. The industrial kraft pulping process generates large amounts of lignin, which is currently used to generate heat for the process. The subject of this thesis is the valorization of kraft lignin into valuable chemicals. The work started with the depolymerization of kraft lignin, followed by studies on efficient separation processes to obtain low-molecular-weight (LMW) compounds. Finally, the microbial conversion of lignin-derived monomers for the production of fine chemicals was investigated.</p> <p>A lab-scale continuous-flow reactor system was developed for the depolymerization of two different Kraft lignin samples: Indulin AT and black liquor retentate (BLR) using NaOH as the only catalyst. The depolymerization experiments were performed under mild conditions at temperatures less than 250 °C and residence times less than 4 min. The effect of the depolymerization temperature, residence time, NaOH concentration, and the lignin substrate loading on depolymerization was studied. Depolymerization of Indulin AT and BLR resulted in a mixture of lower molecular weight compounds. Temperature was found to be an important factor in the depolymerization of lignin, and the main phenolic compounds obtained after depolymerization were guaiacol and vanillin.</p> <p>An ultrafiltration GR95PP membrane with a cut-off of 2 kDa was used in membrane filtration studies. The combination of base-catalyzed depolymerization and membrane filtration was investigated in an attempt to obtain more lignin-derived LMW compounds without severe repolymerization. Ultrafiltration of the depolymerized BLR sample removed 60% of the LMW compounds. A separation process starting with membrane filtration followed by acid precipitation and low-temperature evaporation under vacuum is suggested to obtain pure guaiacol from the depolymerized lignin samples. Concentrated vanillin and other monomers were also separated and collected.</p> <p>Finally, bioconversion of guaiacol using an engineered strain of <i>Pseudomonas putida</i> KT2440 was studied. The strain had been modified for guaiacol consumption with a cytochrome P450 enzyme and ferredoxin reductase and also modified to produce muconic acid by deletion of the downstream enzymes encoded by catBC. Both pure and kraft lignin-derived guaiacol was quantitatively converted into muconic acid, which is a promising precursor to adipic acid or terephthalic acid used in the production of nylon-6,6 or bio-plastics e.g bio-PET.</p>			
<b>Key words</b> Kraft lignin, Depolymerization, CFR, Membrane filtration, Guaiacol, Vanillin, bioconversion			
Classification system and/or index terms (if any)			
Supplementary bibliographical information		<b>Language</b> English	
<b>ISBN (print)</b> 978-91-7422-800-7		<b>ISBN (pdf)</b> 978-91-7422-801-4	
Recipient's notes		<b>Number of pages</b> 262	
		Security classification	
		Price	

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature 

Date 2021-03-26

# Base-catalyzed depolymerization of kraft lignin for valuable chemical production

Kena Li

Department of Chemical Engineering  
Lund University, Sweden  
2021



**LUND**  
UNIVERSITY

Coverphoto by Kena Li and Xianshao Zou

Copyright pp 1-62 (Kena Li)

Paper I © The authors (Open Access)

Paper II © 2019 by MDPI, Open Access

Paper III © by the Authors (Manuscript, submitted for publication)

Paper IV © by the Authors (Manuscript, submitted for publication)

Paper V © by the Authors (Manuscript, submitted for publication)

Paper VI © The authors (Open Access)

Paper VII © by the Authors (Manuscript, submitted for publication)

Faculty of Engineering  
Department of Chemical Engineering

ISBN 978-91-7422-800-7 (print)

ISBN 978-91-7422-801-4 (pdf)

Printed in Sweden by Media-Tryck, Lund University  
Lund 2021



Media-Tryck is a Nordic Swan Ecolabel  
certified provider of printed material.  
Read more about our environmental  
work at [www.mediatryck.lu.se](http://www.mediatryck.lu.se)

**MADE IN SWEDEN** 

# Abstract

Lignin is one of the three predominant biopolymers that make up plants together with cellulose and hemicellulose, and it is the only plant component that is based on aromatic units. The industrial kraft pulping process generates large amounts of lignin, which is currently used to generate heat for the process. The subject of this thesis is the valorization of kraft lignin into valuable chemicals. The work started with the depolymerization of kraft lignin, followed by studies on efficient separation processes to obtain low-molecular-weight (LMW) compounds. Finally, the microbial conversion of lignin-derived monomers for the production of fine chemicals was investigated.

A lab-scale continuous-flow reactor system was developed for the depolymerization of two different Kraft lignin samples: Indulin AT and black liquor retentate (BLR) using NaOH as the only catalyst. The depolymerization experiments were performed under mild conditions at temperatures less than 250 °C and residence times less than 4 min. The effect of the depolymerization temperature, residence time, NaOH concentration, and the lignin substrate loading on depolymerization was studied. Depolymerization of Indulin AT and BLR resulted in a mixture of lower molecular weight compounds. Temperature was found to be an important factor in the depolymerization of lignin, and the main phenolic compounds obtained after depolymerization were guaiacol and vanillin.

An ultrafiltration GR95PP membrane with a cut-off of 2 kDa was used in membrane filtration studies. The combination of base-catalyzed depolymerization and membrane filtration was investigated in an attempt to obtain more lignin-derived LMW compounds without severe repolymerization. Ultrafiltration of the depolymerized BLR sample removed 60% of the LMW compounds. A separation process starting with membrane filtration followed by acid precipitation and low-temperature evaporation under vacuum is suggested to obtain pure guaiacol from the depolymerized lignin samples. Concentrated vanillin and other monomers were also separated and collected.

Finally, bioconversion of guaiacol using an engineered strain of *Pseudomonas putida* KT2440 was studied. The strain had been modified for guaiacol consumption with a cytochrome P450 enzyme and ferredoxin reductase and also modified to produce muconic acid by deletion of the downstream enzymes encoded by catBC. Both pure and kraft lignin-derived guaiacol was quantitatively converted into muconic acid, which is a promising precursor to adipic acid or terephthalic acid used in the production of nylon-6,6 or bio-plastics e.g bio-PET.



# Popular scientific summary

When talking about petroleum products, many people naturally think of the fuels used for transportation, such as gasoline and diesel. However, petroleum-based products are used in many ways in daily life. Examples of these products are liquid detergents, shampoo, and shower gel; synthetic materials such as nylon, polyester, and polypropylene fibers; cosmetics, such as perfumes and colorants; and the countless plastics used in daily life. These are currently all produced from petroleum, which can be regarded as the foundation of the chemical industry. However, the depletion of petroleum fuels resulting from increasing demand, and the environmental problems caused by their use mean that it is imperative that we find renewable sources that can replace fossil resources for the production of fuels and other chemicals.

Lignin is an important component of wood, accounting for up to one-third of the dry weight of plants, and is a promising material to replace petroleum resources. If we compare a plant to a building, then the structure of wood corresponds to the ‘reinforced concrete’ structure of a building. Cellulose, another important component of wood, is equivalent to the ‘reinforced steel’ skeleton of the building while lignin can be compared to the ‘concrete’ filler. Cellulose is an essential raw material in the pulp and paper industry for the production of printing paper, paper bags, cartons, etc., that are used in daily life. Cellulose can also be hydrolyzed into sugar and then fermented to produce fuel ethanol that can be used as a transportation fuel. However, lignin is generated as waste in both the paper industry and the bioethanol production process. Although lignin contains different basic aromatic building blocks and has excellent potential for the production of high-value-added chemicals, it is currently used in industry as a fuel for internal energy supply.

One of the challenges in the high-value utilization of lignin is that its complex structure and recalcitrance make it difficult to degrade. Therefore, the first part of this thesis work was focused on lignin degradation. In other words, using a suitable method to break down the large ‘concrete’ blocks into small pieces. Black liquor, a waste stream from papermaking (denoted kraft lignin) was used as the raw lignin material as it is currently the largest source of lignin. Lignin can be degraded under different conditions, for example, high temperature or high pressure, using acids, alkalis, or metallic catalysts. During the pulping process, sodium hydroxide is used to treat the wood chips. The lignin obtained from such a process is thus dissolved in an alkali. Therefore, the degradation of kraft lignin was investigated in an alkaline environment. A newly designed lab-scale tubular continuous reactor was used to study the effects of degradation temperature and time, and the concentration of lignin and the alkaline catalyst on lignin

degradation. The complex structure of lignin was broken down using this method, resulting in many smaller compounds.

Further utilization of these smaller compounds requires methods for their separation and purification. The second part of this work was thus mainly concerned with finding a suitable membrane filter to separate the small fractions from the degraded lignin. During membrane filtration, the feed solution is passed through a membrane with pores of a specific size; components that are smaller than the pores will pass through the membrane together with the solvent (forming the 'permeate'), while larger fractions are retained (the 'retentate'). Repeated degradation and membrane filtration were used to obtain as many small compounds as possible. After this process, most of the small fractions were collected in the permeate. The most abundant monomer (the smallest unit) in the degradation products of lignin is a compound called 'guaiacol'.

In nature, lignin can be degraded by bacteria or fungi. Some of these microorganisms can metabolize various lignin degradation products to produce one or more specific compounds, greatly reducing the complexity of lignin degradation products. Therefore, the third part of this thesis was focused on using microorganisms to further convert lignin degradation products into valuable chemicals. This part relies on bacterial strain screening and genetical engineering carried out by other researchers in our group. Briefly, a number of bacterial strains are 'fed' a series of lignin degradation products in order to identify the strains that can survive and grow on lignin. These strains are then genetically modified so that they produce a desired chemical. For example, a strain of the bacterium '*Pseudomonas putida* KT2440' was modified previously so that it can metabolize guaiacol to produce a substance called muconic acid. The results of this work indicated that this strain could utilize the guaiacol in the lignin degradation products obtained from the second part of the work, to produce high yields of muconic acid. In industry, muconic acid can be further converted to produce terephthalic acid or adipic acid, which are used to produce terephthalic acid or adipic acid to produce plastics, lubricants, nylon-6,6, etc.

In summary, a path for the valorization of lignin waste streams from the paper industry was developed by investigating continuous degradation, membrane filtration, and bioconversion. In the future, new catalysts or additives could be explored to improve the efficiency of degradation and separation. Also, other microorganisms could be investigated or modified for the efficient conversion of different lignin degradation products into valuable chemicals.

# Populärvetenskaplig sammanfattning

När det diskuteras petroleumprodukter tänker många naturligt på transportbränsle så som bensin och diesel. Men det är en väsentligt större andel av människans produktion och liv som är beroende av oljeprodukter än vid första anblicken. Till exempel innehåller tvättmedel, schampo, dusch gel petroleumderivat, precis som textilier, t.ex. nylon, polyester och polypropenfibres och kosmetika i både parfymer och färgämnen. För att inte nämna alla plaster som används varje dag. Alla dessa produkter är oljebaserade och det är inte fel att säga att olja är basen i hela den kemiska industrin. Människans ökade efterfrågan på produkter ökar därför efterfrågan och i förlängningen utarmningen av olja. Användningen av olja leder även till andra miljöföroreningsproblem som gör det nödvändigt att snabbt hitta en förnybar källa som kan ersätta de fossila bränsle- och kemikalieresurserna för transportbränsle och kemisk produktion

Lignin är en av huvudkomponenterna i trä (ämnet utgör ungefär en tredjedel av en växts torrsvikt) och är ett lovande material för att ersätta petroleum. Om vi liknar en växt vid en byggnad motsvarar trästrukturen den stålförstärkta betongen i byggnaden. Cellulosa, en annan av trädets huvudkomponenter är byggnadens stålskelett och ligninet är betongen som håller ihop materialet. Cellulosa är en viktig råvara vid produktion av papper och massa och används till exempel för att producera skrivpapper, papperspåsar, kartonger och så vidare som används i vardagen. Utöver det kan cellulosa hydrolyseras till socker och vidare jäsas till etanol för att producera bränsle till etanolbilar. Hursomhelst, oavsett om det är i pappersindustrin eller i produktionen av bioetanol så betraktas ligninet som en lågvärdig bioprodukt och används för att producera värme. Detta trots att lignin har en aromatisk struktur och vars ingående byggstenar skulle kunna användas i tillämpningar inom kemiindustrin där de skapar mycket högre värde.

En av flaskhalsarna i att ta till vara det högre värdet i lignin är dess komplexa struktur och stora stabilitet, vilket gör materialet svårt att bryta ned. Därför fokuserar första delen av detta arbete på att bryta ner lignin till mindre bitar. Med andra ord handlar det om att hitta lämpliga metoder för att bryta ner de stora "ligninbetongblocken" till mindre, användbara bitar. Det lignin som används i arbetet kommer från den industriella tillverkningen av papper och utvinns ur det vi kallar svartlut. Denna råvara valdes då det är den största nu tillgängliga potentiella källan av lignin (kraftlignin). Nedbrytning av lignin kan ske under olika betingelser, till exempel i sur eller basisk miljö, vid höga temperaturer eller tryck, i närvaro av katalysatorer etc. Vid tillverkning av pappersmassa används natriumhydroxid för att lösa ut ligninet från trästrukturen vilket innebär att det lignin som kommer från den typen av process är lösligt i alkali. Av denna anledning undersöktes ligninnedbrytning under alkaliska betingelser i en ny reaktortyp. I en nydesignd tubreaktor undersöktes effekten av olika parametrar

så som temperatur, uppehållstid och koncentration av lignin och alkali på nedbrytningen av lignin. Studierna visar att genom denna metod så kan den komplexa lininstrukturen brytas och många småbitar av lignin tillverkas, småbitar av olika storlekar och med olika kemisk sammansättning.

För att använda dessa småbitar behöver de separeras och renas. Därför fokuserade en andra del av arbetet på att hitta lämpliga membran för att separera de allra minsta bitarna från resten av blandningen av lignin och alkali. Vid membranfiltrering av den basiska lösningen trycks den basiska lösningen mot ett membran med specifik porstorlek och de mindre molekylerna kan passera igenom membranet tillsammans med en del av vätskan, detta kallar vi för permeat. Den kvarvarande delen kallas för retentat och innehåller de större molekylerna. I den här delen av forskningen undersöktes hur upprepad nedbrytning av ligninet med mellanfiltrering påverkade utbytet till små molekyler som består av en, två eller tre aromatringar. Den molekyl som förekommer i högst halt kallas för guaiacol.

I naturen kan lignin brytas ned av bakterier och svampar. Vissa av dessa mikroorganismer kan även bryta ner nedbrytningsprodukterna från lignin och tillverka andra specifika produkter. Därför handlar den tredje delen av denna avhandling om användning av mikroorganismer för att vidareförädla ligninets nedbrytningsprodukter till användbara kemikalier. Denna del förlitar sig till stor del på screening av bakteriestammar och ingenjörsarbete från projektteamet. För att kort beskriva processen genomförs först en serie nedbrytningsförsök med lignin och den resulterande produkten matas till olika typer av bakterier för att se vilka typer som äter ligninet. Bakterierna som kan metabolisera ligninderivaten modifieras sen genetiskt för att producera en viss målkemikalie. Olika nedbrytningsprodukter kräver således olika bakterier. Till exempel kan bakterien "*Pseudomonas putida* KT2440" ta upp och omvandla guaiacol till mukonsyra efter genetisk modifiering. Detta arbete resulterade i denna bakterie som kan metabolisera guaiacol och producera nästan lika mycket mukonsyra som mängden guaiacol som används. I industrin kan mukonsyra användas för att producera tereftalsyra eller adipinsyra som används i plast, smörjmedel och i nylon. För att sammanfatta så har arbetet resulterat i en process för att kontinuerligt bryta ner lignin, filtrera ut de mindre nedbrytningsprodukterna och omvandla dessa biologiskt till en specifik kemikalie. Detta arbete öppnas därför vägen för ett nytt sätt att skapa värde från en biprodukt från pappers- och massaindustrin. I framtiden kan nya katalysatorer och tillsatsmedel undersökas för att försöka ytterligare förbättra nedbrytningen av ligninet till mindre bitar samt att förbättra filtreringen för att öka återvinningsgraden av de bildade småbitarna. Slutligen kan också andra mikroorganismer väljas och modifieras för att effektivt omvandla nedbrytningsprodukterna från ligninet till värdefulla kemikalier.

# 中文科普简介

当我们谈起石油产品，很多人自然会想到交通运输所使用的燃料，比如汽油柴油。然而，我们的生活对石油的依赖远不止于此。我们用的清洁产品，比如洗涤剂，洗发剂和沐浴露等；服装材料，比如尼龙，聚酯纤维，聚丙烯纤维等；甚至化妆品中的香精，染料；以及日常生活中使用最多的塑料等都是由石油生产而来的，因此石油又被称为是工业的“血液”。随着人们对能源需求的加重，石油资源也被过度开发，然而石油作为不可再生的化石燃料资源，储量有限。同时，石油资源的使用所造成的环境污染问题也日渐明显。因此，寻找可替代石油资源的可再生的绿色资源成为很大的挑战。

木质素作为木材的一种主要成分，在自然界中含量丰富，可以达到植物干重的三分之一，是极有潜力替代石油资源的绿色原料。如果将木材比作建筑的“钢筋混凝土”结构，纤维素就像是木材这座“建筑物”的“钢筋”骨架，而木质素与半纤维素一起，就相当于木材中的“混凝土”，起到粘合与填充的作用。木材中的纤维素是制浆造纸工业的重要原料，我们日常生活中所用到的各类纸张，纸袋，纸箱等都以其为原料生产而来的。此外，我们所知道的乙醇汽车使用的燃料乙醇也可以通过使用微生物降解发酵纤维素的方式来获得。无论是传统的造纸工业还是现代生物乙醇生产过程，木质素都是以废弃物的形式产生。尽管木质素有着很大的潜力生产高附加值的化学品，可目前工业上对其利用也几乎仅限于将其燃烧以提供热能。

造纸废液中含有大量的木质素成分，除了一部分被燃烧供能外，部分木质素伴随着生产过程被提取出来。这类工业木质素化学结构已经遭到一定的破坏，且溶解于碱液。我的论文研究主体是以造纸工业所产生的废液中提取的木质素为原料，研究其在碱性环境下的降解情况。通过使用新设计的管式连续反应器，研究不同降解温度、时间、木质素的浓度，以及碱性催化剂的浓度等对木质素持续降解的影响。我们发现，通过该方法，木质素大分子结构被打破，产生了许多有价值的小分子物质，如酚类。

如果将木材中的木质素继续比作建筑物中的混凝土，制浆造纸过程中，木质素就被破坏成大块的混凝土。经过碱催化持续降解，大块的混凝土被打破成大小不一的小块混凝土以及一些粒状和粉末状的混凝土混合物。想要进一步转化降解后的木质素小分子，就需要选用合适的分离方法对大小不一的分子进行分离纯化。我的论文中第二部分工作就与此有关。我们主要是选用合适的膜对降解后的木质素进行分离。具体地说来，就是通过重复降解和膜分离的方法来尽可能获得更多的木质素小分子化合物富集的液体。经过进一步的分离提取，木质素小分子油产品和木质素单体被分离出来。木质素降解产物中含量最多的单体是一种名为‘愈创木酚’的化合物。

在自然界中，木质素可以被一些细菌或者真菌等降解。其中一些微生物可以代谢多种木质素的降解产物，而产生特定的化合物小分子。因此，想要将木质素小分子进一步转化成有用的化学平台分子化合物还需要借助一些特定微生物的帮助。我的第三部分工作主要是利用微生物对木质素降解产物进行进一步的生物转化而产生所需的化学品。这一部分依赖于同项目组的菌种筛选及微生物基因改造工作。简而言之，我们首先使用一系列的木质素降解产物来‘喂养’各类菌株，从而筛选出‘食用’木质素的菌株；然后再对筛选出来的特定菌株进行基因改造，使其可以代谢特定的木质素降解产物，产生特定所需的化合物。比如，本文所用的就是一种经过改造的名为‘*P.putida* KT2440’的菌株，它就可以代谢“愈创木酚”而定向产生一种名叫“粘康酸”的物质。工业上，粘康酸经过进一步转化可以生产对苯二甲酸或己二酸，前者是生产 PET 塑料的重要化学品，后者则可用来生产尼龙 66 或者润滑油等重要工业产品。

简单地总结一下，我的博士论文工作通过使用连续碱法对造纸工业废弃物中的木质素进行降解，之后通过使用膜分离的方法分离出小分子物质，最后通过微生物代谢将其转化为高附加值的化学品。我的研究为木质素高附加值化利用提供了一些新的可能性，但在深入探索过程中，我们依然发现了更多的挑战。比如化学降解得率低，分离过程中小分子又会重聚成大分子，可降解多种小分子的微生物难以筛选等等。我们相信在未来，有更多的研究将着力解决这些困难，并找到更高效快捷的方法来转化木质素，以开发更低成本高效率的木质素转化路径，获得更多更“绿色”的化学品。

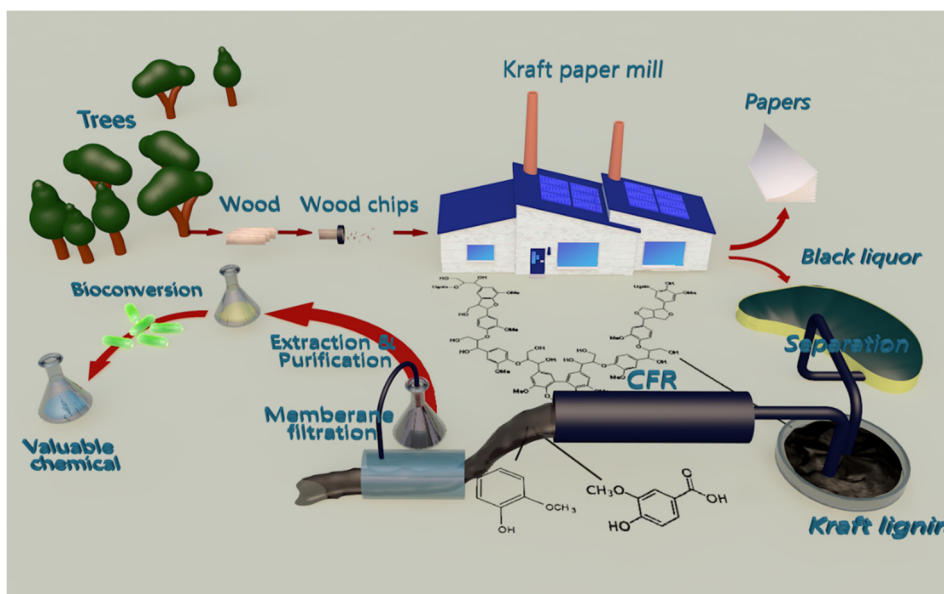


Figure 1. The process at a glance.

# List of publications

This thesis is based on the following papers, which will be referred to in the text by their roman numerals. The author's contributions to each study are also given.

- I. Omar Abdelaziz, **Kena Li**, Per Tunå, and Christian Hulteberg. Continuous catalytic depolymerization and conversion of industrial Kraft lignin into low-molecular-weight aromatics. *Biomass conversion and biorefinery* 2017, 2190-6823.  
*I participated in the design of the study, the performance of the experimental work, and the preparation of the manuscript.*
  
- II. **Kena Li**, Basel Al-Rudainy, Mingzhe Sun, Ola Wallberg, Christian Hulteberg, Per Tunå. Membrane separation of the base-catalyzed depolymerization of black liquor retentate for low-molecular-mass compound production. *Membranes* 2019, 9, 102.  
*I designed the study and performed all of the depolymerization experiments. I performed the analysis with help from my co-authors. I wrote the manuscript with input from my co-authors.*
  
- III. Henrik Almqvist, Henrique Veras, **Kena Li**, Javier Garcia Hidalgo, Christian Hulteberg, Marie Gorwa-Grauslund, Nádia Skorupa Parachin, and Magnus Carlquist. Muconic acid production using engineered *Pseudomonas putida* KT2440 and guaiacol-rich fraction derived from kraft lignin. Under review.  
*I participated in designing the study and in performing the lignin depolymerization, separation and purification experiments, and drafted this section of the manuscript. I critically reviewed the whole manuscript.*
  
- IV. **Kena Li**, Henrik Almqvist, Christian Hulteberg. Three-step conversion of Indulin AT to muconic acid under mild condition. Submitted for publication.  
*I designed the study and performed the chemical conversion experiments, as well as sample preparation and analysis experimental work. I wrote the manuscript with input from the co-authors.*

- V. **Kena Li**, Linnéa Kollberg, Henrik Almqvist, Bin Xu, and Christian Hulteberg. Maximizing yield of liquid-lignin separation from kraft black liquor. Under review.  
*I designed the study, performed the experimental work, and data analysis together with Linnéa Kollberg. I wrote the manuscript based on a draft by Linnéa Kollberg.*
- VI. Jens Prothmann, **Kena Li**, Christian Hulteberg, Peter Spégel, Margareta Sandahl, and Charlotta Turner. Non-targeted analysis strategy for the identification of phenolic compounds in complex technical lignin samples. ChemSusChem 2020, 13, 4605-4612.  
*I participated in the lignin sample preparation work and critically reviewed the manuscript.*
- VII. **Kena Li**, Jens Prothmann, Margareta Sandahl, Sara Blomberg, Charlotta Turner, and Christian Hulteberg. Investigating lignin-derived monomers and oligomers in low-molecular-weight fractions separated from depolymerized black liquor retentate by membrane filtration. Submitted for publication.  
*I designed the study together with Jens Prothmann and performed the depolymerization and sample preparation experiments. I participated in the data analysis. I wrote the manuscript with input from the co-authors.*



# Acknowledgements

I cannot believe that nearly five years have passed since I first arrive in Sweden. From the bottom of my heart, I am grateful to everyone for their help and company. It is you who made me have such a wonderful journey.

Prof. Christian Hulteberg, my fantastic supervisor: I am who and where I am today thanks to your continuous guidance, positive comments, encouragement, and support over the years. It has been an honor to have such a wonderful supervisor.

Dr. Per Tunå, my former co-supervisor, you taught me so much on experimental techniques and answered all my questions so patiently. Your constant encouragement helped banish my self-doubt and gave me confidence.

Dr. Sara Blomberg, my dear co-supervisor, who always give me the greatest support when I needed it most. Your encouragement, feedback and inspiration helped me to improve so much, especially during the writing of this thesis.

Special thanks to Prof. Mats Galbe, Prof. Ola Wallberg, Prof. Gunnar Lidén, Prof. Frank Lipnizki, Dr. Helena Svensson, and Dr. Johan Thuvander for their help and professional suggestions and guidance.

I would like to thank all my co-authors. Special thanks to Dr. Henrik Almqvist and Dr. Basel Al-Rudainy for their expertise and collaboration, and for all their help with lab techniques. I would also like to thank everyone in the Lignin Group for their contributions and interesting discussions on the project. Special thanks to Dr. Jens Prothmann, Dr. Krithika Ravi, Dr. Mingzhe Sun, Dr. Henrique Veras, Dr. Javier García Hidalgo, and Dr. Daniel P. Brink for their help.

Many thanks to Paula Leckius, Zhiwen Wang, Lu Chen, and Xianshao Zou for all their help and professional suggestions during my thesis writing and printing.

The Swedish Energy Agency and the Swedish Foundation for Strategic Research are gratefully acknowledged for financial support.

I would like to thank our administrative and technical staff for their support. Thank you, Gertrud Persson, Gity Yahoo, Herje Schagerlöf, Leif Stanley, Maria Messer, Mattias Alveteg, Krisztina Kovacs, Michaël Grimsberg, and dear Lena for all your help.

A big thank you to all my former and current colleagues, for the friendly atmosphere, and company in the lab and fika room. Omar, thank you for all the help you gave me at the beginning of my PhD, and for the pleasant times on courses and at conferences. Special thanks to Linnéa, both my colleague and my best Swedish friend, for your warm support and love. To Hanna, my dear office roommate and friend, you know everything and took care of me in every way. Thank you, Helena, Henrik, Laura, Karin, Sara, Gregor, Meher, Adnan, Joana, Bin, Lucy, Simon, Maja, Astrid, Mikael, Mariona, Miguel, Bori, Balázs, Anton, Michael, and Johanna.

Finally, I would like to thank all my friends and family for their love and support. Special thanks to the Ståhlberg family for all their care and love. Thank you Fredrik K, Fredrik N, Gustav, Amanda, Vera, Daoba, Ruiyu, Hongduo, Hong C, Xiaoting, Haoran, Xin, Qi, Junhao, Lili, Lulu, Mengze, Feifei, Jian, Weimin, Haotian, Haiyue, Wenjun, Gaowa, Hong J, Zhiwei, Jun, Siyu, Qingshuang, Xuwei, Shuai, Yanfei, Hongyan, Xuan, Yubin, Zhendong, Jingwen, Yangxurui, Weishan, Shan, Yang Lv, Lu Y, Yutang, Chao, Ka, Andreas, Anna, and Claire for the wonderful times we have shared during the past five years. Thank you, dear Signe, Leah, Sara, Gege, Phil, William, and Edvin.

My dear Jason, your sparkling warm soul, unlimited patience, and endless love, nourish me, and make me thrive. I love you! My dear little one, thank you for choosing me to be your mum, and thank you for accompanying me throughout this whole thesis writing journey. We are looking forward to meeting you!

亲爱的爷爷奶奶爸爸妈妈和弟弟，我爱你们！

# Abbreviations

ACD	Acid-catalyzed depolymerization
BCD	Base-catalyzed depolymerization
BLR	Black liquor retentate
CFR	Continuous flow reactor
GGM	Galactoglucomannan
HMW	High-molecular-weight
HPLC	High-performance liquid chromatography
HRMS <sup>n</sup>	High-resolution multiple stage tandem mass spectrometry
HSQC	Heteronuclear single-quantum coherence
LMW	Low-molecular-weight
M <sub>w</sub>	Weight average molecular weight
M <sub>n</sub>	Number average molecular weight
MS	Mass spectrometry
m/z	Mass-over-charge ratio
NMR	Nuclear magnetic resonance spectroscopy
OTR	Oscillatory tubular reactor
QTOF-MS	Quadrupole-time-of-flight mass spectrometry
SEC	Size exclusion chromatography
SLRP	Sequential liquid-lignin recovery and purification
UF	Ultrafiltration
UPLC	Ultra-high-performance liquid chromatography
UHPSFC	Ultra-high-performance supercritical fluid chromatography

# Contents

<b>Abstract</b> .....	<b>i</b>
<b>Popular scientific summary</b> .....	<b>ii</b>
<b>Populärvetenskaplig sammanfattning</b> .....	<b>iv</b>
<b>中文科普简介</b> .....	<b>vi</b>
<b>List of publications</b> .....	<b>viii</b>
<b>Acknowledgements</b> .....	<b>x</b>
<b>Abbreviations</b> .....	<b>xii</b>
<b>Contents</b> .....	<b>xiii</b>
<b>1. Introduction</b> .....	<b>1</b>
1.1 Background .....	1
1.2 Scope and outline of this thesis .....	2
<b>2. Lignin and its valorization</b> .....	<b>5</b>
2.1 Lignin resources and its structure .....	5
2.1.1 Lignin resources .....	5
2.1.2 The structure of lignin .....	6
2.2 Lignin production from industrial processes .....	7
2.2.1 Kraft pulping .....	8
2.2.2 Sulfite pulping .....	9
2.2.3 Soda pulping .....	10
2.2.4 Other industrial processes for lignin production .....	10
2.3 The valorization of technical lignin .....	11
2.3.1 Potential utilization of lignin .....	11
2.3.2 Technical lignin depolymerization .....	11
<b>3. Kraft lignin depolymerization</b> .....	<b>15</b>
3.1 Chemical composition of the two kraft lignins .....	15
3.1.1 Indulin AT .....	15
3.1.2 Black liquor retentate .....	17
3.2 Depolymerization reactor .....	19
3.3 Base-catalyzed depolymerization of Indulin AT .....	20
3.3.1 The effects of the reaction conditions .....	20
3.3.2 Characterization of the depolymerization products .....	23
3.4 Base-catalyzed depolymerization of BLR .....	29
3.4.1 Depolymerization of BLR at different temperatures .....	29

3.4.2 Multiple BLR depolymerization.....	32
<b>4. Separation and characterization of the lignin products.....</b>	<b>35</b>
4.1 Lignin separation from BLR.....	35
4.2 Separation of LMW lignin compounds.....	38
4.2.1 Acid precipitation.....	38
4.2.2 Membrane separation.....	40
4.2.3 Characterization of the permeate.....	42
<b>5. Bioconversion of depolymerized kraft lignin.....</b>	<b>45</b>
5.1 Growth assessment and guaiacol conversion.....	46
5.2 Bioconversion of depolymerized Indulin AT.....	47
<b>6. Conclusions and future work.....</b>	<b>51</b>
6.1 Conclusions.....	51
6.2 Outlook on future research.....	53
<b>References.....</b>	<b>55</b>

# 1. Introduction

## 1.1 Background

Lignocellulosic biomass is the most abundant bio-renewable resource on Earth. It captures solar energy through photosynthesis and stores it in the form of cell wall polymers. It is increasingly being considered as an alternative to fossil resources for the production of fuels, fine chemicals and biomaterials, as this would contribute significantly to the development of a sustainable industrial society and mitigates greenhouse gas emissions (Huber et al., 2006; Ragauskas et al., 2006; Zhou et al., 2011).

Lignocellulose is composed of three types of polymers, cellulose, hemicellulose, and lignin, together with small amounts of extractives. While cellulose and hemicellulose are polysaccharides, lignin is a three-dimensional polymer with phenylpropanoid units that act as cellular glue, providing structural support, mechanical resistance, and defense against microbial attack (Faraco, 2013; Guragain et al., 2015; Rubin, 2008). The first step in the utilization of lignocellulose to provide biofuels and chemicals is to ensure a suitable and widely available biomass feedstock, taking both economic and environmental perspectives into consideration. Lignocellulosic biomass from the paper and pulp industry, agriculture, forestry, the food industry, and municipal solid waste are all considered to be promising resources (Gavrilescu, 2014; Huber et al., 2006). Valorizing low-quality biomass residues or by-products for existing processes could also be an interesting option.

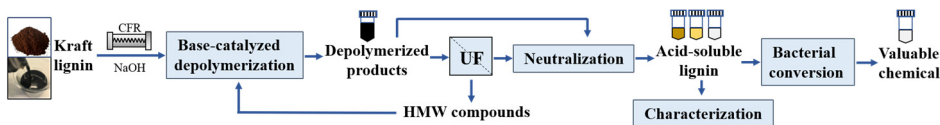
The pulp and paper industry is one of the largest and most important industries in Sweden. The dominant chemical pulping process used in Sweden, as well as worldwide, is the kraft process, which is used in the production of 76% of the world's paper pulp (Triantafyllidis et al., 2013). NaOH and Na<sub>2</sub>S are used to digest lignocellulosic materials in order to free the cellulose fibers by removing lignin, hemicellulose, and other extractives. This leads to the production of black liquor, which contains over 90% of the lignin from pulpwood. The kraft lignin in black liquor is currently burned to provide an internal source of energy for the recovery of pulping chemicals and for steam and power generation (Ragauskas et al., 2014). However, the extraction of 25-50% of the lignin does not disturb the operation of a modern Kraft pulp mill (Wallberg and Jönsson, 2003). Lignin is the most abundant natural source of aromatic polymers, and can provide a series

of aromatic compounds once it is broken down into low-molecular-weight (LMW) compounds. Thus, depolymerizing the lignin obtained from the kraft process into small molecules for the production of valuable chemicals or fuels, is potentially as important as the use of wood fibers in producing pulp and paper in modern kraft mills.

## 1.2 Scope and outline of this thesis

The structural complexity and high stability of intramolecular bonds make lignin depolymerization the greatest challenge in lignin valorization. A lignin depolymerization process suitable for industrial implementation must be a techno-economically feasible process that can be integrated into existing systems. Since NaOH is used as the digesting chemical in the pulping process, base-catalyzed depolymerization is considered a promising method for kraft lignin depolymerization. Repolymerization is a common and serious problem during lignin depolymerization, which leads to the reformation of high-molecular-weight insoluble structures and coke. Therefore, continuous depolymerization processes with a short residence time are desirable to reduce repolymerization and avoid coke formation.

The aim of the work described in this thesis was to develop a process including continuous depolymerization, membrane filtration, extraction, and final bioconversion, to obtain valuable chemicals from kraft lignin. The process is illustrated in Figure 1.1.



**Figure 1.1.** Simplified overview of the process used to obtain valuable chemical from kraft lignin. CFR: continuous flow reactor, UF: ultrafiltration, HMW: high-molecular-weight.

Chapter 2 provides a background on both native lignin and different industrial lignins, as well as their structures and potential for valorization. Chapter 3 discusses the potential of base-catalyzed depolymerization of two different kraft lignin samples using a continuous flow reactor (CFR) under mild conditions, by studying the influence of important process parameters such as temperature, reaction time, substrate loadings, and NaOH concentration. Once the depolymerized lignin degradation products have been obtained, separation methods, such as acid precipitation and membrane filtration were studied as a means of separating the valuable aromatic compounds. This is described in

Chapter 4. An advanced non-targeted UHPSFC/HRMS<sup>n</sup> analysis method was used to characterize the lignin products. Chapter 5 describes the work carried out on the biological conversion of the separated aromatic compounds for muconic acid production. The final chapter presents the conclusions drawn based on the results, together with recommendations for future work.





## 2. Lignin and its valorization

Among three main polymers making up lignocellulosic biomass, cellulose and hemicellulose are carbohydrate polymers, which are used as the raw materials in the pulp and paper industry. Research on the conversion of polysaccharides into monomeric sugars for the production of bioethanol has also led to significant breakthroughs in recent decades. The third polymer in lignocellulose is lignin, which is a phenolic polymer with a highly complex and recalcitrant structure, and its utilization is limited. This chapter describes the structural characteristics, industrial sources, and potential uses of lignin, as well as the current status on research into other uses of lignin.

### 2.1 Lignin resources and its structure

#### 2.1.1 Lignin resources

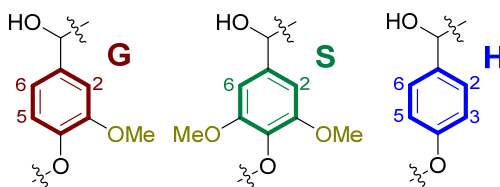
Lignin is one of the three predominant biopolymers that make up plants, besides cellulose and hemicellulose. Among the three main biomass constituents, lignin is the only component that is based on aromatic units (Holladay et al., 2007). Lignin accounts for about 15-40% of the total biomass in plants (Geun Yoo and Ragauskas, 2021). The amount of lignin varies from one plant species or parts to another. As can be seen from Table 2.1, softwood and hardwood contain more lignin than grass (Gellerstedt and Henriksson, 2008; Ragauskas et al., 2014). In addition to the biological and environmental elements, the lignin content in biomass is also affected by the factors such as harvesting time, method, and storage conditions (Welker et al., 2015). The lignin in plant cell walls acts as a cement to bind the cellulose and hemicellulose, thus providing structural support and a mechanical barrier against biological and chemical degradation (Guragain et al., 2015; Rubin, 2008).

**Table 2.1.** Amounts of lignin in various types of plants (adapted from Gellerstedt and Henriksson, 2008).

Plant	Lignin content (%)
<b>Softwood</b>	
Monterey pine	27
Scots pine	28
Norway spruce	28
Douglas fir	29
Eastern hemlock	31
<b>Hardwood</b>	
European aspen	19
Silver birch	20
Blue gum eucalyptus	22
Rose eucalyptus	25
<b>Grass</b>	
Rice straw	6.1
Bagasse	14

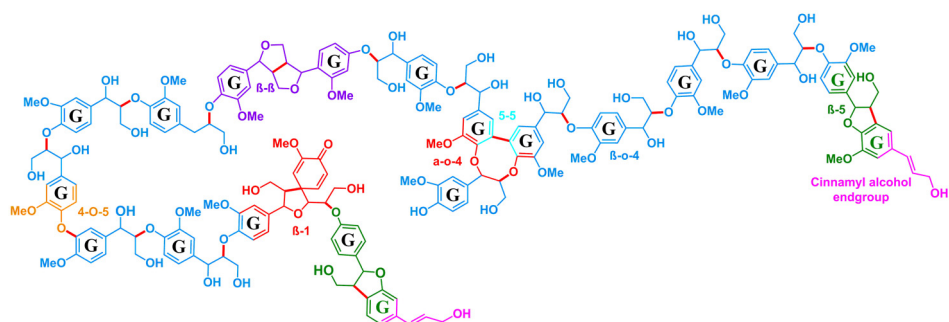
### 2.1.2 The structure of lignin

Lignin is synthesized through the enzymatic dehydrogenation and polymerization of three phenylpropanoid monomers, coniferyl alcohol (G-unit), sinapyl alcohol (S-unit), *p*-coumaryl alcohol (H-unit), together with the formation of C-O and C-C bonds, leading to a heterogeneous three-dimensional structure (Katahira et al., 2018). Figure 2.1 shows the three building units: the G-unit having one methoxyl group, the S-unit having two methoxyl groups, while the H-unit lacks a methoxyl group. Softwood lignin is made up of mainly coniferyl alcohol units (>90%), while hardwood lignin contains both coniferyl and sinapyl alcohol units. Herbaceous plant lignin consists of all three units incorporated with *p*-coumarate and ferulate (Katahira et al., 2018).



**Figure 2.1.** The three lignin building blocks described above: coniferyl alcohol (G-unit), sinapyl alcohol (S-unit), and *p*-coumaryl alcohol (H-unit).

A representative structure of softwood lignin is shown in Figure 2.2 (Ralph et al., 2019). The common lignin interunit linkages include  $\beta$ -O-4, 4-O-5,  $\beta$ -5,  $\beta$ -1,  $\beta$ - $\beta$ , and 5-5 structures. The dominant linkage is the  $\beta$ -O-4 linkage, which accounts for approximately half of the lignin linkage structures. The second methoxyl group on the fifth carbon atom of the aromatic rings of the S-unit prevents the formation of the  $\beta$ -5 and 5-5 carbon-carbon bonds. Therefore, there are less  $\beta$ -5 and 5-5 linkages in softwood lignin, and the  $\beta$ -O-4 linkage is completely dominant (Chakar and Ragauskas, 2004; Zakzeski et al., 2010). Many of the structures of lignin and their linkages have been elucidated using advanced methods such as nuclear magnetic resonance (NMR). However, the heterogeneous structures of lignin, the lower abundance of some linkages, as well as the structure alterations induced by the isolation or analytic process make the identification and precise quantification of lignin structures and linkages a challenge job. The exact structure of native lignin has yet to be determined (Chakar and Ragauskas, 2004).



**Figure 2.2.** Representative structure motifs and common linkages of softwood lignin (adapt from Ralph et al. 2019).

## 2.2 Lignin production from industrial processes

The global paper and cardboard production in 2018 was approximately 420 million metric tons (Tiseo, 2021). In pulp mills, after liberating the cellulosic fibers from wood chips, lignin is separated as a by-product (Sjöström, 1993). It has been estimated that more than 70 million tons of industrial lignin are extracted from pulping processes around the world annually (Lora, 2008). Currently, lignin is almost exclusively used for generating energy, although it holds great potential as a resource for high-value chemicals, fuels, and other bioproducts (Zevallos Torres et al., 2020). Different kinds of technical lignin are obtained depending on the chemical pulping process, such as the kraft process, the soda process, and the sulfite process.

## 2.2.1 Kraft pulping

The kraft process is the dominant chemical pulping process worldwide. It is used in the production of 76% of the world's paper pulp (Triantafyllidis et al., 2013). In the kraft process, the wood chips are treated with a solution of NaOH and Na<sub>2</sub>S, known as white liquor, at a temperature of about 170 °C for about 2 hours, in order to digest the lignocellulosic material and release the cellulose fibers (Rinaldi et al., 2016). During the cooking process, as shown in Figure 2.3, NaOH and Na<sub>2</sub>S react with the lignin in the wood chips, converting it into smaller fractions that dissolve in water/alkali; more than 90% of the lignin is dissolved in this process (Chakar and Ragauskas, 2004; Hamaguchi et al., 2012). The kraft lignin obtained from the acidification of the kraft pulping liquor has a molecular weight in the range from around 150 to 200,000 Da (Gellerstedt and Henriksson, 2008).

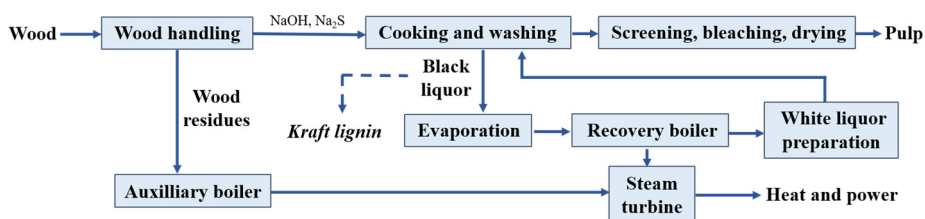
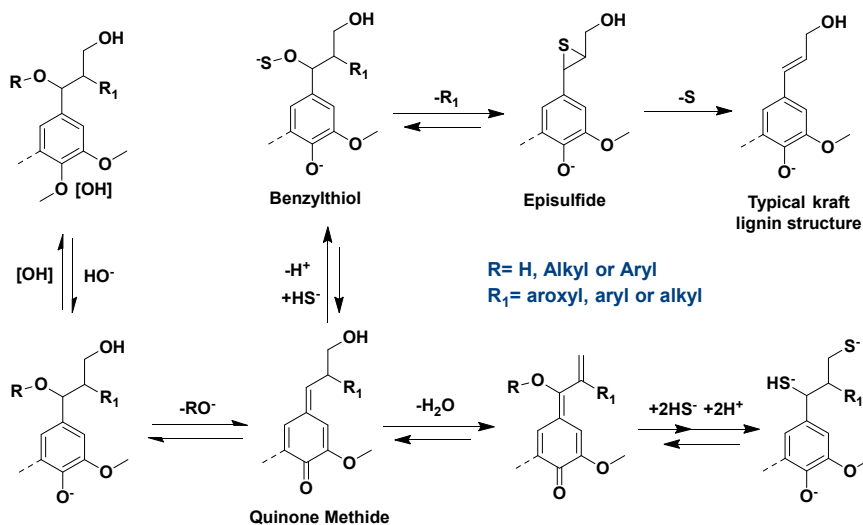


Figure 2.3. Overview of a Kraft process (adapt from Hamaguchi et al., 2012).

The degradation of lignin and lignin-carbohydrate linkages take place during digestion. The dominating lignin degradation reaction during kraft pulping is induced by the cleavage of aryl ether bonds (Lora, 2008). As can be seen in Figure 2.4, a quinone methide structure is formed as the intermediate. The hydrosulfide, sulfide, and hydroxyl ions react with the  $\alpha$ -carbon of quinone methide structures forming the benzyl mercaptide structure. An episulfide intermediate is generated after the nucleophilic species displaces the aryl ether attached to the  $\beta$ -carbon, which causes degradation of the lignin molecule and the formation of a phenolic hydroxyl group in the displaced aryl group. This is the reason why the solubility of kraft lignin in alkali is high (Lora, 2008). Alternatively, the quinone methide intermediate can be converted into an enol ether without cleavage of the  $\beta$ -ether bond (Chakar and Ragauskas, 2004; Gellerstedt and Lindfors, 1984). In addition to the ether bonds,  $\beta$ -5 and  $\beta$ -1 linkages are partially reacted forming stilbene structures (Gellerstedt, 2015). Condensation reactions also take place. The quinone methide sites and formaldehyde can act as acceptors reacting with the carbanions from lignin phenolic structures thus forming condensed lignin structures.



**Figure 2.4.** The dominating reaction for the formation of Kraft lignin (adapt from Lora, 2008).

### 2.2.2 Sulfite pulping

The sulfite process is another widely applied pulping process that produces relatively large amounts of lignin. In this process, the wood is treated by sulfurous acid and various sulfurous acid salts such as calcium sulfite and sodium sulfite at different pH levels. The lignin from biomass is sulfonated mainly at the  $\alpha$ -carbon position. The main condensation reaction during the sulfite process is between the  $\alpha$ -carbon and C6, and can be restrained by adding a higher concentration of bisulfite ions (Gellerstedt and Henriksson, 2008). The lignin fraction recovered from the sulfite process is called lignosulfonates, which are completely water soluble (Areskog et al., 2010). The molecular weight distribution of lignosulfonates is similar to that of kraft lignin, and the amount of sulfur in lignosulfonates is about 3-8%, which is higher than in kraft lignin (Aro and Fatehi, 2017). Due to their solubility in water, lignosulfonates can be purified from other substances in the sulfite pulping liquor by ultrafiltration using membranes with different molecular weight cut-offs (Gellerstedt and Henriksson, 2008). The main application of lignosulfonates is as a dispersant for cement particles in concrete (Ansari and Pawlik, 2007). Recently, some novel methods were reported to produce lignin-based absorbents by using lignosulfonates (Li et al., 2016). Lignosulfonates can also be oxidized to produce vanillin. Borregaard is currently the only company in the world producing vanillin from lignosulfonates (Bjørsvik and Minisci, 1999).

### 2.2.3 Soda pulping

Soda pulping is another industrial pulping process, in which a NaOH solution is used as the digesting solvent at a temperature of 160 °C or lower. Similar to the Kraft process, the main reactions during the soda process are also the cleavage of lignin-carbohydrate linkages, the depolymerization of lignin, and condensation (Shuai and Saha, 2017). Cleavage of the non-phenolic  $\beta$ -aryl ether unit is the main depolymerization reaction, as shown in Figure 2.5. Compared to the kraft process, in which the benzylthiol and episulfide intermediate structures can block the condensation of the quinone methides, the condensation reaction during soda pulping can be more severe. Soda lignin is a sulfur-free lignin, which is also insoluble in water, and has an average molecular weight in the range 6900 to 8500 Da. The industrially available soda lignin is from non-wood biomass. Soda lignin is now mainly used to partially replace phenol in the production of phenol formaldehyde resins, in the manufacture of dispersants, and in the production of animal feed (Lora, 2008).

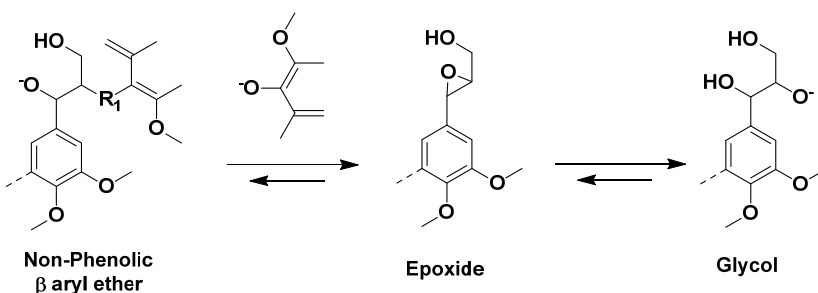


Figure 2.5. The main reaction in the soda process for the production of soda lignin (adapt from Lora, 2008).

### 2.2.4 Other industrial processes for lignin production

In addition to the kraft, sulfite, and soda processes, other pulping processes can be used to isolate different kinds of lignin. One example is the organosolv pulping process, in which an organic solvent (e.g. ethanol) in water is used for wood treatment, with typical cooking temperatures of 180-195 °C for 30 to 90 min. Such a process can remove more than 70% of the lignin from the biomass, with a minimal loss of cellulose (Rinaldi et al., 2016). Steam explosion is a chemical-free process in which the biomass is pretreated with high-temperature steam followed by a rapid pressure release. This degrades wood to different degrees and produces steam-explosion lignin (Ibrahim et al., 2010). So-called refinery lignin is produced in the modern biorefinery process for bio-ethanol production, and is recovered after pretreatment and the enzymatic hydrolysis of the biomass (Ragauskas et al., 2014).

## 2.3 The valorization of technical lignin

### 2.3.1 Potential utilization of lignin

At present, the high-value utilization of technical lignin is very limited. Lignosulfonates can be used as pesticides, stabilizers in colloidal suspension, plasticizers in concrete admixtures, and in vanillin production (Aro and Fatehi, 2017). Kraft lignin is still mainly used as a fuel to provide process heat and for chemical recovery, despite the more economically profitable alternative of producing high-value products. Holladay et al. reviewed the opportunities for converting lignin into different types of value-added products (Holladay et al., 2007). In the short-term, lignin could be gasified to produce syngas for the synthesis of ethanol, methanol, or mixed alcohols. In the medium term, the macro-molecular properties of lignin could be exploited to produce polymeric materials such as carbon fibers, polymer modifiers, and adhesives. In the longer term, lignin could be broken down to produce high-value aromatic compounds such as benzene, toluene, and xylene (often referred to as BTX chemicals), phenols, vanillin, vanillic acid, and aromatic polyols (Holladay et al., 2007).

### 2.3.2 Technical lignin depolymerization

In order to convert lignin into different value-added aromatic compounds, it must first be depolymerized. Various depolymerization processes are discussed below.

Regardless of the way in which lignin is extracted from wood, its structure will have been damaged to varying degrees. In other words, some depolymerization will already have occurred during the process of obtaining lignin. The main aim of the process is often to extract the fibers, and little attention is paid to the effects on lignin, especially in the case of technical lignin. For example, during the kraft process, almost all of the  $\beta$ -ether bonds in lignin are destroyed (Rinaldi et al., 2016). This leads to the formation of highly stable C-C bonds, which make depolymerization more difficult. The structural changes in lignin during different delignification processes have been reviewed by Rinaldi et al. (Rinaldi et al., 2016).

Lignin can be depolymerized using chemical/thermochemical methods (such as, pyrolysis, hydrogenolysis, or hydrolysis) or biological methods, using different microorganisms. Gasification is a severe thermochemical process in which coal, oil, or biomass reacts with air, oxygen, or steam to produce syngas for the production of fuels and chemicals, or producer gas for the production of heat and electricity. During recent decades, the gasification of kraft black liquor has been intensively studied for the purpose of developing more efficient power production systems on the one hand, and for producing synthetic liquid biofuels on the other. A development plant for black liquor gasification was built in Sweden in 2005,



in which the operating conditions of the gasifier are <30 bar and 1000 °C. The synthesis gas generated consists of H<sub>2</sub>, CO, CO<sub>2</sub>, CH<sub>4</sub>, and H<sub>2</sub>S (Wiinikka et al., 2010). When the heat required to drive gasification is generated by exothermic combustion, it is called direct gasification, while when the heat is needed, it is called indirect gasification. The indirect gasification process without oxygen or steam in an inert gas atmosphere is called pyrolysis (Huber et al., 2006). The main products of the pyrolysis of lignin are bio-oil (mainly alkoxy-phenols and oxygenated aromatics), gases (such as H<sub>2</sub>, CO, and CO<sub>2</sub>), and char (Lazaridis et al., 2018). The products of pyrolysis vary considerably, depending on the type of lignin.

Another approach to lignin depolymerization is catalytic hydro-pyrolysis, for example, hydrocracking, hydrodeoxygenation, and hydrogenolysis, which are carried out at much lower temperatures (up to 500-600 °C) than pyrolysis (up to 1000-1300 °C), using hydrogen or a hydrogen donor in the presence of a heterogeneous catalyst. The main products of lignin hydrogenolysis are a bio-oil that is similar to pyrolysis oil, but has a lower oxygen content, which means it is more chemically stable. The pyrolysis and catalytic hydro-pyrolysis of different types of lignin have been reviewed by Azadi et al. (Azadi et al., 2013).

Depolymerization with water, also called hydrolysis, can be categorized according to the type of chemical or catalyst applied: base-catalyzed, acid-catalyzed, ionic liquid-assisted-catalyzed, supercritical fluid-assisted-catalyzed, and metallic-catalyzed depolymerization (Chio et al., 2019; Wang et al., 2013).

Base-catalyzed depolymerization (BCD) is performed at temperatures up to 350 °C at high pressure, usually with commercially available bases such as NaOH, KOH or LiOH as the catalytic reagent. Evan et al. reported that more depolymerized lignin products could be obtained using strong bases (NaOH and KOH) than with weak bases (Ca(OH)<sub>2</sub> and LiOH) (Miller et al., 1999). Following BCD, lignin is usually degraded into a series of aromatic monomers (such as guaiacol, syringol, and catechol), dimers, and oligomers (Rößiger et al., 2018).

Acid-catalyzed depolymerization (ACD) of lignin using hydrochloric acid, dilute sulfuric acid, or formic acid as the catalytic reagent, has also been studied for many years. The main products of ACD of lignin obtained at temperatures of 360 to 400 °C have been reported to be methoxy-phenol, catechol, and phenol (Forchheim et al., 2012). Several studies that using very strong triflic acid as the acid catalyst with ethylene glycol as the protecting agent to selectively depolymerize lignin into aromatic acetals have also been reported (Deuss et al., 2017, 2015). The disadvantages of ACD are the relatively severe reaction conditions (i.e., the high temperature and long reaction time), the use of corrosive chemicals, and repolymerization (Chio et al., 2019).

An ionic liquid is usually used as a solvent to assist other catalysts when depolymerizing lignin (Constant et al., 2016). An ionic liquid (1-ethyl-3-

methylimidazolium trifluoromethanesulfonate) has been used together with  $\text{Mn}(\text{NO}_3)_2$  for the depolymerization of organosolv beech lignin, giving a yield of 2,6-dimethoxy-1,4-benzoquinone of 11.5% after 24 hours' depolymerization at 100 °C and 8.4 MPa (Stärk et al., 2010). However, the high cost of ionic liquids limits their widespread use in lignin depolymerization. In recent years, supercritical fluids have also been used to assist the solvent for the depolymerization of lignin, but high cost also limits their use (Wang et al., 2013). Apart from the depolymerization methods mentioned above, lignin can also be oxidized using hydrogen peroxide, resulting in products such as formic acid, acetic acid and succinic acid (Hasegawa et al., 2011).

The use of NaOH as a catalyst for lignin depolymerization is favorable due to its low cost, its widespread use in Kraft pulp mills, and the water solubility of Kraft lignin under alkaline conditions. Promising results have been reported from base-catalyzed depolymerization of Kraft lignin in recent years. BCD of Indulin AT lignin with a CFR at 270-315 °C has been reported, and under optimal conditions, up to 8.4 wt.% of the monomers was identified from the depolymerization products (Beauchet et al., 2012). Mahmood et al. have reported the depolymerization of softwood Kraft lignin using NaOH as the catalyst at 250 °C for 45-90 min, and 80-90% of the lignin-derived compounds were in the size range of 3000-5000 Da (Mahmood et al., 2013). Rößiger et al. reported that up to 14.5 wt.% lignin oil was obtained from the BCD of Kraft lignin in a pilot-scale flow reactor system at 340 °C and a pressure of 25 MPa (Rößiger et al., 2017). Repolymerization is also a common problem during the BCD process, which would result the formation of stable C-C bonds (Li et al., 2007; Shimada et al., 1997). The longer the depolymerization process, the more severe repolymerization, resulting in the production of coke.

Bioprocessing using microorganisms or enzymes to depolymerize lignin or transform the lignin-derived products has been increasingly explored. Compared to chemical process, it typically requires milder conditions, such as lower temperature, atmospheric pressure and neutral pH, which leads to a reduction in the use of energy as well as the use of environmentally hazardous and non-renewable chemicals (Eltis and Singh, 2018). Some microorganisms have evolved metabolic pathways that enable the utilization of a wide range of different lignin-derived aromatic molecules, and the funneling of these compounds into a few central intermediates, such as protocatechuate and catechol, which are then further metabolized through the cleavage of carbon-carbon bonds in the aromatic rings by dioxygenase enzymes to produce ring-opened species (Beckham et al., 2016; Fuchs et al., 2011; Linger et al., 2014). This provides the opportunity of solving the problem of the heterogeneity of chemically depolymerized lignin products by using microbial bioconversion to produce a homogenous product from a heterogenous mix of aromatic substrates.



# 3. Kraft lignin depolymerization

It is important to use an appropriate depolymerization process to ensure the efficient valorization of kraft lignin. This chapter describes the depolymerization of two different commercial kraft lignin samples using the internal reagent NaOH as the only catalyst under mild conditions in a CFR. The two different kraft lignin samples used in this work, Indulin AT and black liquor retentate (BLR), are described in Section 3.1, while Section 3.2 presents the CFR developed for the depolymerization experiments. The results obtained from the depolymerization of both kinds of lignin are presented in Sections 3.3 and 3.4. In addition, the results are used to discuss potential reaction pathways that may have occurred.

## 3.1 Chemical composition of the two kraft lignins

### 3.1.1 Indulin AT

The Indulin AT used in this work was a softwood kraft lignin precipitated from the black liquor of linerboard-grade pulp, produced by the MeadWestvaco Corporation, Charleston, SC (USA). Table 3.1 gives the chemical composition of the Indulin AT used in the studies described in Papers I, III, and IV. Hu et al. have previously reported the total lignin content, the total sugar content, and the ash content of Indulin AT, to be 92.9%, 1.53%, and 2.01%, respectively (Hu et al., 2017). The moisture content of Indulin AT is about 4.5%.

The molecular weight distribution of Indulin AT obtained with the tetrahydrofuran-based advanced polymer chromatography system has been reported previously; the weight average molecular weight (Mw) and number average molecular weight (Mn) values being 3118 Da and 400 Da, respectively (Sulaeva et al., 2017). In the present work, the molecular weight distribution of Indulin AT was obtained from an alkali-based size exclusion chromatography (SEC) system. The Mw and of the tested Indulin AT was 5036 Da, and the Mn value was 1526 Da. The SEC results will be discussed in more detail in Section 3.3.

**Table 3.1.** Chemical composition and elementary analysis of Indulin AT (adapt from Hu et al. 2017 and Paper I).

Indulin AT	
Total lignin <sup>a</sup>	92.9
Klason lignin	88.8
Acid-soluble lignin	4.10
Total sugars <sup>a</sup>	1.53
Ash <sup>a</sup>	2.01
Elementary analysis <sup>b</sup>	
C	63.9
H	5.80
N	0.70
S	1.50
O	24.6

Two-dimensional heteronuclear single-quantum coherence (2D HSQC) NMR spectroscopy is widely used to elucidate the structure of lignin. The 2D HSQC NMR spectra of Indulin AT in the linkage region and the aromatic region are shown in Figure 3.1, revealing important structural information on Indulin AT. As was mentioned in the previous chapter, the  $\beta$ -ether bond is the main lignin linkage. Most of the  $\beta$ -ether bond was cleaved during the kraft process (Rinaldi et al., 2016). Some  $\beta$ -O-4 bonds can be observed in the structure of Indulin AT (Figure 3). The  $\beta$ - $\beta$  and  $\beta$ -5 C-C structure can also be seen in the inter-unit aliphatic region ( $\delta_C/\delta_H$  52.5-90.0/2.8-5.7 ppm). The spectrum for the aromatic region ( $\delta_C/\delta_H$  105-135/6.0-8.0 ppm) shows that the main CH cross peaks of Indulin AT correspond to the G-type ring.

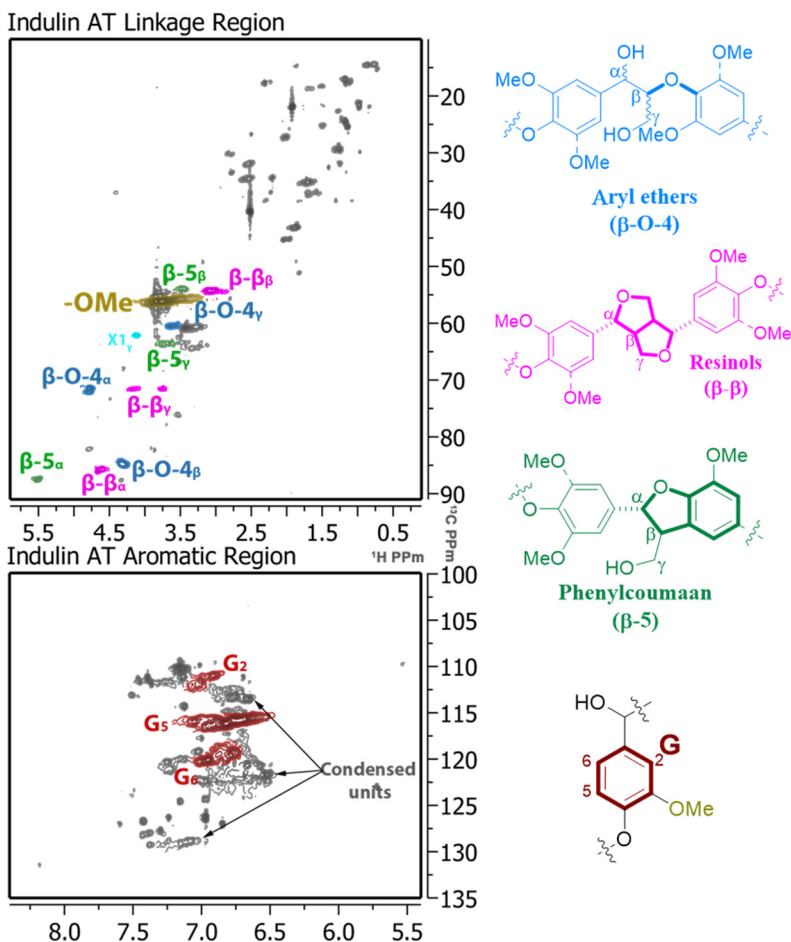


Figure 3.1. HSQC NMR spectra of Indulin AT for the linkage region and the aromatic region (from Paper IV).

### 3.1.2 Black liquor retentate

The BLR used in this work was obtained from a membrane filtration pilot plant in a pulp and paper mill in northern Sweden. The starting material for pulping was a mixture of 70% softwood and 30% hardwood. The black liquor had been filtered continuously at 110 °C using a 1 kDa cut-off ceramic membrane. BLR was obtained as the retentate after filtration. It contained 32.9% total solids, 22.4% total lignin, and 6.5% ash. The BLR had a much higher molecular weight than the Indulin AT. It is due to the membrane filtration removed components with lower molecular weight and kept polysaccharides such as xylan, glucan, mannan, galactan, and arabinan with higher molecular weight in the retentate (Paper II). The 2D HSQC NMR spectra for BLR are shown in Figure 3.2. As in

the case of Indulin AT, weak  $\beta$ -O-4 signals and clear  $\beta$ - $\beta$  and  $\beta$ -5 signals can be seen in the inter-unit aliphatic region. A clear polysaccharide aromatic region can also be seen at  $\delta_C/\delta_H$  90-105/5.5-4.0 ppm, indicating the presence of some sugars in the BLR. Since the BLR sample was produced from a mixture of hardwood and softwood, both G- and S-units from the aromatic region were seen ( $\delta_C/\delta_H$  100-135/8.0-6.0 ppm). Clear signals were seen from the C<sub>2</sub>-H<sub>2</sub>, C<sub>5</sub>-H<sub>5</sub>, and C<sub>6</sub>-H<sub>6</sub> bonds related to G-units at  $\delta_C/\delta_H$  112.5/6.64, 115.7/6.68, and 119.1/6.76 ppm, respectively. Signals from the S-units and the  $\alpha$ -oxidized S-units related to C<sub>2,6</sub>-H<sub>2,6</sub> can be seen at  $\delta_C/\delta_H$  = 104.0/6.62 ppm and 106.2/7.3 ppm, respectively.

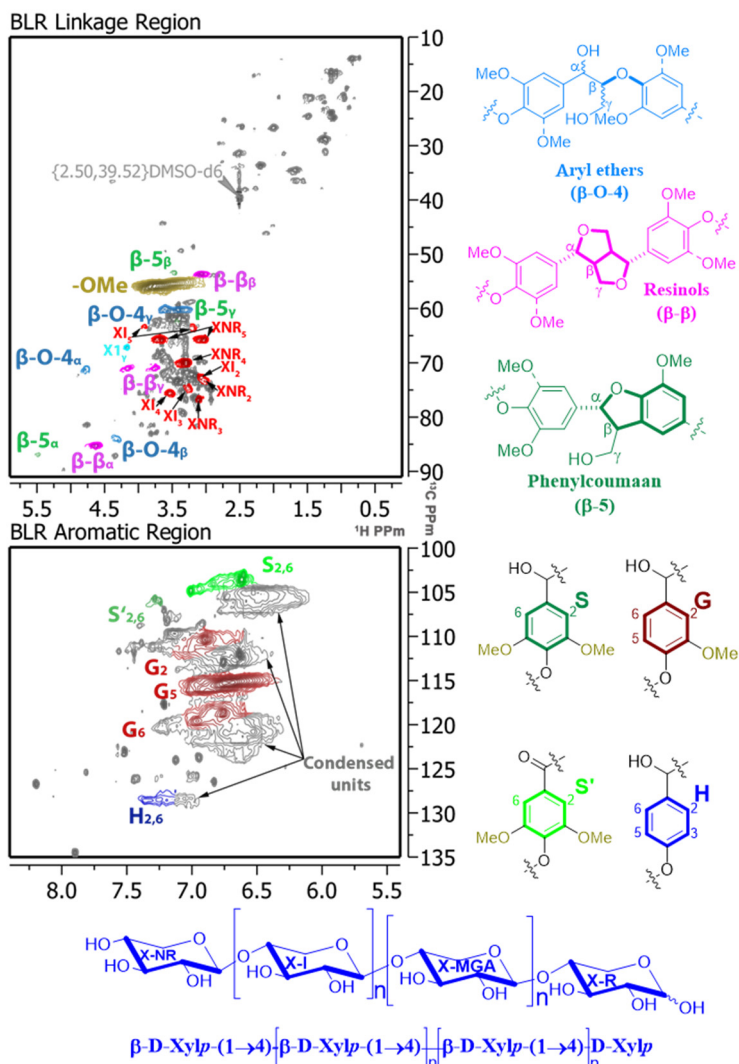
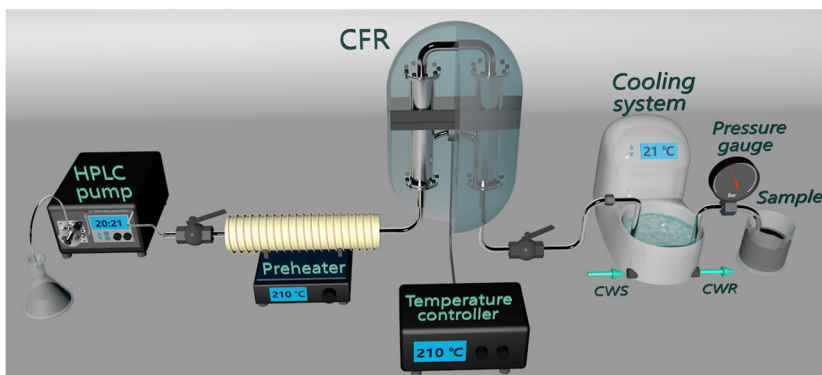


Figure 3.2. HSQC spectra of BLR for the linkage region and the aromatic region (from Paper II).

## 3.2 Depolymerization reactor

Batch reactors are widely used for various chemical processes including lignin depolymerization. They have the advantage of allowing the reaction to be run with a broad range of temperatures and pressures, and the experimental conditions can be easily controlled. However, cooling can take a very long time, which is a disadvantage as it allows repolymerization. This will lead to a decrease in the yields of the depolymerized products.

A CFR was therefore designed for the depolymerization of lignin samples to ensure a short residence time, thus avoiding repolymerization. A schematic of the CFR setup is presented in Figure 3.3. The system consists of a Gilson 307 HPLC piston pump, a tubular reactor, and a pressure control valve (for further details, see Paper I).



**Figure 3.3.** Schematic of the continuous flow reactor experimental setup. CWS: cooling water supply; CWR: cooling water return (from paper I).

In the original system, a flow rate of 10 mL/min corresponded to a residence time of 1 min. After upgrading the system by connecting two tubular reactors in series, the residence time was doubled with the same flow rate. It means that the production amount is doubled by doubling the flow rate. In addition, a filter was installed in the original system after the cooler in the first stage to remove any solid products. However, if coke is formed during the reaction, it will block the filter and cause a rapid increase in pressure in the system. The filter was therefore removed in the upgraded system.

The maximum pressure in the system is 150 bar, above which safety valves open. The pressure used in the current depolymerization experiments was 100-130 bar. The feed was prepared by mixing the lignin sample with NaOH solution in various proportions. When not in use, the system was filled with deionized water.



Before each experiment, the equipment was heated and pressurized to the required conditions with a continuous flow of deionized water at the set flow rate. Once stable conditions had been achieved, a 1% NaOH solution was pumped through the system for several minutes in case the precipitation of the lignin in the system. After flushing the system with NaOH, the feed was pumped into the system. Details of the experimental parameters and conditions can be found in Papers I and IV. The collection of products started after 10 min of stable pumping under the set conditions to ensure that all the water and NaOH had been removed from the system.

### 3.3 Base-catalyzed depolymerization of Indulin AT

#### 3.3.1 The effects of the reaction conditions

Papers I and IV describe the studies performed on the continuous depolymerization of Indulin AT under base conditions. The first studies were carried out to determine the suitable conditions for the depolymerization of Indulin AT samples using the new CFR reactor. The effects of temperature, time, lignin loading, and NaOH concentration on depolymerization were studied. Hydrogen peroxide was also added to the feed to investigate the possibility of oxidizing Indulin AT under base conditions. In addition, the stability of the depolymerized lignin products was investigated.

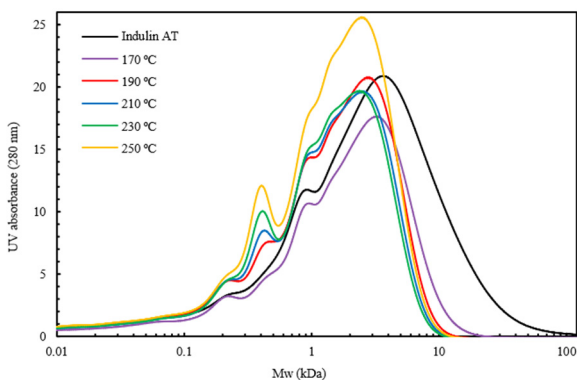
Molecular weight is one of the key indicators in understanding the structural properties of lignin. SEC can be used to determine a wide range of molecular weights from 200 to  $3 \times 10^6$  Da without the need for pre-fractionation (Baumberger et al., 2007). Although the accuracy of SEC is reduced due to the lack of ideal standards representing the composition of lignin, it can still be used to compare the molecular weight distribution of lignin samples after different treatments. There are two types of SEC system, one based on tetrahydrofuran as solvent, and the other on alkali. The alkali-based SEC system was used in this work as the original lignin samples and the products obtained after depolymerization were dissolved in NaOH.

Ultra-high-performance supercritical fluid chromatography coupled to quadrupole-time-of-flight mass spectrometry (UHPSFC/QTOF-MS) was used for the qualitative analysis of the depolymerized aromatic products (Prothmann et al., 2017). An ultra-high-performance liquid chromatography (UPLC) system was used to analyze the main monomer compounds identified in the depolymerized Indulin AT samples.  $^1\text{H}$ - $^{13}\text{C}$  2D HSQC NMR spectroscopy was used to analyze the structure of Indulin AT and the products after depolymerization under different conditions.

### *The effects of depolymerization temperature and reaction time on the molecular weight distribution of Indulin AT*

The depolymerization of Indulin AT using the CFR was initially studied at various temperatures from 170 °C to 250 °C with 5 wt.% lignin, 5 wt.% NaOH, and 90% of water in the feed. As mentioned in Section 3.1, Indulin AT has a wide molecular weight distribution of 0.1 to 100 kDa. With the very short reaction time of 1 min, the molecular weight distribution of Indulin AT gradually shifted towards lower molecular weights when increasing the depolymerization temperature. Peaks at around 200, 400, and 900 Da that represent lignin monomers and oligomers gradually appeared as the temperature approached 220 °C. The highest temperature, 250 °C, yielded the lowest molecular weight distribution, and the highest peak was seen at 200-400 Da.

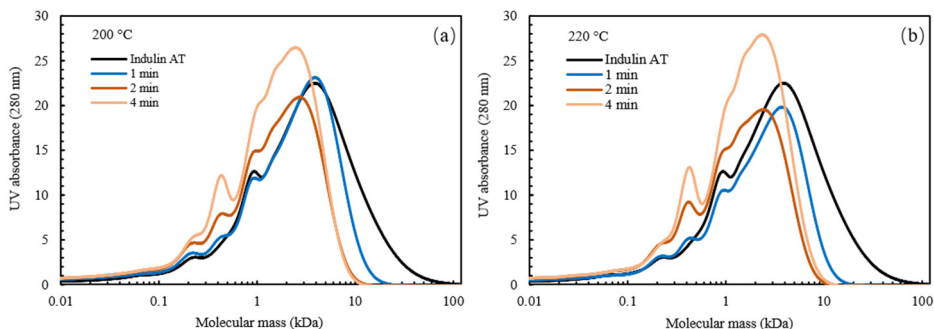
The molecular weight of Indulin AT lignin was further reduced when the residence time was increased to 2 min. As can be seen in Figure 3.4, there was a noticeable decrease in the molecular weight distribution of Indulin AT at all the depolymerization temperatures studied, especially at temperatures above 190 °C. Peaks representing LMW compounds can be clearly seen around 200-900 Da (Paper I).



**Figure 3.4.** Effect of temperature effect on the molecular mass distribution of Indulin AT after depolymerization with residence time of 2 min (from Paper I).

The effect of residence time was also investigated, and the results of depolymerization with residence times of 1, 2, and 4 min at temperatures of 200 and 220 °C are shown in Figure 3.5. The most obvious result is that, at the same depolymerization temperature, longer residence times yielded more LMW compounds. Lignin components with molecular weights over 10 kDa were more depleted after longer residence times. For example, molecules larger than 10 kDa almost completely disappeared when the residence time was increased from 1 to 2 min at a temperature of 200 °C (Figure 3.5a). The efficiency of depolymer-

ization after 4 min was similar to that after 2 min. A remarkable increase was seen in the proportion of components with molecular masses between 1 and 5 kDa after a residence time of 4 min. In addition, below 240 °C, more distinct peaks were seen in the range 0.2-1 kDa after 4 min. However, at this residence time, the mass distribution curves moved towards slightly higher molecular weight, indicating possible repolymerization.



**Figure 3.5.** The effect of residence time on the molecular weight distribution after depolymerization of Indulin AT at 200 and 220 °C (adapted from Figure 3-5 in Paper I).

### *Repolymerization and coke formation*

As mentioned in Chapter 2, lignin depolymerization is always accompanied by repolymerization. Depolymerization breaks down the complex lignin structure, decreasing the molecular weight, but the cleaved radical moieties can recombine to form more stable C-C bonds. The highly polymeric lignin species called coke is ultimately generated (Rößiger et al., 2018). The use of the CFR system designed in this work considerably reduced the residence time, especially the cooling time after the reaction, thus significantly reducing the degree of repolymerization. Some coke was still formed, especially at higher depolymerization temperatures and longer depolymerization times. For example, accompany with the decreasing of the molecular weight, the coke was formed with a residence time of 2 min at 250 °C. Figure 3.6 shows the coke formed in the CFR system after depolymerization. The problem of coke formation in a batch reactor is technically solvable, while it can be a serious problem in a CFR, as it would plug the system, leading to termination of the reaction.

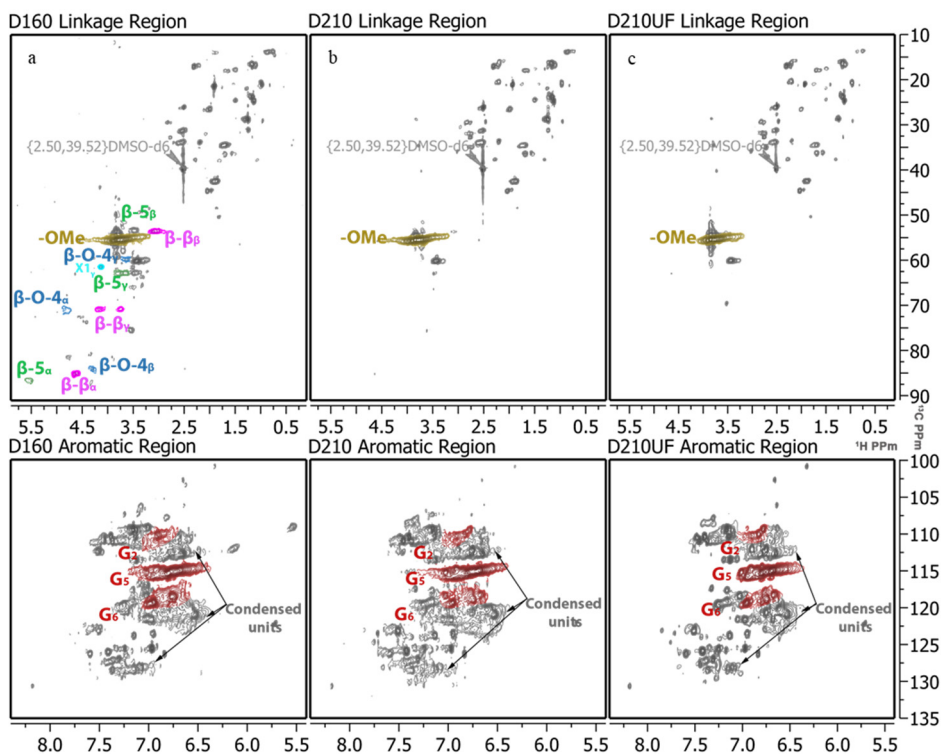


**Figure 3.6.** The coke formed in the CFR system after Indulin AT depolymerization at 250 °C.

### 3.3.2 Characterization of the depolymerization products

#### *Changes in the lignin structure*

The chemical structure of the heavy fraction from the depolymerization of Indulin AT at 160 and 220 °C, after acid precipitation, centrifugation, and washing, was analyzed using 2D HSQC NMR spectroscopy, the results are shown in Figure 3.7a and b. No clear structural changes were observed in the aromatic regions at either temperature. In the lignin side-chain region ( $\delta_C/\delta_H$  50-90/2.5-5.5 ppm), the  $\beta$ -O-4,  $\beta$ - $\beta$ , and  $\beta$ -5 linkages that were seen in the raw Indulin AT sample were also observed in the heavy fractions of the Indulin AT depolymerized at 160 °C. However, these linkages disappeared when the temperature was increased to 190 °C, indicating the cleavage of the C-O and C-H bonds under these conditions. These results illustrate the effect of temperature on Indulin AT depolymerization. A temperature of 160 °C was thus deemed too low for Indulin AT degradation, given these residence times. These results are in accordance with the results on the molecular weight distribution discussed in Section 3.3.1, and the monomer production after depolymerization at different temperatures, which will be discussed below.



**Figure 3.7.** The 2D HSQC NMR spectra of the heavy fractions from Indulin AT after depolymerization at 160 and 210 °C (a and b), and the permeate after membrane filtration of the 210 °C depolymerized Indulin AT (c).

### Identification of lignin monomers

Due to the complexity of the depolymerized lignin products, it is essential to use efficient methods to identify and quantify them. Gas chromatography with flame ionization detection is usually used for the identification of lignin depolymerization products as it has the advantages of higher resolution, easier coupling with MS and lower cost, compared with LC-based techniques. However, a derivatization step is required before analysis, which will lengthen the analysis time. Furthermore, insufficient or discriminative derivatization may lead to unreliable results (Prothmann et al., 2017). Therefore, the UHPSFC/QTOF-MS method was used to analyze the depolymerized compounds in this work.

The products of Indulin AT depolymerization were first acidified by the addition of HCl to pH 1, and after centrifugation, the supernatant was collected and extracted with ethyl acetate. After evaporating the ethyl acetate, the monomer-rich lignin oil obtained was dissolved in methanol for analysis. Some of the results of this analysis are given in Table 3.2. Guaiacol, vanillin, acetovanillone, p-hydroxyacetophenone, p-hydroxybenzaldehyde, p-hydroxybenzoic acid, and vanillic acid were identified in the Indulin AT depolymerized for 2 min at 240 °C (Paper I).

**Table 3.2.** Most abundant chemical compounds identified by UHPSFC/QTOF-MS in monomeric-rich fractions from Indulin AT depolymerized for 2 min at 240 °C (from Paper I).

Compound	Retention time (min)	Calculated m/z ([M-H] <sup>-</sup> )	Measured m/z ([M-H] <sup>-</sup> )	Base peak intensity	MS <sup>2</sup> fragmentation	
					MS <sup>2</sup> transition	Lost fragment
Guaiacol	0.51	123.0446	123.0446	4.3E5	123 → 108	CH <sub>3</sub>
Vanillin	1.27	151.0395	151.0397	5.1E5	151 → 136	CH <sub>3</sub>
					151 → 108	CH <sub>3</sub> + CO
					165 → 150	CH <sub>3</sub>
Acetovanillone	1.27	165.0552	165.0556	1.1E6	165 → 122	CH <sub>3</sub> + CO
					165 → 108	CH <sub>3</sub> + COCH <sub>2</sub>
<i>p</i> -Hydroxybenzaldehyde	2.51	121.0290	121.0293	3.3E5	121 → 92	CHO
<i>p</i> -Hydroxyacetophenone	2.55	135.0446	135.0445	4.8E5	135 → 120	CH <sub>3</sub>
					135 → 108	C <sub>2</sub> H <sub>3</sub>
					167 → 152	CH <sub>3</sub>
Vanillic acid	3.29	167.0345	167.0356	2.2E6	167 → 123	CO <sub>2</sub>
					167 → 108	CH <sub>3</sub> + CO <sub>2</sub>
<i>p</i> -Hydroxybenzoic acid	4.05	137.0239	137.0251	2.5E5	137 → 93	CO <sub>2</sub>

### *Quantification of the main lignin monomers – guaiacol and vanillin*

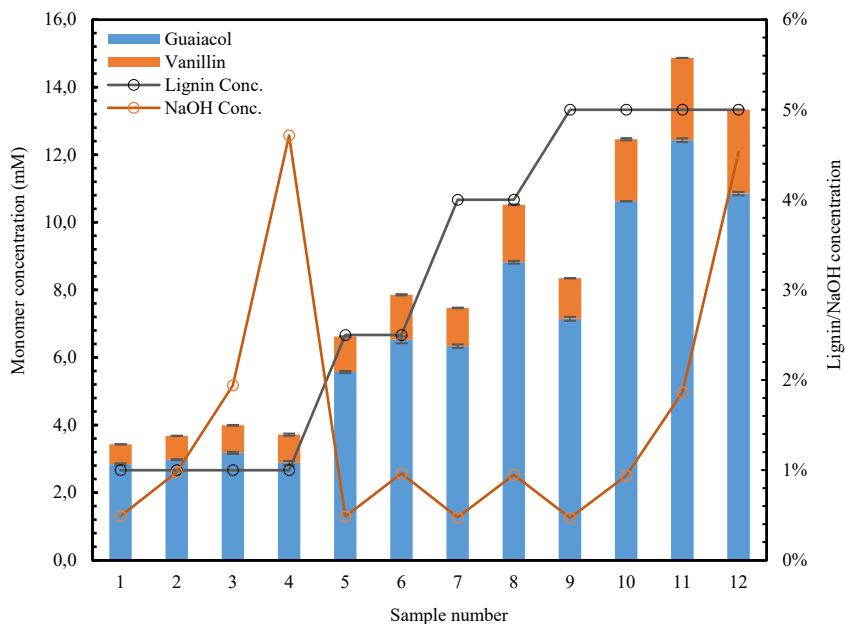
Among the monomers, guaiacol and vanillin were the main products generated. In this work, the guaiacol and vanillin contents in the Indulin AT lignin samples were determined using a UPLC system with the detection done by an RI-detector, and used as a measure of the depolymerization efficiency. The samples were neutralized by the addition of 5 M HCl, and then centrifuged. The supernatant was collected for analysis. In order to investigate all the monomers produced by depolymerization, the precipitate obtained after centrifugation was washed with distilled water twice. After washing and further centrifugation, the second and third supernatants were also collected for guaiacol and vanillin analysis.

The difference between depolymerization of Indulin AT in a batch reactor and a CFR was compared in this work. The batch reactor used in this work was an OTR, which is described in detail in Papers IV and V. In short, the volume of each tubular reactor is 23 mL, and a maximum of four can be placed on an oscillatory shelf inside an oven. The maximum temperature obtainable in the oven is 180 °C, thus limiting the depolymerization temperature. Depolymerization of the same lignin feed was compared in the OTR for 30 min at 180 °C and in the CFR for 2 min at 200 °C. The results showed that the latter reactor produced almost 5 times more guaiacol and 2 times more vanillin, than the former, showing that the CFR is more efficient for depolymerizing lignin than the OTR (Paper IV).

The effect of  $\text{H}_2\text{O}_2$  on the depolymerization of Indulin AT at 120 °C was also investigated (Paper IV). Adding  $\text{H}_2\text{O}_2$  to the alkaline lignin solution led to the consumption of the guaiacol and vanillin, and almost no vanillin was detected at a  $\text{H}_2\text{O}_2$  concentration of 6% (Figure 2 in Paper IV). Under the experimental conditions, the guaiacol and vanillin concentrations after the depolymerization with the addition of  $\text{H}_2\text{O}_2$  in both OTR and CFR were lower than those from the Indulin AT alone without the addition of  $\text{H}_2\text{O}_2$ . Therefore, the addition of  $\text{H}_2\text{O}_2$  does not promote depolymerization (Paper IV).

When performing the initial experiments on the effects of temperature and residence time, a 5 wt.% NaOH solution was chosen as the solvent for the depolymerization of Indulin AT in order to ensure sufficient alkalinity during depolymerization. The lower the alkali requirement, the better in an industrial process. Therefore, the effects of the lignin substrate loading and NaOH concentration on depolymerization were investigated.

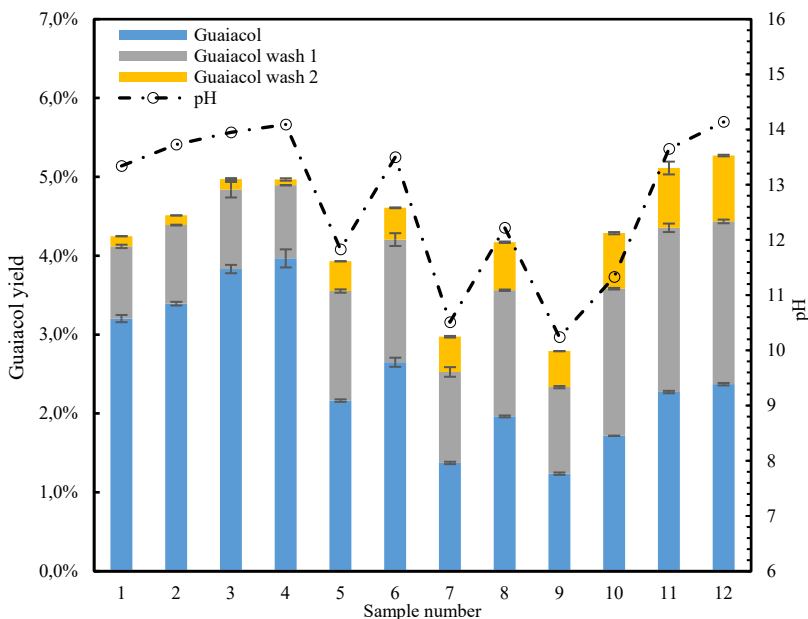
Figure 3.8 shows the concentrations of guaiacol and vanillin after depolymerization under different conditions. As can be seen from the figure, at the same lignin dose, the guaiacol content increased with increasing amount of NaOH, and this effect is especially clear when the lignin content was high. At 1 wt.% Indulin AT, increasing the NaOH concentration from 0.5 wt.% to 5 wt.% increased the guaiacol and vanillin concentrations slightly. The result demonstrates that 1 wt.% NaOH is sufficient to catalyze this low dose of Indulin AT, and that it is meaningless to simply increase the NaOH concentration. When the amount of Indulin AT was increased to 5 wt.%, a clear increase was seen in the monomer yield with increasing NaOH concentration from 0.5 wt.% to 2 wt.%. However, increase the NaOH concentration further from 2 wt.% to 5 wt.%, led to a decrease in the guaiacol concentration (compare samples 11 & 12 in Figure 3.8). Too high NaOH concentration would cause the polymerization of guaiacol, forming char (Zhou et al., 2020).



**Figure 3.8.** The effect of the concentration of Indulin AT substrate and NaOH (1-5 wt.% and 0.5-5 wt.%, respectively) on the production of guaiacol from depolymerized Indulin AT. The depolymerization conditions for samples no. 1-12 are reported in Table 1, Paper IV (from Paper IV).

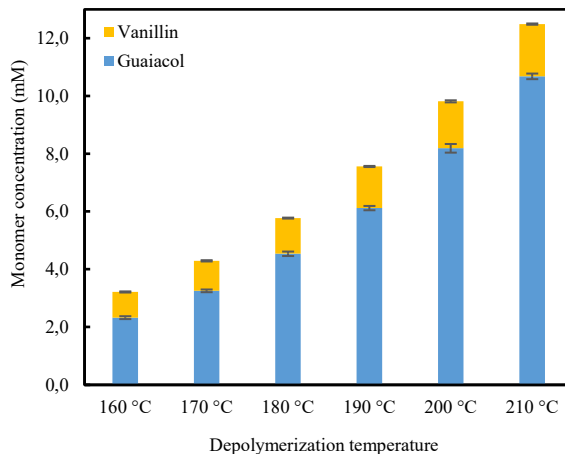
The guaiacol and vanillin concentrations shown above are the concentrations in the supernatant after centrifugation of the neutralized products. The precipitate obtained after centrifugation was washed twice with distilled water in order to calculate the total guaiacol and vanillin yields. The total yields of guaiacol and vanillin were calculated by adding the guaiacol or vanillin contents in the first supernatant, and the two washing liquids. Figure 3.9 shows the total guaiacol yield after depolymerized Indulin AT at various lignin and NaOH concentration. At the low Indulin AT loading (1 wt.%), washing the precipitate once led to the collection of about 30% of the guaiacol found in the supernatant after the first centrifugation (samples 1-4); at higher lignin loading (samples 5-12), more monomers were produced, and thus more guaiacol was adsorbed on the surface of the precipitate. Therefore, to recover more guaiacol and vanillin from the depolymerized Indulin AT samples, it is necessary to wash the precipitate at least once after neutralization.





**Figure 3.9.** The total yields of guaiacol after BCD of Indulin AT at various lignin and NaOH concentrations. The depolymerization conditions for samples no. 1-12 are reported in Table 1.

The highest total yield of guaiacol was 5.3% from sample no. 12 with 5 wt.% of Indulin AT and 5 wt.% of NaOH. The guaiacol yield from sample no. 11 (5.1%) was very close to the highest value, although with half NaOH loading. with 5 wt.% of Indulin AT and 2 wt.% of NaOH. Therefore, 2 wt.% of the NaOH concentration was suggested to catalyze the depolymerization of Indulin AT with loading of lower than 5 wt.%. The general trend for the total vanillin yield is similar to that for guaiacol (see Figure 4b from Paper IV). The pH of the Indulin AT samples after depolymerization is positively correlated with the production of both guaiacol and vanillin. A pH value of at least 12.5 is important for efficient depolymerization of Indulin AT. The lignin and NaOH concentration studies show that a suitable lignin-to-NaOH ratio be chosen depending on the application. To obtain a neutralized solution with high guaiacol or vanillin concentrations for further conversion, a relatively high lignin loading (4-5 wt.%) and a high NaOH concentration (2-4 wt.%) should be chosen. To obtain high total yields of monomers and achieve a higher degree of depolymerization, a low lignin loading (e.g. 1 wt.%) with 1-2 wt.% NaOH should be chosen (Paper IV).



**Figure 3.10.** The guaiacol and vanillin concentration from the depolymerization of Indulin AT at 160 to 210 °C with 4 wt.% of Indulin AT and 2 wt.% of NaOH, for 2 min at 120 bar (from Paper IV)

Based on the results of these studies, as well as taking the operational capability of the pump in the CFR system into consideration, 4 wt.% Indulin AT and 2% NaOH were deemed to be the optimal combination for Indulin AT depolymerization. Having determined the optimal feed proportions, depolymerization was performed at temperatures of 160-210 °C. As expected, the yields of guaiacol and vanillin increased with increasing temperature (Figure 3.10). The guaiacol and vanillin concentrations after depolymerization at 210 °C are 10.7 and 1.81 mmol/L, respectively. The experimental condition of 2 min of the residence time at 210 °C with 4 wt.% Indulin AT and 2 wt.% NaOH was chosen for the further bioconversion step that will be discussed in Chapter 5. The storage condition of the depolymerized Indulin AT was also investigated in this study. The temperature of 4 °C was suggested for long-term storage while room temperature is sufficient for short-term storage (Paper I and IV).

## 3.4 Base-catalyzed depolymerization of BLR

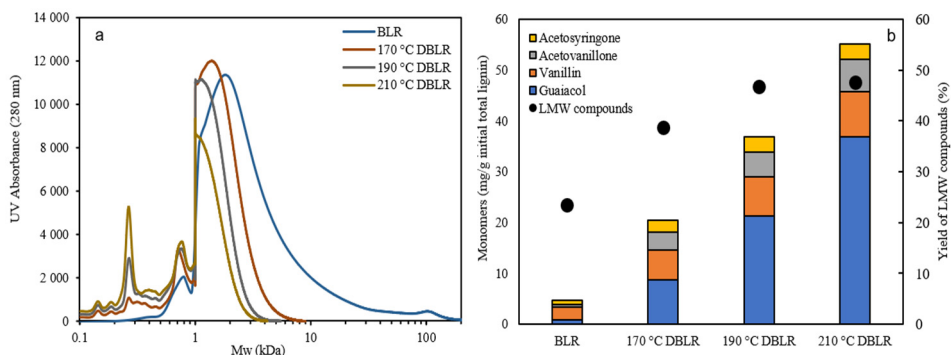
### 3.4.1 Depolymerization of BLR at different temperatures

The BLR is a lignin-rich stream obtained from membrane filtration of black liquor, as described in Section 3.1.2. BLR has been developed commercially for hydrocarbon production after a series of treatments such as hydrothermal treatment, acid washing, solvent extraction, and hydrogenation (Jafri et al., 2020). Introducing a simple depolymerization process to produce small aromatic

products for fine chemical production would increase the chain value. The possibility of base-catalyzed depolymerization of BLR was therefore investigated in this work (Paper II).

The high lignin content of BLR together with residual polysaccharides means BLR has a relatively high viscosity, which will cause problems in pumping when using the CFR. Therefore, the BLR was diluted 5 times with 2% NaOH and filtered before depolymerization. The molecular weight distribution of BLR depolymerized at 190-210 °C with a residence time of 2 min is shown in Figure 3.11a, where a clear decrease in the size of the molecules can be seen. The base-catalyzed depolymerization of BLR yielded better results than Indulin AT depolymerization, as more lignin macromolecules were depolymerized under the same conditions. For example, all the molecules with molecular weights of about 4 kDa to 10 kDa in BLR were degraded at a temperature of 190 °C, while some remained after Indulin AT depolymerization. The highest molecular weight of BLR products shifted to a value below 3 kDa when the temperature was increased to 210 °C, indicating a high degree of depolymerization. As a result, small fractions with molecular weights below 900 Da were produced.

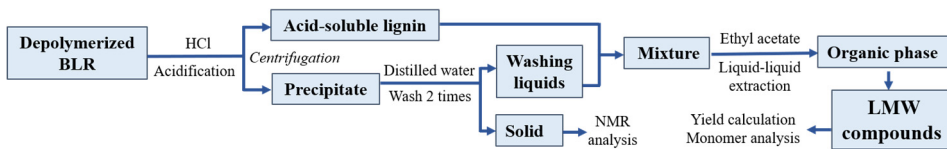
The polysaccharides in BLR contribute to the higher molecular weight, and are almost completely degraded by depolymerization at temperatures above 190 °C. This may explain the greater decrease in the molecular weight of BLR than that of Indulin AT following depolymerization (Paper II).



**Figure 3.11.** (a) Molecular weight distribution of BLR and the products of its depolymerization at various temperatures (measured as UV absorbance at 280 nm), and (b) the amounts of LMW compounds and monomers before and after depolymerization. Depolymerization was performed for 2 min at 170-210 °C and 120 bar (from Paper II).

The LMW compounds extraction process is shown in Figure 3.12. About 23.4% (based on initial lignin) of the LMW compounds could be extracted from the BLR feed, and increased to 47.7% after depolymerization at 210 °C. The results of UPLC showed that guaiacol was the main monomer produced by

depolymerization, followed by vanillin, acetovanillone, acetosyringone, and other monomers. As can be seen in Figure 3.11b, very small amounts of monomers from the BLR starting material were detected. A clear increase in all the monomers (especially guaiacol, from 0.825 mg/g in the feed to 36.8 mg/g at 210 °C) was observed after depolymerization.

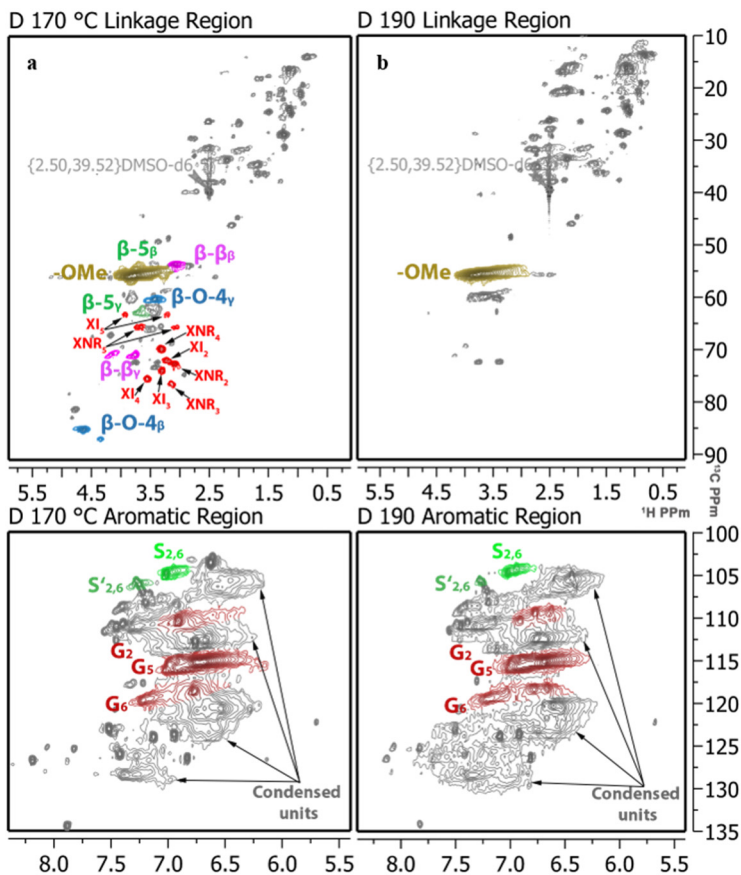


**Figure 3.12.** A schematic of the extraction of LMW compounds from depolymerized BLR.

Figure 3.13 shows 2D HSQC spectra from the linkage region and the aromatic region for the heavy fractions from BLR depolymerized at 170 and 190 °C. The most conspicuous observations are the disappearance of the  $\beta$ -O-4,  $\beta$ - $\beta$ , and  $\beta$ -5 linkages as well as the polysaccharides anomeric regions in the lignin side-chain region ( $\delta_C/\delta_H$  50-90/2.5-5.5ppm) after depolymerization at 190 °C. As reported previously, the aryl-alkyl-ether bonds are the weakest, and can easily be cleaved under base conditions. A small amount of the remaining  $\beta$ -O-4 linkages in BLR is broken down completely after depolymerization. No obvious change could be seen in the aromatic region after depolymerization at 190 °C. The structural changes in lignin are discussed in more detail in Paper II.

Base-catalyzed depolymerization is usually performed at temperatures higher than 250 °C when carried out in a batch reactor. In a study by Schmieidl et al., organosolv lignin was depolymerized for 10 min at 250-300 °C and 250 bar. They found that at 250 °C 8.8% guaiacol (based on the extracted oil) and its derivatives were produced, which increased to 22.9% at a temperature of 350 °C (Schmieidl et al., 2012). This demonstrates that increasing the depolymerization temperature has a positive effect on the guaiacol monomer production. In the present work, BLR was depolymerized for 2 min at 210 °C, the amount of guaiacol generated could be as high as 11.5% (based on the extracted LMW compounds). As mentioned in the previous section, coke is usually formed during depolymerization due to the generation of stable C-C bonds. Toledano et al. reported that the presence of a base prevented coke formation, (Toledano et al., 2012). They found that compared with the hydrolysis of organosolv lignin in water (around 35% of coke was formed), only about 5% coke was produced when depolymerizing 5 wt.% organosolv lignin with 4 wt.% NaOH for 40 min at 300 °C and 90 MPa. Depolymerization temperatures higher than 220 °C were also tested in the present work. However, the coke gradually collected inside the tubes,

blocking the system. Therefore, the maximum depolymerization temperature was restricted 210 °C and attempts were made to find other ways of increasing the monomer yields, such as multiple depolymerization of BLR.



**Figure 3.13.** 2D HSQC NMR spectra (of both the linkage region and the aromatic region) of the residual heavy fractions from BLR depolymerized at (a) 170 °C and (b) 190 °C (from Papers II and V).

### 3.4.2 Multiple BLR depolymerization

Multiple depolymerization of BLR was carried out for 2 min at 190 °C in an attempt to depolymerize lignin to a greater extent and produce more LMW compounds, while avoiding the formation of char. The experiments were performed by pumping the depolymerized BLR product directly into the system for a second and third time under the same conditions. The results showed that repeated depolymerization decreased the molecular weight of the components slightly, and gradually increased

the LMW compounds yield, while there was no significant decrease in the molecular weight of the BLR. Only a slight increase in the peak at 200-500 Da was observed after the second depolymerization, and a further slight increase after the third depolymerization (Figure 5 in Paper II). Compare with the yield of LMW of 46.8% after the first depolymerization, a slight increase after the third depolymerization was observed (51.5%). More monomers were produced after multiple depolymerization, and guaiacol was still the dominant monomer product, which increased from 21.3 mg/g initial lignin in the first depolymerization to 28.5 mg/g after the third depolymerization. This is lower than the amount of guaiacol produced by the first depolymerization at 210 °C.

The 2D HSQC NMR spectra for the heavy fractions from the third depolymerization of BLR at 190 °C was shown in Figure 4 from Paper II. No obvious structural differences could be seen compared with single depolymerization, except the more noticeable peaks at  $\delta_C/\delta_H$  29.3/3.85,  $\delta_C/\delta_H$  35.4/3.83, and  $\delta_C/\delta_H$  40.8/3.85, which could correspond to the methylene bridge isomers (*o-o'*, *o-p'*, and *p-p'*) (Huang et al., 2015). This indicates that repolymerization occurs during multiple depolymerization. In summary, although the production of LMW compounds increased slightly after repeated depolymerization, the increases after the multiple depolymerization were less. The repolymerization of small aromatics to form oligomers and polymers during multiple depolymerization could be the reason for this (Paper II).



## 4. Separation and characterization of the lignin products

Both the kraft lignin from black liquor and the depolymerized kraft lignin contain molecules with a wide range of molecular weight. In order to utilize these components, a suitable method of separation must be applied. The most common ways of separating kraft lignin from black liquor are precipitation and ultrafiltration (Wallberg, 2005).

### 4.1 Lignin separation from BLR

Acid precipitation has been used commercially for a long time in pulp and paper mills for the extraction of kraft lignin from black liquor using sulfuric acid, other acids, or carbon dioxide as the hydrogen source. Black liquor usually has a pH above 12.5, and at this alkalinity, the lignin molecules are negatively charged due to the dissociation of the phenolic groups and are thus dissolved.

Upon the addition of an acid, the phenolic groups on lignin are protonated by the hydrogen ions, which reduces the repulsive forces between the lignin molecules. This leads to the agglomeration of lignin molecules and precipitation (Öhman, 2006; Wallberg, 2005; Zhu, 2015). Sulfuric acid is used to precipitate lignin at many mills because of the ease of handling in the mixing stage, the low cost, and the ready availability. However, this disturbs the chemical balance in the mill due to the excess sulfur, which would affect the recovery of cooking chemicals (Loutfi et al., 1991). Using carbon dioxide for acidification would thus reduce the influence on the chemical balance. The pH of 8-9 obtained after carbon dioxide acidification has been found to be the most suitable pH for lignin separation (Loutfi et al., 1991).

The techniques based on precipitation for lignin separation in which progress has been made in recent years are the LignoBoost process, the LignoForce process, and the Sequential Liquid-lignin Recovery and Purification (SLRP) process (Öhman et al., 2007; Tomani, 2010; Velez and Thies, 2013). The LignoBoost process employs carbon dioxide as the first acidifying agent, followed by sulfuric acid. After precipitation, filtration is performed and the lignin is obtained in the

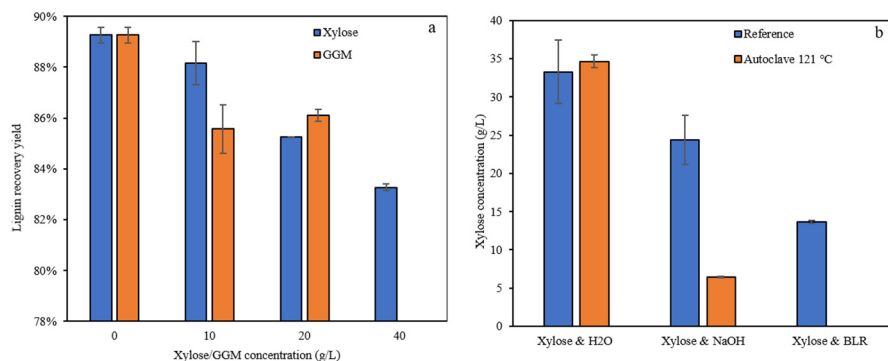


form of a filter cake. A wet lignin solid is then obtained after washing. The LignoForce process also uses carbon dioxide for precipitation. It differs from the LignoBoost process in that there is an extra oxidation stage before acidulation to oxidize the hemicelluloses, reducing the sulfuric compounds, and decreasing the carbon dioxide demand. Lake and Blackburn studied the SLRP process for 'liquid-lignin' recovery (Lake and Blackburn, 2011). Carbon dioxide is also used for acidification during SLRP at temperatures above 100 °C and pressures of around 6 bar. Under such conditions, the lignin remains in the bottom of the separation column in the liquid phase and can be separated from the black liquor based on differences in density. The SLRP process is a promising method for lignin recovery as it has less impact on the sulfur balance, and a lower cost than the LignoBoost process.

Ultrafiltration can also be used to separate lignin from kraft black liquor based on the molecular weight difference between lignin and the cooking chemicals. Ultrafiltration can be applied directly to filter black liquor to obtain concentrated lignin in the retentate, or to recover the lignin agglomerates after acidification of the black liquor. Wallberg et al. have described the ultrafiltration of black liquor using both polymeric and ceramic membranes with different cut-offs and operational parameters (Wallberg et al., 2003; Wallberg and Jönsson, 2003). They reported that the lignin recovery was 66% and 28% for 5 and 15 kDa ceramic membranes, respectively (Wallberg and Jönsson, 2003). The use of membrane filtration to preconcentrate the lignin in the black liquor could be one method to reduce the amount of acid required during the acidification.

Arkell et al. proposed a process for the extraction of lignin from black liquor using a combination of membrane filtration and precipitation. The black liquor was first filtered through the membrane, allowing the cooking chemicals to pass through. After membrane filtration, the volume reduction was 80% and the lignin retention 90%, which means that 80% of the liquid volume, containing large amount of alkaline cooking chemicals, could be returned to the pulp mill. This process thus had significantly lower impact on the Na/S balance (Arkell et al., 2013). The BLR sample used in the present work was obtained from such a membrane filtration process. The BLR was subjected to acidification with carbon dioxide at elevated temperature and pressure, similar to the SLRP process.

During the industrial process that was operated by our collaborators, the SLRP-like process was performed using BLR in order to obtain the kraft lignin. The BLR was processed both untreated and after depolymerization. The results showed that a higher lignin recovery yield was obtained using the depolymerized BLR than the raw BLR sample. Since the treatment was carried out under mild conditions, the effect of the treatment lies in the removal of a certain amount of the hemicelluloses, and the degradation of carbohydrates was suspected of being the main reason for this positive effect (Paper V).



**Figure 4.1.** (a) Influence of xylose and galactoglucomannan (GGM) concentration on lignin recovery yield during carbon dioxide liquid-liquid separation (xylose concentration: 0–40 g/L; GGM concentration: 0–20 g/L). (b) Stability of xylose (the starting concentration: 40 g/L) in water, in a 2% NaOH solution, and in BLR heat treated for 1 hour at 121 °C in an autoclave (from Paper V).

To investigate the effects of carbohydrates on lignin extraction, different amounts of the monosaccharide xylose and the polymeric hemicellulose galactoglucomannan (GGM) were added to the 170 °C depolymerized BLR, which were then subjected to the liquid-lignin separation. The GGM was recovered from the process water during thermomechanical pulp processing of softwood, containing mainly arabinan, galactan, glucan and mannan (Thuvander et al., 2019). The results showed that xylose and GGM had a negative effect on the recovery of liquid-lignin. Higher xylose addition led to lower liquid-lignin recovery (Figure 4.1a). This trend was clearer in the material that was subjected to continuous heat treatment prior to liquid-lignin separation than in that subjected to batch heat treatment. One possible explanation of this difference could be a difference in the efficiency of hemicellulose removal from the starting material. To obtain a better understanding of the lignin-sugar interaction, the xylose content was analyzed after lignin separation with xylose addition. Surprisingly, no detectable amounts of xylose were found in any of the treated lignin samples, neither in the aqueous phase nor in the lignin phase, indicating that the interactions between xylose and lignin are very strong.

Samples of xylose in water, in NaOH, and in the depolymerized BLR were subjected to similar conditions as in the separation process. The concentration of xylose in water was found to be constant after 1 hour of treatment at 121 °C., About 27% of the xylose was degraded directly when adding 40 g/L xylose to the NaOH solution. After heat treatment, the xylose content decreased to 6.4 g/L. More than half of the xylose was degraded when it was added to BLR before heating, and no xylose could be detected after treatment (Figure 4.1b).

Previous studies have suggested that the interaction between lignin and hemicellulose can be explained by the sorption of hemicellulose on the surfaces

of solid lignin particles (Sewring et al., 2019; Wallmo et al., 2009). Since the lignin was in liquid phase during the whole process in the present work, this explanation is irrational. The ratio of the surface area to the volume of liquid lignin is far smaller than that of precipitated lignin, so sorption on liquid surfaces is unlikely. The most likely explanation is that the xylose and hemicellulose interact with lignin on a molecular level, possibly forming covalent bonds between lignin and hemicellulose, so-called lignin-carbohydrate complex (LCC) linkages. This could explain not only the trend of decreasing liquid-lignin yield with increasing sugar content, but also the fact that xylose was undetectable after treatment. The lignin dissolved in the water phase interacts with xylose under the process conditions of liquid-lignin separation, and the resulting LCC is more polar than the original lignin molecule, thus making the complex more prone to remain in the water phase rather than transitioning to the less polar liquid-lignin phase.

Based on the experimental work performed, it can be concluded that the base-catalyzed heat treatment improves two parts of the overall process. First of all, it reduces the molecular weight of the lignin and produces more LMW compounds that can be valorized, chemically or biologically. Secondly, the heat treatment reduces the amount of hemicellulose compounds in the BLR, which significantly improves the lignin recovery. This last part is a key in reducing membrane surface area in a full-scale process and thus result in a lower investment cost.

## 4.2 Separation of LMW lignin compounds

The depolymerization of lignin into smaller molecular weight fractions is the first step in the valorization of kraft lignin. The separation and purification of these lignin-derived LMW compounds is necessary in order to analyze them and then use them to make a range of valuable products.

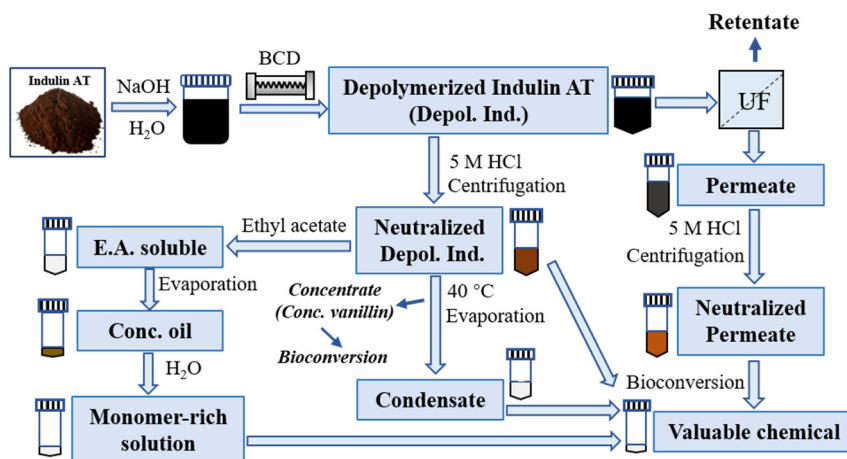
### 4.2.1 Acid precipitation

The depolymerization in the present work was performed under base conditions. Therefore, similar to the extraction of lignin from black liquor, adding acid to the depolymerized lignin solution causes the precipitation of high-molecular-weight (HMW) polymers and thus the separation of the dissolved LMW compounds. In this work, depolymerized BLR and Indulin AT were neutralized with hydrochloric acid. A process was developed for the extraction of lignin-derived LMW compounds and monomers from base-catalyzed depolymerized Indulin AT, as shown in Figure 4.2. After neutralizing the depolymerized lignin to pH 6-7, the solution could be used directly for analysis and further experiments.

The neutralized sample was evaporated under vacuum at low temperature (40 °C) to concentrate the lignin monomers. After 10-fold concentration, the concentrations of vanillin (one of the two main monomers) as well as other monomers increased tenfold. However, guaiacol was not concentrated, but evaporated proportionally with the water (the condensate sample in Figure 4.2). Vigneault et al. reported similar results. Over 70% and 30% of the guaiacol could be distilled from a mixture of synthetic monomers and the acid-soluble phase from base-catalyzed depolymerized lignin, respectively (Vigneault et al., 2007). The vapor pressure of guaiacol over a guaiacol/water mixture at 40 °C has been reported to be about 6 kPa (Pereira et al., 2018). This is significantly higher than that of vanillin, which has been reported to be about 0.25 Pa at 40 °C for the crystalline compound (Almeida et al. 2018), or 0.22 Pa at 25 °C for vanillin dissolved in water (Camera-roda et al., 2014). Furthermore, the vapor pressure of water is 7.38 kPa at 40°C (Wexler, 1976), which is similar to that of guaiacol. Computer simulations have indicated that in water solutions guaiacol has a tendency to accumulate at the water-air interface, thus increasing its rate of evaporation (Karlsson and Friedman, 2017).

A highly complex mixture of compounds are usually resulted from the depolymerization of technical lignin, which makes the separation of a single compound in a single step challenging. The results in the present work show that the guaiacol could be separated from the depolymerized Indulin AT sample with a high purity in one simple step. This demonstrates the potential of this method as a means of extracting pure guaiacol from lignin. The only drawback of this method is the energy required to evaporate the water (Paper III).

It has been reported that liquid-liquid extraction followed by vacuum distillation is an efficient method of separating monomers from depolymerized lignin (Vigneault et al., 2007). Liquid-liquid extraction of the neutralized Indulin AT sample using ethyl acetate as solvent was therefore investigated in an attempt to obtain high concentrations of the lignin-derived monomers. Most of the guaiacol and vanillin were obtained in the ethyl acetate phase, demonstrating that this is a suitable extraction solvent. After evaporating the ethyl acetate, a concentrated brown oil was obtained (*'conc. oil'* in Figure 4.2). The ethyl acetate could be evaporated and reused without loss of guaiacol since the boiling point of ethyl acetate is much lower than those of water and guaiacol. The concentrated oil was dissolved in a small amount of distilled water to obtain a monomer-rich solution for further bioconversion experiments (Paper III). For example, a volume of 100 mL neutralized *depol. Ind.* (depolymerized Indulin AT) solution (9.15 and 1.64 mmol/L of guaiacol and vanillin, respectively) was extracted by ethyl acetate followed by vacuum distillation of the ethyl acetate soluble fraction at 45 °C. The solution with the guaiacol and vanillin concentration of about 62 and 13 mmol/L are obtained after the redissolution of the *conc. oil* into 10 mL of distilled water. This can be an ideal substrate for further bioconversion (Paper IV).



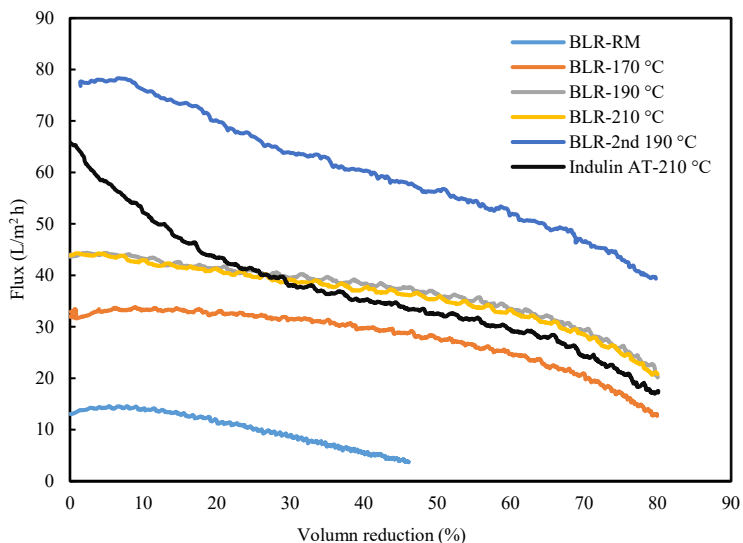
**Figure 4.2.** Schematic of the process used for lignin depolymerization and fractionation (Conc.: concentrated; depol.: depolymerization; E.A.: ethyl acetate; Ind.: Indulin AT) from Paper III and IV.

In conclusion, in these lab-scale experiments, acidification was necessary to obtain soluble LMW compounds. The neutralized lignin sample could be easily analyzed and was also suitable for bacterial cultivation for further chemical production. Evaporation of the neutralized lignin at low temperature under vacuum could be a promising method for the production of pure guaiacol for utilization by bacterial species to produce other value-added chemicals. Liquid-liquid extraction of the neutralized sample could improve the concentration of LMW compounds, and also reduce the amounts of undesirable compounds, since not all the LMW compounds are soluble in ethyl acetate. The concentrated oil obtained could be redissolved in a fixed amount of distilled water depending on the concentration of monomers required for the bioconversion process.

#### 4.2.2 Membrane separation

As mentioned above, membrane filtration has considerable potential for separating lignin from black liquor. In this work, membrane filtration was also investigated for the separation of LMW compounds from depolymerized BLR. Three membranes from Alfa Laval (UF5pHt, GR95PP, and ETNA01) with molecular weight cut-offs of 5, 2, and 1 kDa, respectively, were investigated. The original BLR solution was filtrated at constant temperature, pressure, and diafiltration factor of 50 °C, 5.5 bar, and 1 g/g, respectively, in a stirred cell with a calculated crossflow velocity of 0.5 m/s. The flux of the ETNA01 and GR95PP membranes was approximately the same, while the flux of the UF5pHt membrane was twice as high as the other two. Despite this, the results of SEC showed that the amounts of LMW compounds were higher, and approximately

equal, for the ETNA01 and GR95PP membranes. Although these two membranes showed the same SEC peak maximum, the polydispersity of the permeate produced by the ETNA01 membrane was slightly higher, so the GR95PP membrane was chosen for further studies.



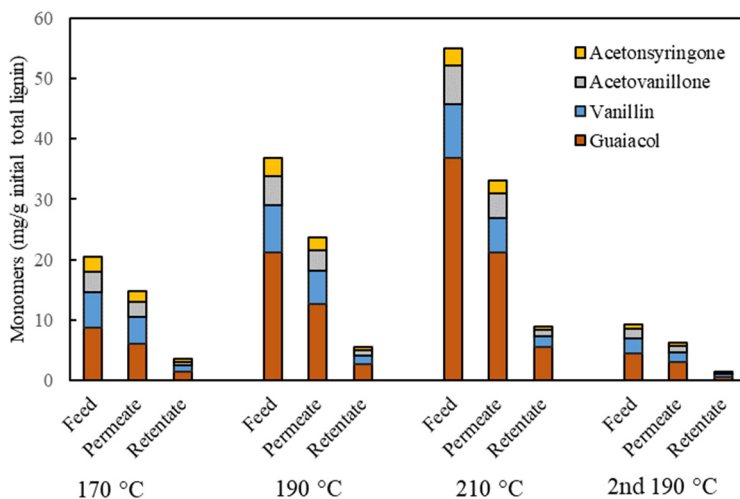
**Figure 4.3.** Flux during the ultrafiltration of BLR raw material (RM), BLR depolymerized at 170-210 °C, BLR the second round depolymerized BLR at 190 °C, and Indulin AT depolymerized at 210 °C using the GR95PP membrane. The temperature was 50 °C, the transmembrane pressure 5.5 bar, and the crossflow 0.5 m/s (Paper II and IV).

BLR samples, untreated and depolymerized at 170-210 °C, were filtered using the GR95PP membrane in order to separate the LMW compounds. The initial flux of the untreated BLR sample was 13.1 L/(m<sup>2</sup> h), and decreased to 3.7 L/(m<sup>2</sup> h) at a volume reduction of 46% (Figure 4.3). The flux for samples depolymerized at 190 and 210 °C were similar, both being higher than that of the sample depolymerized at 170 °C. The degradation of polysaccharides and depolymerization of the high molecular weight lignin fractions (at 170-210 °C) contributed to an increase in the flux across the membrane (Paper II). The Indulin AT sample, depolymerized at 210 °C was also ultrafiltered with the same membrane. The initial flux was about 65.8 L/(m<sup>2</sup> h), and decreased to 17.2 L/(m<sup>2</sup> h) as the volume reduction increased to 80% (Figure 4.3). The flux of the Indulin AT sample is higher than BLR can be attributed to its lower molecular weight (Paper IV).

### 4.2.3 Characterization of the permeate

The molecular weight distribution of the depolymerized Indulin AT samples before and after ultrafiltration were determined using SEC. After membrane filtration of Indulin AT depolymerized at 210 °C, a decrease in both Mw and Mn can be seen in the permeate sample (Figure 9, Paper IV). The UPLC results show that higher guaiacol and vanillin concentrations in the permeate (14.5 and 3.1 mmol/L, respectively) were obtained, compare with 9.15 and 1.64 mmol/L, respectively in the feed before membrane filtration. Compared with the feed, the neutralization of this permeate stream consumes less acid, and obtained higher volume of the neutralized solution, which can be used as a guaiacol-enriched substrate for further bioconversion step (Paper IV).

After membrane filtration of the BLR samples, the majority of the LMW compounds passed through the membrane and were found in the permeate (Figure 7 and 8, Paper II). The main monomers in the depolymerized BLR samples, both before and after membrane filtration, are shown in Figure 4.4. Most of the main monomers (guaiacol, vanillin, acetovanillone, and acetosyringone) were recovered in the permeate after ultrafiltration. The retentate after membrane filtration of 190 °C depolymerized BLR was collected for the second round of depolymerization. This sample was then ultrafiltrated under the same conditions as the first time to separate the LMW compounds from the second depolymerized BLR. Despite the low monomer content after the second round of depolymerization, the ultrafiltration efficiency was relatively high. Most of the four main monomers were found in the permeate (Figure 4.4) (Paper II).



**Figure 4.4.** Monomer contents in Feed (D170, D190, D210, and 2D190) and both of permeate and retentate after membrane filtration (Adapt from Figure 8, Paper II).

The identification of lignin-derived monomers from the kraft lignin samples after depolymerization relies on the targeted UHPSFC/QTOF-MS method that was developed by other researchers in our group as mentioned in Section 3.3.2 (Prothmann et al., 2017). Identification of oligomers is also essential for understanding the depolymerization and membrane filtration efficiency. However, due to the lack of commercially available reference standards, the targeted analysis method is not useful for the identification of the oligomers in the lignin samples. In the present work, a nontargeted UHPSFC-high resolution multiple stage tandem mass spectrometry (HRMS<sup>n</sup>) analysis method was developed for the identification of both monomers and oligomers from the lignin samples (analysis strategy, Figure 5 in Paper VI).

This nontargeted method was used to evaluate the permeate samples after ultrafiltration of BLR depolymerized at 170-210 °C as well as the permeate after ultrafiltration of the 190 °C depolymerized retentate after the first round of ultrafiltration (also at 190 °C) (Paper VII). An overview of the obtained analysis results of the four different BLR samples is shown in Table 4.1. It can be seen that the total number of identified and validated lignin monomers, dimers and trimers are increasing with an increased reaction temperature during the depolymerization process. Most of the identified and validated m/z values in the samples (especially BDM190, BDM210 and 2BDM190 samples) showed more than one retention time, meaning the presence of structural isomers. Including the structural isomers, 77 of the lignin-related phenolic compounds were identified in the BDM170 sample, 135 in the BDM190 sample and 186 in BDM210. The number of m/z values with more than one retention time in the extracted ion-chromatogram increases with an increased depolymerization temperature.

**Table 4.1.** The number of validated m/z values identified as lignin monomers and oligomers, the number of m/z values with more than one retention time, and the number of identified phenolic compounds being lignin monomers, dimers or trimers in the BLR samples. BDM170-210: the permeate samples after the ultrafiltration of the 170-210 °C depolymerized BLR; 2BDM190: the permeate after ultrafiltration of the 190 °C depolymerized retentate after the separation of BDM190 (Adapt from Table 1, Paper VII).

Sample	BDM170*	BDM190	BDM210	2BDM190
Identified and validated m/z values	44	65	79	58
Monomers	10	17	27	6
Dimers	34	48	51	48
Trimers	0	0	1	4
m/z values with more than one retention time	20	63	79	58
Identified phenolic compounds	77	135	186	101
Monomers	14	33	63	9
Dimers	63	102	122	84
Trimers	0	0	1	8



The aromatic compounds that are produced from sample 2BDM190 is similar to the BDM190 sample (Figure 4 in Paper VII), demonstrating that similar products can be generated under the same temperature during further depolymerization of the HMW fraction. Depolymerizing the retentate after membrane filtration of the 190 °C depolymerized BLR produces a lower number of monomers and dimers than the first time but generates a high number of identified trimers. This is most likely caused by repolymerization reactions after repeated thermal treatment (Paper VII).

The results in this Chapter show that a simple membrane filtration step can provide a monomer- and oligomer-enriched permeate, facilitating further extraction and conversion. Ultrafiltration thus appears to be promising for the separation of small molecules from depolymerized technical lignin samples under alkaline conditions. For further utilization, the LMW compounds could probably be separated from the NaOH solution by nanofiltration. After extracting the LMW compound, the heavy fractions could be acidified using the SLRP process to obtain a kraft lignin starting material for the production of value-added hydrocarbon fuels via the more severe hydrogenation process. Since membrane filtration is performed at 50 °C, the depolymerized lignin components could repolymerize. Therefore, protection reagents or additives will be required during ultrafiltration to prevent this, ensuring that high amounts of LMW compounds are produced.

## 5. Bioconversion of depolymerized kraft lignin

Muconic acid, which contains two carboxylic acid groups, is a promising platform molecule. It can, for example, be catalytically converted to adipic acid, which is in high demand industrially, and is currently produced from petroleum (Beckham et al., 2016). Nylon-6,6 production consumes around 85% of all available adipic acid (Vardon et al., 2016). In addition, muconic acid can also be converted to terephthalic acid through the isomerization of cis,cis-muconic acid to trans,trans-muconic acid, followed by a Diels-Alder reaction with ethylene and dehydrogenation (Beckham et al., 2016). Terephthalic acid is also an important industrial chemical used as a precursor in the production of polyethylene terephthalate (commonly known as PET) (Becker et al., 2018). The use of muconic acid instead of fossil-based sources to produce adipic acid and terephthalic acid would have a considerable positive effect on the environment.

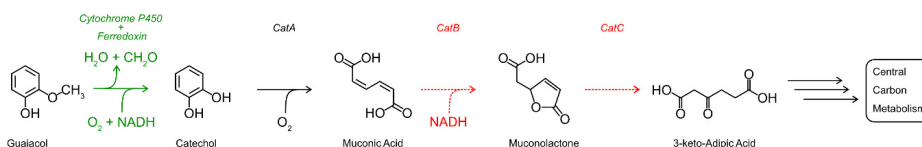
Chemical lignin degradation, for example, using the base-catalyzed depolymerization process described in Chapter 3, is the first step in the production of valuable chemicals from technical lignin. Separation and purification processes such as those described in Chapter 4 provide access to a series of small aromatic water-soluble compounds that can be used as carbon and energy sources for different microbes. Muconic acid, for instance, can be then produced from these lignin-derived aromatic compounds by microbial conversion first to central intermediates such as catechol, and then cleaving by the ring-opening dioxygenases (Beckham et al., 2016).

In recent years, a number of studies have demonstrated the significant potential of microorganisms for the bioconversion of lignin degradation products into value-added chemicals (Becker and Wittmann, 2019). There are many known bacteria and fungi capable of degradation of lignin and aromatic compounds (Brink et al., 2019). Among them, *Pseudomonas putida* KT2440 has been reported to have high potential to upgrade aromatic compounds to targeted molecules (Beckham et al., 2016; Ravi et al., 2017). Guaiacol, the main monomer product from the depolymerized Indulin AT lignin in this work also attracted much attention as a substrate for microbial conversion, since it can be converted to muconic acid in two enzymatic reactions (García-Hidalgo et al., 2019). The assimilation of guaiacol has been treated as a key target to increase the efficiency

of lignin biological valorization (Beckham et al., 2016), and the genes responsible for this conversion had until recently not been identified (García-Hidalgo et al., 2019; Tumen-Velasquez et al., 2018). Guaiacol is not a natural substrate for *P. putida* KT2440, but the insertion of genes coding for a cytochrome P450 and a ferredoxin reductase from *Rhodococcus rhodochrous* J3 or *Amycolatopsis* sp. allowed *P. putida* KT2440 to metabolize and grow on guaiacol as the sole carbon source (García-Hidalgo et al., 2019; Tumen-Velasquez et al., 2018). The application of engineered *P. putida* KT2440 for stoichiometric conversion of the guaiacol derived from Kraft lignin to muconic acid is discussed in this chapter.

## 5.1 Growth assessment and guaiacol conversion

As can be seen from Figure 5.1, the cytochrome P450 and ferredoxin reductase from *Rhodococcus rhodochrous* were introduced into *P. putida* by other researchers in our group to enable O-aryl-demethylation of guaiacol to catechol (García-Hidalgo et al., 2019). The *catB* and *catC* genes encoding cis,cis-muconate-lactonizing enzyme and muconolactone isomerase were deleted, thereby stopping the pathway at muconic acid (Paper III).



**Figure 5.1.** Guaiacol to muconic acid pathway in *P. putida*  $\Delta$ catBC+pG. Modifications from wildtype are the inclusion of a cytochrome P450 and a ferredoxin (green) the deletion of *catBC* (red) (Paper III).

Cultivation of the control strain *P. putida* KT2440 and the constructed strain *P. putida* TMBHV002 (Table 1, Paper III) was performed in shake flasks with M9 medium supplemented with 10 g/L glucose as carbon source to sustain growth during evaluation of the bioconversion of 5 mM guaiacol to muconic acid. No muconic acid was produced from the control strain KT2440 despite a slight decrease in the amount of guaiacol. In the engineered strain TMBHV002, guaiacol was converted to a stoichiometric amount of muconic acid, indicating a lack of side-reactions from native enzymes acting on guaiacol and a successful engineering strategy.

The cultivation of microorganisms usually relies on carbohydrates such as glucose as the main carbon source which are not present in depolymerized lignin, and thus lignin-derived compounds capable of sustaining growth are attractive as an alternative carbon source for growth (Shinoda et al., 2019). *P. putida* KT2440

can grow on vanillin using a parallel pathway to the muconic acid pathway (García-Hidalgo et al., 2020), meaning that despite the introduced deletions, TMBHV002 is still capable of growth on vanillin. The glucose was therefore replaced with 5-20 mM vanillin as the carbon source during the conversion of 5 mM guaiacol in M9 medium. Growth was only observed with 5 mM vanillin, and 1 mM muconic acid was produced accompanied by consumption of the same amount of guaiacol. This was much lower than that when glucose was used as the carbon source. Neither growth nor guaiacol conversion was observed at higher vanillin concentrations, illustrating the strong inhibitory effect of vanillin. Guaiacol itself did not inhibit growth in these strains below concentrations of 20 mM (Paper III).

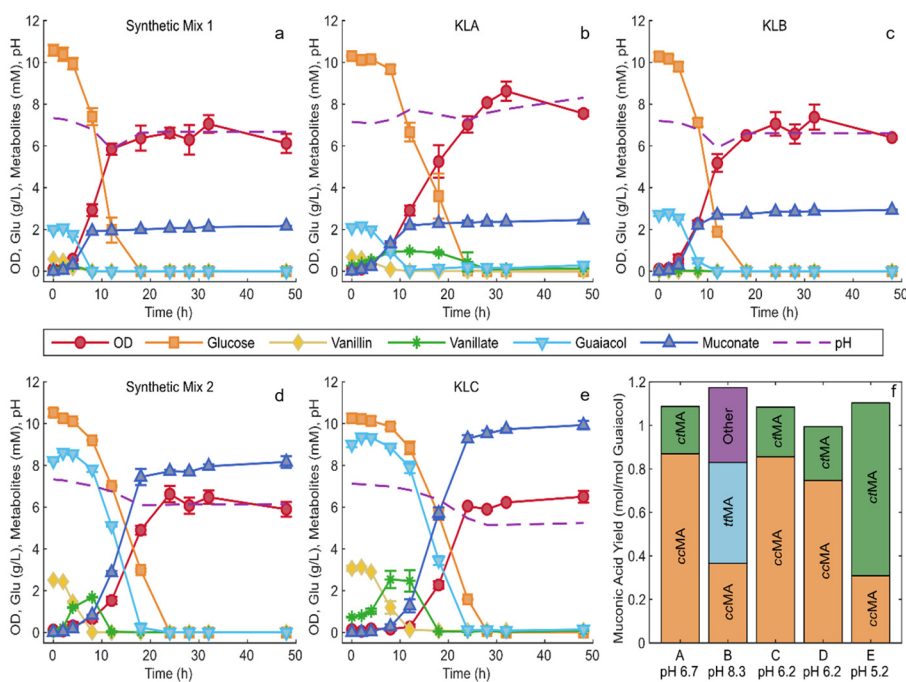
## 5.2 Bioconversion of depolymerized Indulin AT

Under suitable conditions, the tested strains grew on the synthetic medium, while the depolymerized lignin samples contained a mixture of different aromatic compounds, even after separation. The previous chapter describes how the neutralized *depol. Ind.* (sample KLA, guaiacol and vanillin contents of 2.5 mM and 0.8 mM respectively), the condensate (sample KLB, 2.7 mM guaiacol only), and the concentrated lignin oil (sample KLC, 10 mM guaiacol and 3.2 mM vanillin) were obtained. The potential of these three depolymerized lignin-based fractions as substrates for the production of muconic acid was evaluated in whole-cell bioconversion experiments. Two synthetic media mimicking the substrate concentrations in KLA and KLB (Synthetic medium 1: 2.0 mM guaiacol and 0.6 mM vanillin), and the concentrations in KLC (Synthetic medium 2: 8.6 mM guaiacol and 2.5 mM vanillin) were also evaluated to eliminate the effects of unknown depolymerized lignin residues remaining in the separated fractions. All media were supplemented with glucose to sustain cell growth and maintain redox homeostasis (Paper III).

Fast glucose consumption and growth were seen in both the synthetic media and the depolymerized lignin sample. However, growth was slightly slower, and there was a short lag phase in glucose consumption in the depolymerized lignin sample (Figure 5.2a and b). The reduction in growth rate could be due to inhibitors in the medium, other than vanillin and guaiacol. However, within 12 hours, the guaiacol was completely consumed and converted to muconic acid. The results show that guaiacol in an unrefined depolymerized kraft lignin sample can be efficiently converted into muconic acid by the TMBHV002 strain. Vanillin was quantitatively converted to vanillate before being consumed.

Since KLB contained mainly guaiacol, the result of the fermentation was similar to that in synthetic medium 1, indicating that guaiacol purification through evap-

oration provides a pure stream with no inhibitors, making it suitable for fermentation (Figure 5.2a and c). The growth rate of guaiacol was in the range from 0.3 to 0.5 h<sup>-1</sup> depending on the guaiacol concentration (ranging 2-9 mM). The maximum specific growth rate during bioconversion of a lignin-derived guaiacol sample with higher guaiacol concentration (around 8 mM after adjustment) (Figure 1, Paper IV) was 0.42 ± 0.03 h<sup>-1</sup>, and the consumed guaiacol was stoichiometrically converted to muconic acid at a yield of 1.06 ± 0.18 mol/mol (Figure 11, Paper IV). The purification of guaiacol from depolymerized lignin sources for bioconversion was efficient, apart from the energy required for evaporation.



**Figure 5.2.** Cultivation of *P. putida* ΔcatBC+pG in five different media (a-e) and a comparison of the yields of muconic acid (f) (a, Synthetic mix 1; b, KLA; c – KLB; d, Synthetic mix 2; e, KLC) (Paper III).

The growth rate was slower in Synthetic medium 2 (8.6 mM guaiacol and 2.5 mM vanillin), than in Synthetic medium 1 (Figure 5.2a and d). The reduction in growth rate was attributed to inhibition by the higher concentrations of guaiacol and 2.5 mM vanillin in Synthetic medium 2. The growth rate in the KLC medium was similar as that in Synthetic medium 2 and the inhibiting effect was much weaker (Figure 5.2d and e). Within 24 hours of growth, guaiacol in KLC was converted to muconic acid completely. The KLC preparation process is relatively

simple and not only increased the concentration of the desired substrates, but also reduced the inhibitory effect of the substrate, which is promising for the preparation of feedstocks for muconic acid production. The muconic acid yields in several cases exceeded 1 can be explained by evaporation of water during the experiment concentrating the solutes (Figure 5.2f). Other lignin-derived aromatic compounds in the KLA and KLC samples may also contribute to the muconic acid production. For example, catechol, which is an intermediate in the guaiacol pathway, was however not detected during analysis.

In conclusion, guaiacol in both the Synthetic media and the depolymerized Indulin AT sample was quantitatively converted to muconic acid. Barton et al. previously reported on the use of an engineered *Amycolatopsis* sp. ATCC 39116 for the conversion of 5 mM guaiacol to cis,cis-muconic acid with a yield of 0.9 mol/mol, which is comparable to the results obtained with Synthetic medium 1 in the present work. A muconic acid concentration of 1.7 mM at a yield of 0.72 mol/mol was resulted from the depolymerized lignin sample (Barton et al., 2018). The result obtained using the KLA substrate in the present work (2.46 mM, ~1.0 mol/mol) is comparable to this (Table 5.1). Furthermore, the fermentation of the permeate after ultrafiltration of the depolymerized Indulin AT (Figure 4.2 in Chapter 4) that with higher guaiacol concentration (around 6.5 mM after adjustment) was also performed. Although the maximum specific growth rate ( $0.19 \pm 0.01 \text{ h}^{-1}$ ) was lower, the muconic acid yield ( $0.97 \pm 0.21 \text{ mol/mol}$ ) was similar as the other samples (Figure 11, Paper IV).

**Table 5.1.** Growth rate and muconic acid yield of *P. putida* TMBHV002 in shake flasks containing depolymerized lignin or synthetic media with different concentrations of guaiacol, vanillin, and vanillate (from Paper III).

	Synthetic 1	KLA	KLB	Synthetic 2	KLC
Guaiacol (mM)	1.99 ± 0.05	2.09 ± 0.00	2.71 ± 0.06	8.21 ± 0.04	9.00 ± 0.13
Vanillin (mM)	0.59 ± 0.01	0.68 ± 0.01	<0.05	2.50 ± 0.00	3.07 ± 0.13
Vanillate (mM)	0	<0.05	0	0	0.73 ± 0.01
Maximum specific growth rate ( $\text{h}^{-1}$ )	0.51 ± 0.02	0.31 ± 0.02	0.46 ± 0.02	0.31 ± 0.00	0.28 ± 0.04
Yield (mol muconic acid / mol guaiacol)	1.09 ± 0.02	1.17 ± 0.01	1.08 ± 0.01	0.99 ± 0.04	1.10 ± 0.01

The results presented in this work are especially important in the field since few studies have been performed on the bioconversion of guaiacol (in part due to the previous lack of knowledge of guaiacol degrading strains and the biochemical mechanisms of guaiacol metabolism), which is one of the most abundant lignin degraded products. It has been reported in previous studies that catechol can be converted to high concentrations of muconic acid by *P. putida* KT2440-MA9 (64.2 g/L, 452 mM) and by *Corynebacterium glutamicum* MA2 (85 g/L, 745

mM) (Becker et al., 2018; Kohlstedt et al., 2018). Although the product concentrations in the present work was much lower, guaiacol is closely related to catechol, and only one reaction step is needed to convert it to catechol. Therefore, the production of muconic acid from guaiacol has considerable potential to be an industrially relevant process.

# 6. Conclusions and future work

## 6.1 Conclusions

The research presented in this thesis explored the possibility of continuously depolymerizing kraft lignin samples to produce LMW compounds under base conditions for the production of valuable platform chemicals. The complex structure and high molecular weight of industrial lignin pose considerable challenges to its upgrading. Furthermore, traditional batch depolymerization of lignin suffers from repolymerization, increasing the difficulty of degrading lignin into smaller molecules.

The first studies described in this thesis focused on base-catalyzed depolymerization of two different kraft lignin samples with a continuous flow reactor developed for this purpose. Acid precipitation and membrane filtration were then used to separate the LMW compounds from the depolymerized lignin samples, and a non-targeted method was developed and used for the analysis of lignin-derived monomers and oligomers. Finally, the extracted monomers were fermented with engineered microorganisms for the production of platform chemicals. The specific conclusions of this work were summarized below.

A lab-scale CFR system was developed for the depolymerization of two different kraft lignin samples (Indulin AT and BLR), which were dissolved in a NaOH solution and treated for very short times. This system enabled a very short cooling time after reaction, largely suppressing repolymerization. The results of SEC measurements showed that the molecular weight of the kraft lignin samples could be considerably reduced by depolymerization. The main phenolic compounds obtained after the depolymerization of Indulin AT and BLR were guaiacol and vanillin.

The effect of NaOH concentration, depolymerization temperature and residence time on the production of LMW compounds from Indulin AT was investigated. Since the depolymerization process consumed NaOH, increasing the substrate concentration increased the demand for NaOH. A guaiacol yield of 5.1 wt.% was obtained after depolymerization of 5 wt.% of lignin with 2 wt.% of NaOH at 200 °C with a residence time of 2 min. When taking the whole operation, including the cost and depolymerization efficiency, into consideration, the concentration, lignin loading of 4 wt.% of lignin and NaOH concentration of 2 wt.% were chosen



as the optimal conditions for the production of a guaiacol-rich fraction. Increasing the depolymerization temperature (from 170 to 250 °C) and the residence time (from 1 to 4 min) led to a decrease in the molecular weight of the kraft lignin degradation products. However, the rate of repolymerization increased with the increasing reaction severity, resulting in the formation of coke. Although only small amounts were formed, the production of solid char is quite detrimental to the continuous flow reaction process. Therefore, the depolymerization temperatures under 230 °C were used in this work to reduce the risk of coke formation.

The BLR could also be depolymerized effectively with 2 min residence time and a temperature of 210 °C, leading to the production of about 48% LMW compounds and 55 mg monomers/g initial lignin. Recycling the product and repeating depolymerization resulted in higher yields of LMW compounds, but the increase following the second and the third rounds was rather small.

Acid precipitation by using hydrochloric acid was suggested as a method of separating the LMW compounds on lab-scale for the purpose of analysis and research, due to the advantage of easy handling. Pure guaiacol could easily be separated by evaporating the neutralized *depoly. Ind.* sample at a low temperature (40 °C) in vacuum.

Membrane filtration was found to be a promising method for the separation of lignin-derived LMW compounds from depolymerized lignin. Using a GR95PP ultrafiltration membrane, 60% of the LMW compounds could be separated from the depolymerized BLR lignin. The utilization of membrane filtration in the multiple depolymerization experiments means that the separated low-molecular weight fractions do not participate in the following depolymerization rounds, greatly reducing the risk of repolymerization.

The *P. putida* strain KT2440, which was metabolically engineered for stoichiometric conversion of guaiacol to muconic acid, by other researchers in our group was studied in this work for the bioconversion of depolymerized Indulin AT after treatment and purification. The guaiacol in depolymerized kraft lignin at neutral pH, that in a sample of pure guaiacol extracted from depolymerized kraft lignin, and that in a concentrated sample of small aromatic compounds obtained by liquid-liquid extraction was quantitatively converted to the value-added platform chemical muconic acid.

## 6.2 Outlook on future research

Work on base-catalyzed continuous depolymerization of kraft lignin under mild conditions has been presented in this thesis. A promising process for valorizing kraft lignin into valuable platform chemical was developed through further separation, purification and biological conversion. However, several problems such as the low yield of aromatic compounds, the formation of coke, and repolymerization during storage and membrane filtration, must be addressed. Some suggestions for future work are given below.

Highly efficient continuous depolymerization can only be successfully achieved under the appropriate conditions. The formation of solid coke under severe conditions would block the system, stopping the reaction. The CFR system should therefore be designed to cope with a small amount of coke, so that the sample can be pumped into the reactor continuously. Also, the reactor should be designed so that it is easy to open it for coke removal. This would allow for higher operating temperatures or more severe conditions to be employed to obtain a higher degree of depolymerization and thus higher yields of small aromatic compounds.

NaOH was the only catalyst used for the depolymerization of kraft lignin, and even under optimal conditions, the monomer yield was still below 10%. In the future, more alkali-resistant catalysts should be developed to improve the yield of aromatic compounds. The mechanisms of kraft lignin depolymerization under base conditions with different catalysts should also be considered, especially regarding the formation of the main monomers.

Ultrafiltration appears to be a very promising method for separating small fractions from depolymerized kraft lignin without affecting the chemical balance. Studies should be carried out to identify appropriate surfactants or protective agents to prevent repolymerization during membrane filtration. Future work could be focused on further purification of the permeate using nanofiltration to separate LMW compounds from the alkali solution. This would allow the NaOH to be reused, and no acid, or only very small amounts of acid, would be needed.

The final step of lignin valorization, biological conversion, would benefit considerably from genetic engineering of microorganisms for the conversion of different aromatic compounds. This would allow the conversion of not only guaiacol, but also vanillin and other monomers, or even dimers, produced from kraft lignin to valuable chemicals.

The lignin-derived LMW compounds obtained from Kraft lignin constitute only a small fraction of the total mass; the remainder consisting of HMW polymers. Other process, such as hydrogenation and hydrodeoxygenation at high temperature and pressure with hydrogen gas or a hydrogen donor, could be studied in the future for the conversion of this fraction into valuable hydrocarbon fuels.



# References

- Ansari, A., Pawlik, M., 2007. Floatability of chalcopyrite and molybdenite in the presence of lignosulfonates. Part I. Adsorption studies. *Miner. Eng.* 20, 600–608. <https://doi.org/10.1016/j.mineng.2006.12.007>
- Areskog, D., Li, J., Gellerstedt, G., Henriksson, G., 2010. Structural modification of commercial lignosulfonates through laccase catalysis and ozonolysis. *Ind. Crops Prod.* 32, 458–466. <https://doi.org/10.1016/j.indcrop.2010.06.016>
- Arkell, A., Krawczyk, H., Jönsson, A.S., 2013. Influence of heat pretreatment on ultrafiltration of a solution containing hemicelluloses extracted from wheat bran. *Sep. Purif. Technol.* 119, 46–50. <https://doi.org/10.1016/j.seppur.2013.09.001>
- Aro, T., Fatehi, P., 2017. Production and Application of Lignosulfonates and Sulfonated Lignin. *ChemSusChem* 10, 1861–1877. <https://doi.org/10.1002/cssc.201700082>
- Azadi, P., Inderwildi, O.R., Farnood, R., King, D.A., 2013. Liquid fuels, hydrogen and chemicals from lignin: A critical review. *Renew. Sustain. Energy Rev.* 21, 506–523. <https://doi.org/10.1016/j.rser.2012.12.022>
- Barton, N., Horbal, L., Starck, S., Kohlstedt, M., Luzhetskyy, A., Wittmann, C., 2018. Enabling the valorization of guaiacol-based lignin: Integrated chemical and biochemical production of cis,cis-muconic acid using metabolically engineered *Amycolatopsis* sp ATCC 39116. *Metab. Eng.* 45, 200–210. <https://doi.org/10.1016/j.ymben.2017.12.001>
- Baumberger, S., Abaecherli, A., Fasching, M., Gellerstedt, G., Gosselink, R., Hortling, B., Li, J., Saake, B., De Jong, E., 2007. Molar mass determination of lignins by size-exclusion chromatography: Towards standardisation of the method. *Holzforschung* 61, 459–468. <https://doi.org/10.1515/HF.2007.074>
- Beauchet, R., Monteil-Rivera, F., Lavoie, J.M., 2012. Conversion of lignin to aromatic-based chemicals (L-chems) and biofuels (L-fuels). *Bioresour. Technol.* 121, 328–334. <https://doi.org/10.1016/j.biortech.2012.06.061>
- Becker, J., Kuhl, M., Kohlstedt, M., Starck, S., Wittmann, C., 2018. Metabolic engineering of *Corynebacterium glutamicum* for the production of cis, cis-muconic acid from lignin. *Microb. Cell Fact.* 17, 1–14. <https://doi.org/10.1186/s12934-018-0963-2>
- Becker, J., Wittmann, C., 2019. A field of dreams: Lignin valorization into chemicals, materials, fuels, and health-care products. *Biotechnol. Adv.* 37, 107360. <https://doi.org/10.1016/j.biotechadv.2019.02.016>
- Beckham, G.T., Johnson, C.W., Karp, E.M., Salvachúa, D., Vardon, D.R., 2016. Opportunities and challenges in biological lignin valorization. *Curr. Opin. Biotechnol.* 42, 40–53. <https://doi.org/10.1016/j.copbio.2016.02.030>

- Bjørsvik, H.R., Minisci, F., 1999. Fine chemicals from lignosulfonates. 1. Synthesis of vanillin by oxidation of lignosulfonates. *Org. Process Res. Dev.* 3, 330–340. <https://doi.org/10.1021/op9900028>
- Brink, D.P., Ravi, K., Lidén, G., Gorwa-Grauslund, M.F., 2019. Mapping the diversity of microbial lignin catabolism: experiences from the eLignin database. *Appl. Microbiol. Biotechnol.* 103, 3979–4002. <https://doi.org/10.1007/s00253-019-09692-4>
- Camera-roda, G., Cardillo, A., Loddo, V., Palmisano, L., Parrino, F., 2014. Improvement of Membrane Performances to Enhance the Yield of Vanillin in a Pervaporation Reactor 96–112. <https://doi.org/10.3390/membranes4010096>
- Chakar, F.S., Ragauskas, A.J., 2004. Review of current and future softwood kraft lignin process chemistry. *Ind. Crop. Prod.* 20, 131–141. <https://doi.org/10.1016/j.indcrop.2004.04.016>
- Chio, C., Sain, M., Qin, W., 2019. Lignin utilization: A review of lignin depolymerization from various aspects. *Renew. Sustain. Energy Rev.* 107, 232–249. <https://doi.org/10.1016/j.rser.2019.03.008>
- Constant, S., Wienk, H.L.J., Frissen, A.E., Peinder, P. de, Boelens, R., van Es, D.S., Grisel, R.J.H., Weckhuysen, B.M., Huijgen, W.J.J., Gosselink, R.J.A., Bruijninx, P.C.A., 2016. New insights into the structure and composition of technical lignins: a comparative characterisation study. *Green Chem.* 18, 2563–2910. <https://doi.org/10.1039/C5GC03043A>
- Deuss, P.J., Lancefield, C.S., Narani, A., De Vries, J.G., Westwood, N.J., Barta, K., 2017. Phenolic acetals from lignins of varying compositions: Via iron(III) triflate catalysed depolymerisation. *Green Chem.* 19, 2774–2782. <https://doi.org/10.1039/c7gc00195a>
- Deuss, P.J., Scott, M., Tran, F., Westwood, N.J., De Vries, J.G., Barta, K., 2015. Aromatic Monomers by in Situ Conversion of Reactive Intermediates in the Acid-Catalyzed Depolymerization of Lignin. *J. Am. Chem. Soc.* 137, 7456–7467. <https://doi.org/10.1021/jacs.5b03693>
- Eltis, L.D., Singh, R., 2018. Chapter 11 Biological Funneling as a Means of Transforming Lignin-derived Aromatic Compounds into Value-added Chemicals, in: Beckham, G.T. (Ed.), *Lignin Valorization: Emerging Approaches*. The Royal Society of Chemistry, pp. 290–313. <https://doi.org/https://doi.org/10.1039/9781788010351-00290>
- Faraco, V., 2013. Lignocellulose Conversion. Enzymatic and Microbial Tools for Bioethanol Production, *Lignocellulose Conversion*. <https://doi.org/10.1007/978-3-642-37861-4>
- Forchheim, D., R. Gasson, J., Hornung, U., Kruse, A., Barth, T., 2012. Modeling the Lignin Degradation Kinetics in a Ethanol/Formic Acid Solvolysis Approach. Part 2. Validation and Transfer to Variable Conditions. *Ind. & Eng. Chem. Res.* 51, 15053–15063. <https://doi.org/10.1021/ie3026407>
- Fuchs, G., Boll, M., Heider, J., 2011. Microbial degradation of aromatic compounds- From one strategy to four. *Nat. Rev. Microbiol.* 9, 803–816. <https://doi.org/10.1038/nrmicro2652>

- García-Hidalgo, J., Brink, D.P., Ravi, K., Paul, C.J., Lidén, G., Gorwa-Grauslunda, M.F., 2020. Vanillin Production in *Pseudomonas*: Whole-Genome Sequencing of *Pseudomonas* sp. Strain 9.1 and Reannotation of *Pseudomonas putida* CalA as a Vanillin Reductase. *Appl. Environ. Microbiol.* 86, 1–17. <https://doi.org/https://doi.org/10.1128/AEM.02442-19>
- García-Hidalgo, J., Ravi, K., Kuré, L.L., Lidén, G., Gorwa-Grauslund, M., 2019. Identification of the two-component guaiacol demethylase system from *Rhodococcus rhodochrous* and expression in *Pseudomonas putida* EM42 for guaiacol assimilation. *AMB Express* 9. <https://doi.org/10.1186/s13568-019-0759-8>
- Gavrilescu, M., 2014. Chapter 14 Biorefinery Systems: An Overview, in: *Bioenergy Research: Advances and Applications*. pp. 219–241. <https://doi.org/10.1016/B978-0-444-59561-4.00014-0>
- Gellerstedt, G., 2015. Softwood kraft lignin: Raw material for the future. *Ind. Crops Prod.* 77, 845–854. <https://doi.org/10.1016/j.indcrop.2015.09.040>
- Gellerstedt, G., Lindfors, E.-L., 1984. Structural Changes in Lignin During Kraft Pulping. *Holzforschung* 38, 151–158. <https://doi.org/https://doi.org/10.1515/hfsg.1984.38.3.151>
- Gellerstedt, G.L.F., Henriksson, E.G., 2008. Chapter 9 Lignins: Major sources, structure and properties, in: *Monomers, Polymers and Composites from Renewable Resources*. pp. 201–224. <https://doi.org/10.1016/B978-0-08-045316-3.00009-0>
- Geun Yoo, C., Ragauskas, A.J., 2021. Opportunities and Challenges of Lignin Utilization, in: *Lignin Utilization Strategies: From Processing to Applications*. pp. 1–12. <https://doi.org/10.1021/bk-2021-1377.ch001>
- Guragain, Y.N., Herrera, A.I., Vadlani, P. V., Prakash, O., 2015. Lignins of bioenergy crops: A review. *Nat. Prod. Commun.* 10, 201–208. <https://doi.org/10.1177/1934578x1501000141>
- Hamaguchi, M., Cardoso, M., Vakkilainen, E., 2012. Alternative technologies for biofuels production in kraft pulp mills-potential and prospects. *Energies* 5, 2288–2309. <https://doi.org/10.3390/en5072288>
- Hasegawa, I., Inoue, Y., Muranaka, Y., Yasukawa, T., Mae, K., 2011. Selective Production of Organic Acids and Depolymerization of Lignin by Hydrothermal Oxidation with Diluted Hydrogen Peroxide. *Energy & Fuels* 25, 791–796. <https://doi.org/10.1021/ef101477d>
- Holladay, J.E., White, J.F., Bozell, J.J., Johnson, D., 2007. Top Value-Added Chemicals from Biomass Volume II — Results of Screening for Potential Candidates from Biorefinery Lignin. Prepared for the U.S. Department of Energy under Contract DE-AC05-76RL01830 II.
- Hu, Z., Du, X., Liu, J., Chang, H., Jameel, H., 2017. Structural Characterization of Pine Kraft Lignin : BioChoice Lignin vs Indulin AT. *J. Wood Chem. Technol.* 36, 432–446. <https://doi.org/10.1080/02773813.2016.1214732>
- Huang, X., Korányi, T.I., Boot, M.D., Hensen, E.J.M., 2015. Ethanol as capping agent and formaldehyde scavenger for efficient depolymerization of lignin to aromatics. *Green Chem.* 17, 4941. <https://doi.org/10.1039/c5gc01120e>

- Huber, G.W., Iborra, S., Corma, A., 2006. Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chem. Rev.* 106, 4044–4098. <https://doi.org/10.1021/cr068360d>
- Ibrahim, M.M., Agblevor, F.A., El-Zawawy, W.K., 2010. Isolation and characterization of cellulose and lignin from steam-exploded lignocellulosic biomass. *BioResources* 5, 397–418. <https://doi.org/10.15376/biores.5.1.397-418>
- Jafri, Y., Wetterlund, E., Mesfun, S., Rådberg, H., Mossberg, J., Hulteberg, C., Furusj, E., 2020. Combining expansion in pulp capacity with production of sustainable biofuels – Techno-economic and greenhouse gas emissions assessment of drop-in fuels from black liquor part-streams 279. <https://doi.org/10.1016/j.apenergy.2020.115879>
- Karlsson, B.C.G., Friedman, R., 2017. Dilution of whisky – the molecular perspective 1–9. <https://doi.org/10.1038/s41598-017-06423-5>
- Katahira, R., Elder, T.J., Beckham, G.T., 2018. Chapter 1: A Brief Introduction to Lignin Structure, in: *Lignin Valorization : Emerging Approaches*. pp. 1–20. <https://doi.org/10.1039/9781788010351-00001>
- Kohlstedt, M., Starck, S., Barton, N., Stolzenberger, J., Selzer, M., Mehlmann, K., Schneider, R., Pleissner, D., Rinkel, J., Dickschat, J.S., Venus, J., B.J.H. van Duuren, J., Wittmann, C., 2018. From lignin to nylon: Cascaded chemical and biochemical conversion using metabolically engineered *Pseudomonas putida*. *Metab. Eng.* 47, 279–293. <https://doi.org/10.1016/j.ymben.2018.03.003>
- Lake, M.A., Blackburn, J.C., 2011. Process for recovering lignin. WO2011037967A2.
- Lazaridis, P.A., Fotopoulos, A.P., Karakoulia, S.A., Triantafyllidis, K.S., 2018. Catalytic fast pyrolysis of kraft lignin with conventional, mesoporous and nanosized ZSM-5 zeolite for the production of alkyl-phenols and aromatics. *Front. Chem.* 6. <https://doi.org/10.3389/fchem.2018.00295>
- Li, F., Wang, X., Yuan, T., Sun, R., 2016. A lignosulfonate-modified graphene hydrogel with ultrahigh adsorption capacity for Pb(II) removal. *J. Mater. Chem. A* 4, 11888–11896. <https://doi.org/10.1039/c6ta03779h>
- Li, J., Henriksson, G., Gellerstedt, G., 2007. Lignin depolymerization/repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour. Technol.* 98, 3061–3068. <https://doi.org/10.1016/j.biortech.2006.10.018>
- Linger, J.G., Vardon, D.R., Guarnieri, M.T., Karp, E.M., Hunsinger, G.B., Franden, M.A., Johnson, C.W., Chupka, G., Strathmann, T.J., Pienkos, P.T., Beckham, G.T., 2014. Lignin valorization through integrated biological funneling and chemical catalysis. *Proc. Natl. Acad. Sci. U. S. A.* 111, 12013–12018. <https://doi.org/10.1073/pnas.1410657111>
- Lora, J.H., 2008. Chapter 10 Industrial commercial lignins: Sources, properties and applications, in: *Monomers, Polymers and Composites from Renewable Resources*. pp. 225–241. <https://doi.org/10.1016/B978-0-08-045316-3.00010-7>
- Loutfi, H., Blackwell, B., Uloth, V., 1991. Lignin recovery from kraft black liquor : preliminary process design. *Tappi J.* 74, 203–210.

- Mahmood, N., Yuan, Z., Schmidt, J., Xu, C., 2013. Production of polyols via direct hydrolysis of kraft lignin: Effect of process parameters. *Bioresour. Technol.* 139, 13–20. <https://doi.org/10.1016/j.biortech.2013.03.199>
- Miller, J.E., Evans, L., Littlewolf, A., Trudell, D.E., 1999. Batch microreactor studies of lignin and lignin model compound depolymerization by bases in alcohol solvents 78, 1363–1366.
- Öhman, F., 2006. Precipitation and separation of lignin from kraft black liquor. Chalmers University of Technology.
- Öhman, F., Wallmo, H., Theliander, H., 2007. A novel method for washing lignin precipitated from kraft black liquor – Laboratory trials. *Filtration* 7, 309–315.
- Pereira, C.G., Féjean, C., Betoulle, S., Ferrando, N., Lugo, R., de Hemptinne, J.C., Mougin, P., 2018. Guaiacol and its mixtures: New data and predictive models part 1: Phase equilibrium. *Fluid Phase Equilib.* 470, 75–90. <https://doi.org/10.1016/j.fluid.2018.01.035>
- Prothmann, J., Sun, M., Spégel, P., Sandahl, M., Turner, C., 2017. Ultra-high-performance supercritical fluid chromatography with quadrupole-time-of-flight mass spectrometry (UHPSFC/QTOF-MS) for analysis of lignin-derived monomeric compounds in processed lignin samples. *Anal. Bioanal. Chem.* 409, 7049–7061. <https://doi.org/10.1007/s00216-017-0663-5>
- Ragauskas, A.J., Beckham, G.T., Biddy, M.J., Chandra, R., Chen, F., Davis, M.F., Davison, B.H., Dixon, R.A., Gilna, P., Keller, M., Langan, P., Naskar, A.K., Saddler, J.N., Tschaplinski, T.J., Tuskan, G.A., Wyman, C.E., 2014. Lignin valorization: improving lignin processing in the biorefinery. *Science (80-. )*. 344, 1246843. <https://doi.org/10.1126/science.1246843>
- Ragauskas, A.J., Williams, C.K., Davison, B.H., Britovsek, G., Cairney, J., Eckert, C.A., Frederick, W.J., Hallett, J.P., Leak, D.J., Liotta, C.L., Mielenz, J.R., Murphy, R., Templer, R., Tschaplinski, T., 2006. The path forward for biofuels and biomaterials. *Science (80-. )*. 311, 484–489. <https://doi.org/10.1126/science.1114736>
- Ralph, J., Lapierre, C., Boerjan, W., 2019. Lignin structure and its engineering. *Curr. Opin. Biotechnol.* 56, 240–249. <https://doi.org/10.1016/j.copbio.2019.02.019>
- Ravi, K., García-Hidalgo, J., Gorwa-Grauslund, M.F., Lidén, G., 2017. Conversion of lignin model compounds by *Pseudomonas putida* KT2440 and isolates from compost. *Appl. Microbiol. Biotechnol.* 101, 5059–5070. <https://doi.org/10.1007/s00253-017-8211-y>
- Rinaldi, R., Jastrzebski, R., Clough, M.T., Ralph, J., Kennema, M., Bruijninx, P.C.A., Weckhuysen, B.M., 2016. Paving the Way for Lignin Valorisation: Recent Advances in Bioengineering, Biorefining and Catalysis. *Angew. Chemie - Int. Ed.* 55, 8164–8215. <https://doi.org/10.1002/anie.201510351>
- Rößiger, B., Röver, R., Unkelbach, G., Pufky-Heinrich, D., 2017. Production of Bio-Phenols for Industrial Application: Scale-Up of the Base-Catalyzed Depolymerization of Lignin. *Green Sustain. Chem.* 07, 193–202. <https://doi.org/10.4236/gsc.2017.73015>



- Rößiger, B., Unkelbach, G., Pufky-Heinrich, D., 2018. Chapter 4 Base-Catalyzed Depolymerization of Lignin: History, Challenges and Perspectives, in: *Lignin-Trends and Applications*. <https://doi.org/10.5772/intechopen.72964>
- Rubin, E.M., 2008. Genomics of cellulosic biofuels. *Nature* 454, 841–845. <https://doi.org/10.1038/nature07190>
- Schmiedl, D., Endisch, S., Pindel, E., Rückert, D., Reinhardt, S., Schweppe, R., Unkelbach, G., 2012. Base catalyzed degradation of Lignin for the generation of oxy-aromatic compounds - Possibilities and challenges. *Erdol Erdgas Kohle incl Oil Gas Eur. Mag.* 128, 357–363.
- Sewring, T., Durruty, J., Schneider, L., Schneider, H., Mattsson, T., Theliander, H., 2019. Acid Precipitation of Kraft Lignin from Aqueous Solutions: The Influence of pH, Temperature, and Xylan. *J. Wood Chem. Technol.* 39, 1–13. <https://doi.org/10.1080/02773813.2018.1488870>
- Shimada, K., Hosoya, S., Ikeda, T., 1997. Condensation Reactions of Softwood and Hardwood Lignin Model Compounds Under Organic Acid Cooking Conditions. *J. Wood Chem. Technol.* 17, 57–72. <https://doi.org/10.1080/02773819708003118>
- Shinoda, E., Takahashi, K., Abe, N., Kamimura, N., Sonoki, T., Masai, E., 2019. Isolation of a novel platform bacterium for lignin valorization and its application in glucose-free cis,cis-muconate production. *J. Ind. Microbiol. Biotechnol.* 46, 1071–1080. <https://doi.org/10.1007/s10295-019-02190-6>
- Shuai, L., Saha, B., 2017. Towards high-yield lignin monomer production. *Green Chem.* 19, 3752–3758. <https://doi.org/10.1039/c7gc01676j>
- Sjöström, E., 1993. Chapter 7 Wood Pulping, in: *Wood Chemistry*. pp. 114–164. <https://doi.org/10.1016/b978-0-08-092589-9.50011-5>
- Stärk, K., Taccardi, N., Bösmann, A., Wasserscheid, P., 2010. Oxidative Depolymerization of Lignin in Ionic Liquids. *ChemSusChem* 719–723.
- Sulaeva, I., Zinoviyev, G., Plankeele, J., Sumerskii, I., 2017. Fast Track to Molar-Mass Distributions of Technical Lignins 629–635. <https://doi.org/10.1002/cssc.201601517>
- Thuvander, J., Lipnizki, F., Jönsson, A.S., 2019. On-Site Recovery of Hemicelluloses from Thermomechanical Pulp Mill Process Water by Microfiltration and Ultrafiltration. *J. Wood Chem. Technol.* 39, 214–223. <https://doi.org/10.1080/02773813.2019.1565865>
- Tiseo, I., 2021. Global paper industry - statistics & facts [WWW Document]. URL <https://www.statista.com/topics/1701/paper-industry/>
- Toledano, A., Serrano, L., Labidi, J., 2012. Organosolv lignin depolymerization with different base catalysts. *J. Chem. Technol. Biotechnol.* 87, 1593–1599. <https://doi.org/10.1002/jctb.3799>
- Tomani, P., 2010. THE LIGNOBOOST PROCESS. *Cellul. Chem. Technol.* 44, 53–58.
- Triantafyllidis, K., Lappas, A., Stöcker, M., 2013. Chapter 9 Catalytic Depolymerization and Deoxygenation of Lignin, in: *The Role of Catalysis for the Sustainable Production of Bio-Fuels and Bio-Chemicals*.

- Tumen-Velasquez, M., Johnson, C.W., Ahmed, A., Dominick, G., Fulk, E.M., Khanna, P., Lee, S.A., Schmidt, A.L., Linger, J.G., Eiteman, M.A., Beckham, G.T., Neidle, E.L., 2018. Accelerating pathway evolution by increasing the gene dosage of chromosomal segments. *Proc. Natl. Acad. Sci. U. S. A.* 115, 7105–7110. <https://doi.org/10.1073/pnas.1803745115>
- Vardon, D.R., Rorrer, N.A., Salvachúa, D., Settle, A.E., Johnson, C.W., Menart, M.J., Cleveland, N.S., Ciesielski, P.N., Steirer, K.X., Dorgan, J.R., Beckham, G.T., 2016. Cis, cis -Muconic acid: Separation and catalysis to bio-adipic acid for nylon-6,6 polymerization. *Green Chem.* 18, 3397–3413. <https://doi.org/10.1039/c5gc02844b>
- Velez, J., Thies, M.C., 2013. Solvated liquid-lignin fractions from a Kraft black liquor. *Bioresour. Technol.* 148, 586–590. <https://doi.org/10.1016/j.biortech.2013.08.097>. Epub 2013 Aug 29. PMID: 24054066.
- Vigneault, A., Johnson, D.K., Chornet, E., 2007. Base-catalyzed depolymerization of lignin: Separation of monomers. *Can. J. Chem. Eng.* 85, 906–916. <https://doi.org/10.1002/cjce.5450850612>
- Wallberg, O., 2005. Extraction of lignin from kraft cooking liquor by ultrafiltration. Lund University.
- Wallberg, O., Jönsson, A.S., 2003. Influence of the membrane cut-off during ultrafiltration of kraft black liquor with ceramic membranes. *Chem. Eng. Res. Des.* 81, 1379–1384. <https://doi.org/10.1205/026387603771339591>
- Wallberg, O., Jönsson, A.S., Wimmerstedt, R., 2003. Fractionation and concentration of kraft black liquor lignin with ultrafiltration. *Desalination* 154, 187–199. [https://doi.org/10.1016/S0011-9164\(03\)80019-X](https://doi.org/10.1016/S0011-9164(03)80019-X)
- Wallmo, H., Theliander, H., Jönsson, A.S., Wallberg, O., Lindgren, K., 2009. The influence of hemicelluloses during the precipitation of lignin in kraft black liquor. *Nord. Pulp Pap. Res. J.* 24, 165–171.
- Wang, H., Tucker, M., Ji, Y., 2013. Recent Development in Chemical Depolymerization of Lignin: A Review. *J. Appl. Chem.* 2013, 1–9. <https://doi.org/10.1155/2013/838645>
- Welker, C.M., Balasubramanian, V.K., Petti, C., Rai, K.M., De Bolt, S., Mendu, V., 2015. Engineering plant biomass lignin content and composition for biofuels and bioproducts. *Energies* 8, 7654–7676. <https://doi.org/10.3390/en8087654>
- Wexler, A., 1976. Vapor pressure equation for water in the range 0 to 100 C. *J. Res. Natl. Bur. Stand. Sect. A Phys. Chem.* 80A, 775–785. <https://doi.org/10.6028/jres.075a.022>
- Wiinikka, H., Carlsson, P., Granberg, F., Löfström, J., Marklund, M., Tegman, R., Lindblom, M., Gebart, R., 2010. Design and methodology of a high temperature gas sampling system for pressurized black liquor gasification. *Fuel* 89, 2583–2591. <https://doi.org/10.1016/j.fuel.2010.02.021>
- Zakzeski, J., Bruijninx, P.C.A., Jongerius, A.L., Weckhuysen, B.M., 2010. The catalytic valorization of lignin for the production of renewable chemicals. *Chem. Rev.* 110, 3552–3599. <https://doi.org/10.1021/cr900354u>

- Zevallos Torres, L.A., Lorenci Woiciechowski, A., de Andrade Tanobe, V.O., Karp, S.G., Guimarães Lorenci, L.C., Faulds, C., Soccol, C.R., 2020. Lignin as a potential source of high-added value compounds: A review. *J. Clean. Prod.* 263. <https://doi.org/10.1016/j.jclepro.2020.121499>
- Zhou, C.H., Xia, X., Lin, C.X., Tong, D.S., Beltramini, J., 2011. Catalytic conversion of lignocellulosic biomass to fine chemicals and fuels. *Chem. Soc. Rev.* 40, 5588–5617. <https://doi.org/10.1039/c1cs15124j>
- Zhou, H., Wang, H., Perras, F.A., Naik, P., Pruski, M., Sadow, A.D., Slowing, I.I., 2020. Two-step conversion of Kraft lignin to nylon precursors under mild conditions. *Green Chem.* 22, 4676–4682. <https://doi.org/10.1039/d0gc01220c>
- Zhu, W., 2015. Precipitation of Kraft Lignin Yield and Equilibrium. Chalmers University of Technology.