Alkaline wet oxidation of biorefinery lignin from wheat straw

by

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Preface

This master thesis has been conducted at the department of Chemical Engineering at Lund University during the academic year 2020/2021. Many people have helped me during the time I have been working with this, so I would like to express by gratitude to them all.

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Abstract

Climate change and depletion of fossil resources have generated a need for energy production based on renewable resources such as lignocellulosic biomass. Agricultural residues, like wheat straw, is lignocellulosic biomass that could contribute to the energy supply if it was used for biogas production. One issue with biogas production from lignocellulosic materials is, however, the high content of recalcitrant lignin. The inhibiting lignin fraction can, nevertheless, become more easily degraded if it undergoes pretreatment. A well-known industrial process to degrade lignin is alkaline wet oxidation, which is examined in this study with biorefinery lignin from wheat straw as raw material. The effect of temperature, dry matter (DM) content, reaction time and NaOH amount is examined, with the objective to contribute to the development of lignin pretreatment methods for subsequent biogas production.

The optimal reaction conditions using 10 bar O_2 were found at 180˚C, 6.5% DM content, 23 minutes reaction time and 9.8 wt% NaOH. The total yield of the identified phenolic compounds, including vanillin, vanillic acid and guaiacol, was 0.197%. The approximate yield of all organic acids was 35.5%, including formic acid, acetic acid and seven other unidentified compounds. pH stability is a critical factor in the outcome of the oxidation and is very influenced by the relation of DM content and NaOH loading.

In this study, it has been shown that alkaline wet oxidation can be applied to degrade wheat straw lignin, although many improvements should be made in the experimental procedure. In future studies, alternative wet oxidation processes with more benefits for subsequent biogas production could, however, be examined.

Sammanfattning

Klimatförändringar och överkonsumtion av fossila naturresurser har skapat ett behov av en energiproduktion som baseras på förnybara energikällor, däribland biomassa innehållandes lignocellulosa. Restavfall från jordbruket, som vetehalm, är material innehållandes lignocellulosa som kan bidra till energitillförseln om de används till biogasproduktion. Ett problem med biogasproduktion från sådana material är emellertid den höga halten av svårnedbrytbart lignin. Den inhiberande ligninfraktionen kan dock göras mer nedbrytbar med lämplig förbehandling. En välbeprövad industriell process för att bryta ned lignin är alkalisk våtoxidation, vilket undersöks i denna studie med vetehalmslignin utvunnet från bioraffinaderiprocesser som råmaterial. Effekten av temperatur, torrhalt, reaktionstid samt mängd NaOH undersöks, med målet att bidra till utvecklingen av metoder för förbehandling av lignin för efterföljande biogasproduktion.

De optimala reaktionsförhållandena med 10 bar O_2 hittades vid 180˚C, 6.5% torrhalt, 23 minuter reaktionstid och 9.8% NaOH. Det totala utbytet av de identifierade fenoliska ämnena, innefattandes vanillin, vanillinsyra samt guajakol, var 0.197%. Det approximativa utbytet av alla organiska syror var 35.5%, där inräknat myrsyra, ättiksyra samt sju andra ämnen som inte kunde identifieras. Stabiliteten av pH är en kritisk faktor för utfallet av oxideringen och påverkas starkt av förhållandet mellan torrhalt och mängd NaOH.

I denna studie har det visats att alkalisk våtoxidation kan appliceras för att bryta ned vetehalmslignin, även om många förbättringar behöver implementeras i den laborativa proceduren. För framtida studier kan alternativa våtoxidationsmetoder, med fler fördelar för efterföljande biogasproduktion, undersökas.

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1 Introduction

1.1 Project description

This study has been conducted as a part of the project Lignogas at the department of Chemical Engineering, Lund University. The aim of the Lignogas project is to develop methods to use lignin from residual streams in biorefineries and to convert it to methane through anaerobic digestion, a conversion that also is known as biogas production. The residual lignin streams, commonly referred to as biorefinery lignin, of interest in this project are black liquor and the undigested fraction from anaerobic digestion of lignocellulosic biomass. The general concept is that lignin is recovered from the residual stream, transformed to water-soluble derivatives, and then (re-)introduced to anaerobic digestion. It is, thus, a means to valorize a non-degraded, residual fraction from lignocellulosic biomass, which can improve the overall energy output and economy of biorefinery processes.

This master thesis is focused on the step where the lignin fraction is transformed to water-soluble compounds. More specifically, alkaline wet oxidation is applied as a method to degrade the recalcitrant lignin to smaller and more water-soluble compounds, also referred to as oxidation products. Wheat straw is used as material, but it is exposed to different pretreatments to increase its similarity to the residual lignin fraction that would be obtained after anaerobic digestion. This study contributes to the Lignogas project by increasing the general understanding for how a lignin-rich residue responds to degradation through alkaline wet oxidation.

1.2 Aim

The overall objective of this study is to examine an oxidation method of lignin from wheat straw and to identify the most favourable conditions for a high yield of oxidation products. The research questions are as follows:

1. *How can an experimental procedure, with appropriate analytical methods, for alkaline wet oxidation of biorefinery lignin be constructed?*

2. *What products are formed during alkaline wet oxidation of biorefinery lignin from wheat straw?*

3. *How is the outcome of alkaline wet oxidation affected by varying the parameters temperature, dry matter (DM) content, reaction time and amount of NaOH?*

4. *What reaction conditions give the highest yield of oxidation products?*

1.3 Scope

The scope of this study is to examine the outcomes of alkaline wet oxidation at reaction conditions within predefined ranges. The different outcomes of the oxidation experiments are analysed in several ways, from quantitative measures and response-surface modelling to qualitative observations. Only stable systems are, however, examined in more detail. The composition and characteristics of the untreated material is also analysed, whenever applicable. Since not many trials of alkaline wet oxidation have been conducted before this study, the applied experimental procedure is also evaluated and improvements for future studies are suggested.

This master thesis can be seen as an initial study of alkaline oxidation to convert lignin to water-soluble compounds. As a result, not all formed compounds are yet known. Due to time limitations, the identification of all oxidation products is not included in this study. Furthermore, the potential to produce biogas from the oxidized materials is not evaluated either.

1.4 Disposition

This master thesis is organized in five chapters. In the first chapter, an introduction to the project and the research questions are presented. In the second chapter, a background for the studied topic is given. A general introduction to energy production and lignocellulosic biomass is first presented, then lignin and alkaline oxidation in particular are described in more detail. In the third chapter, the methodology used in this study is specified, followed by a presentation and analysis of the outcomes of the study in the fourth chapter. Lastly, the answers to the research questions are summarized in the fifth and concluding chapter.

2 Background

In this chapter, a background to the studied topic is provided. In the first part, the context of energy production from biomass is briefly presented. Then, in the second part, a general description is provided of what lignocellulosic biomass is and why wheat straw, in particular, is an interesting feedstock. In the third part, a more in-depth description of lignin is given, so that the subsequent, and fourth, part about lignin degradation through oxidation can be more easily understood. In the last, and fifth, part, the changes that can arise when going from raw wheat straw to biorefinery lignin are outlined.

2.1 Energy production from biomass

The ongoing climate change, along with the depletion of fossil resources, have created an urgent need to reduce the greenhouse gas emissions and to transition to an energy production based on renewable resources.[1] Biomass is both renewable and is seen as the fourth largest energy source in the world after oil, coal, and natural gas.[2] However, one of the obstacles with using biomass as a feedstock for energy production is that it often competes with food production.[3] Beside being a concern with ethical dimensions, the competition to food production can also induce an indirect land use change (iLUC). Essentially, iLUC implies that food production might need to move somewhere else when arable land is used for cultivating biomass meant for energy production. As more agricultural land has to be created, on the expense of forests and other natural ecosystems, a significant increase of green house gas emissions can arise.[4]

During the past decade, focus has thus shifted from using food crops for the production of energy to the use of waste and residual materials instead.[3] Lignocellulosic biomass, such as agricultural residues or forestry waste, and energy crops are of special interest in the context of energy production.[2] The very nature of lignocellulosic biomass makes it inedible for humans, therefore there is no direct competition between food and energy production when using that type of materials.[5] The more the market for value-added products from agricultural residues grows, however, the more likely is it indirect land use changes eventually arise. Nonetheless, agricultural residues and straw in particular can for the time being be considered as low iLUC-risk feedstock.[6-7]

2.1.1 Energy carriers derived from biomass

Biomass can be converted to a variety of energy carriers in all different aggregation states. The final products, the energy carriers, can for instance be solid biocoal, liquid bioethanol, gaseous biogas or electricity from combustion.[1, 8] A number of different processes exists for achieving the energy carrier of interest, including thermochemical processing, physical-chemical transformation, biochemical transformation and combustion. Choosing the most appropriate process depends on the properties of the specific biomass to be processed, such as its content of water or problematic trace elements.[8] Deublein and Steinhauser (2011) present an overview of the energy balance for some biomass-based energy carriers. Biogas stands out compared to ethanol, electricity and heat due to its high energy output in relation to the energy input required to produce it.[8] That is a promising indication, as lignocellulosic biomass often requires different pretreatment steps[9], thereby lowering the overall ratio of energy output over energy input.

2.1.2 Biogas as an advantageous energy carrier

Biogas is a mixture of methane and carbon dioxide, but where methane is the energy carrier sought for. It is naturally produced in multiple steps by microorganisms in an oxygen-free environment, a process known as anaerobic digestion.[8] In the first step of the anaerobic digestion – the hydrolysis –

complex molecules such as carbohydrates, proteins and lipids are broken down by enzymes to sugar-, amino acid- or fatty acid monomers. Subsequently, these monomers are utilized by microbes in a fermentation process – the acidogenesis. In the third step – the acetogenesis – the intermediate products from the fermentation are converted to acetate, hydrogen gas and carbon dioxide. In the last step – the methanogenesis – microbes transform the products from the acetogenesis to methane and carbon dioxide.[9] In nature, this occurs for instance in swamps, flooded rice fields and through the digestion system of ruminants.[8]

There are several uses of biogas. It can be used as an energy source to generate electricity and heat, as fuel for vehicles or be fed in the natural gas grid.[8] The biogas must, however, be upgraded before being used in vehicles or being fed in the natural gas grid. The upgrading is basically a purification process, where corrosive substances, particles and water are removed. The carbon dioxide must also be removed to increase the energy value of the gas.[10]

2.2 Lignocellulosic biomass – an overview

Since lignocellulosic biomass is deemed as a good feedstock for energy production, a brief description of its main components is given. There are, however, challenges associated to the use of lignocellulosic materials. These challenges are outlined, and anaerobic digestion is highlighted as a solution with much potential to handle the most problematic component in the lignocellulosic material – the lignin. Lastly, wheat straw is presented as a particularly interesting lignocellulosic feedstock for energy production through anaerobic digestion.

2.2.1 Main components of lignocellulosic material

The main constituents of lignocellulosic biomass are cellulose, hemicellulose and lignin. Cellulose consists of chains of glucose, resulting in a stable and tightly packed polymer structure. These cellulosic chains are held together by van der Waals forces and hydrogen bonds but contain both crystalline and amorphous areas. The more disorganized amorphous fractions are more easily degraded by enzymes than the crystalline parts, as the former fractions are less densely packed. The crystalline fractions are, however, the reason to why cellulose withstand thermal degradation to a greater extent than hemicellulose.[9]

Hemicellulose consists of a variety of different sugar units, which makes it a much more disorganized polysaccharide compared to cellulose. The sugars that are present in hemicellulose are pentoses (five-carbon sugars), hexoses (six-carbon sugars), deoxyhexoses and hexuronic acid. Xylose is said to be the most common type of sugar present.[9] However, the exact composition of hemicellulose does not only vary between different types of plants, but also within different parts of the plant. As previously mentioned, the variety of sugars gives a variety of bonds between the different sugars units and thus give hemicellulose an amorphous structure.[11] Because of this, its structure is relatively easily degraded by addition of enzymes or dilute acids and bases.[9] Hemicellulose connects cellulose to lignin as it interacts with cellulose through hydrogen bonds and binds with covalent ether- or ester-type of linkages to lignin.[11]

Lignin has a very complex structure compared to cellulose and hemicellulose.[12] It is a three-dimensional polymer, consisting of aromatic units that are linked together randomly.[9] In fact, lignin is the largest renewable source of aromatic compounds found in nature.[9, 13] In plants, it serves as a glue and structural support by filling the emptiness between cellulose and hemicellulose.[14] Lignin also provides vascular channels in the plant where water and nutrients can be transported, due to its hydrophobic nature.[9] Since the lignin is strongly cross-linked with cellulose[9], it also protects the more easily degraded fractions in the material against microbial and chemical attack.[12]

The chemical structure of lignin varies greatly between different types of biomasses. Even within the same kind of plant species there can be a significant variation in the lignin structure. The formation of the lignin is greatly affected by the conditions in which the plant has been growing, such as the climate, the access to nutrition and illumination.[12] A more in-depth description of the structure and characteristics of lignin is given in section 2.3.

2.2.2 Challenges with lignocellulosic materials in biorefineries and the role of lignin

In a biorefinery context, it is primarily sugars from the cellulose and hemicellulose fractions that are exploited for production of biofuels or other biobased products through microbial or chemical transformation. However, in lignocellulosic biomass, all the components are intertwined in a so-called lignin-carbohydrate complex. This complex has a somewhat different structure in herbaceous plants, further described in section 2.3.3. Regardless of the exact structure of this lignin-carbohydrate complex, it is the main obstacle for an efficient utilization of lignocellulosic biomass as the resistant lignin protects the easily degradable carbohydrates.[9] In fact, the more lignin there is in a lignocellulosic material, the larger is the resistance towards degradation of that material.[15] Pretreatment steps are required to degrade the lignin and make the more desirable cellulose exposed for further processing. Most often, the lignin is removed and then left as a waste stream.[9] This residual lignin stream from different kinds of biorefineries is commonly referred to as biorefinery lignin.

The pretreatment of lignocellulosic materials has a very large influence on the economy of biorefineries. In fact, the pretreatment of lignocellulosic biomass represents the highest operational cost in biofuel production, which contributes to a costlier production of biofuel compared to fossil fuel. Therefore, it can be useful to make use of the entire lignocellulosic material to improve the economics of the biorefinery. Valorization of biorefinery lignin, otherwise being the residues of a biorefinery process, can be an important step to make use of the whole lignocellulosic material and to obtain an additional product stream.[9]

2.2.3 Why anaerobic digestion of biorefinery lignin is advantageous

Usually, the residual lignin is combusted for production of heat or power.[5] In the paper and pulp industries, the leftover lignin can be used for steam production.[9] Beside these applications, lignin can also be used for production of other low-value products such as binders or dispersants. The conversion of lignin to bio-oil and other chemicals through pyrolysis has also been extensively studied, but the yields of that process are low and the aftertreatment is both expensive and energy intensive. In contrast, anaerobic digestion of lignin is both cheap and provides much higher conversion rates. The obtained biogas can, for instance, be used as an energy source to operate the biorefinery or be used as raw material for hydrogen gas production.[16] However, some issues need to be resolved for a reliable biogas production from lignin. For instance, the amount of lignin in a material decreases the amount of methane that can be produced through anaerobic digestion[17], due to the natural resistance of lignin towards microbial degradation.[9] When more lignocellulosic biomass is solubilized, including its lignin fraction, the higher does the methane production rate become, though.[17] This is why the development of appropriate pretreament methods of lignocellulosic biomass in general, and of biorefinery lignin in particular, is an important step to make use of the available materials and, consequently, obtain more profitable biorefineries. Some pretreatment methods for valorization of lignin are presented in section 2.4.

2.2.4 Wheat straw – a lignocellulosic feedstock with great potential

Lignocellulosic biomass is a very broad classification and includes many different types of materials. It can be hard to draw any specific conclusions due to the heterogeneity of biomass and that different materials usually require different types of handling. Therefore, it is crucial to narrow down to a specific type of lignocellulosic material in order to evaluate its potential.

One of the main agricultural residues in the world is wheat straw.[18] When wheat is harvested, the grain is separated from all the other parts of the plant and further processed for food or animal feed applications, thereby leaving the straw and impurities as residual waste.[19] The straw accounts for approximately half of the total wheat biomass produced.[20] If wheat straw is not used as animal feed or returned to the soil as natural fertilizer, it is either burnt or just left unused. In the latter cases, none of its inherent energy potential is thus recovered.[1, 5, 8, 14] In fact, straw is listed in the EU directive 2018/2001 among feedstocks that are encouraged to be used for production of biogas and advanced biofuels.[21]

Approximately 2.9 million tons of wheat straw is produced in Sweden each year. If around 1.6 million ton of that was used for biogas production, it could provide 5.8 TWh.[20] In 2019, the domestic production of biogas in Sweden corresponded to 2.1 TWh. Including imported biogas, the total amount that was used in the same year has been estimated to 4 TWh.[10] These numbers show that even if only wheat straw would be used for biogas production, then it would still meet the current demand for biogas in Sweden by far.

The major obstacle for digesting wheat straw anaerobically is its lignocellulosic structure, as briefly described in section 2.2.3.[22] The exact content of cellulose, hemicellulose and lignin varies depending on the origins of the wheat straw, but some general conclusions can still be drawn. From the values in Table 2.1, it can be concluded that the fraction of lignin and hemicellulose each correspond to approximately one fifth of the dry matter in wheat straw. Moreover, the amount of cellulose roughly corresponds to the amount of hemicellulose and lignin combined. In general, the ash content in wheat straw does not seem to exceed 8%.

Table 2.1: Chemical composition of wheat straw on a dry matter basis (%).

Lignin	Cellulose	Hemicellulose	Ash	Country	Reference
5-17	32-49	27-39	1.8-6	China, Greece, UK, USA	[23]
18-24	37	22-28	1-6	Canada	[24]
22[a)], 18[b)]	35	22	7.5	Spain	[1]
20	34-40	20-25	-	-	[14]

a) Total lignin. b) Acid insoluble lignin

Even though wheat straw has a lower lignin content compared to other biomass feedstocks, as for instance corn stover[23], the lignin-carbohydrate complex reduces the availability to the more easily degraded carbohydrates for enzymatic and microbial transformation[22]. In fact, a higher energy output is achieved when wheat straw is combusted instead of anaerobically digested for biogas production. The drawbacks with combustion are, however, that problematic fly ash and boiler corrosion arise due to the high content of potassium chloride in the straw. Furthermore, combustion does not make it possible to recycle nutrients and use them as soil fertilizer. This recycling is possible with the digested material after biogas production. Anaerobic digestion of wheat straw is also more energetically efficient than only using the material for bioethanol production. A combination of both ethanol and biogas production can, however, be energetically favourable.[25]

2.3 Lignin

Only a brief introduction to lignin was given in the previous section. This section will, hence, provide a deeper description of this component. After some more general characteristics of lignin, its structure and main building blocks are presented. A short description of lignin in wheat straw is then provided, followed by methods to break down the complex lignin structure. At last, some characteristics of lignin when being exposed to ultraviolet (UV) measurements are outlined.

2.3.1 General characteristics

As mentioned in section 2.2.1, lignin is a heterogenous and very complex component of lignocellulosic biomass[12] It is insoluble in water and have a high stability in nature[26], as it is an amorphous polymer consisting of aromatic units.[9] Nonetheless, lignin can be degraded by white-rot fungi through the action of their oxidative enzymes. Otherwise, biological degradation of unmodified lignin (so-called native lignin) require oxygen.[9] The two types of lignin present in lignocellulosic materials are acid soluble lignin (ASL) and acid insoluble lignin (AIL). The latter is, however, the most predominant type in lignocellulosic biomass. [9] The AIL has a higher molecular weight, usually being above 850kD.[16]

2.3.2 Structure and interunit linkages

Lignin mainly consists of the elements carbon, hydrogen and oxygen. These elements are combined in three different phenylpropane units, also known as monolignols.[12] The monolignols are formed by a metabolic pathway in plants where water is irreversibly removed from sugars to form aromatic structures.[19] Another way to call these monolignols is methoxylated hydroxycinnamyl alcohols.[11] The monolignols serve as precursors when the lignin polymer is synthesized in nature, meaning that they are the components that become the main building blocks of lignin. These precursors are *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. In the final lignin polymer, these monolignols correspond to the structural units *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), respectively.[26]

The positions of carbon atoms on the propane side chain in the monolignols are labeled with Greek letters, starting with α for the carbon closest to the aromatic structure. The aromatic ring, on the other hand, is numbered from 1 to 6. Position 1 is assigned to the carbon being connected to the propane side chain, whereas the carbon with the -OH group is assigned number 4.[27]

The difference between the structural units in the lignin is the number of methoxy ($-OCH_3$) groups. The H unit is the most condensed unit as it only contains a hydroxy (-OH) group. Apart from that hydroxy group, the G unit also contain an methoxy group, whereas the S unit contains two additional methoxy groups.[9] Since the H unit completely lacks methoxy groups, it has more reactive sites and less steric hindrance than G and S units.[28-29] The overall lignin structure is, however, more easily degraded when it has a higher degree of methoxylation, meaning a more S-like structure. This is due to an interunit linkage that is more easily cleaved for S units compared to H and G units.[9] Regarding the biodegradability of lignin, many anaerobic microbes also use the methoxy groups as their main point of attack.[9]

The main interunit linkages in lignin connect carbon with oxygen (C-O bonds, or ether bonds) or carbon with carbon (C-C bonds). Some C-O bonds are the β-O-4, α-O-4 and 4-O-5 linkages, whereas some C-C bonds are the 5-5, β-5, β-β and β-1 linkages. The β-O-4 linkage is the most common bond found in lignin[11, 30], which is why it can be used as an indicator to evaluate the lignin structure.[2] In fact, the reactivity of lignin is determined by its interunit linkages [5], where the β-O-4 linkage is the interunit bond that is most easily cleaved.[9] C-C linkages have high bond dissociation energy and are, therefore, more resistant towards cleavage.[11]

When the positions involved in the β-O-4 linkage are evaluated, it becomes clear why it is the most common bond that connects lignin units together. Common for all structural units in lignin is that the carbon at the β position has a double bond to the adjacent carbon. A double bond is weaker than a single bond, thus making the carbon on that position more prone to interact and be reactive.[31] Furthermore, phenols are known to be acidic[31], meaning that the OH-group at position 4 in the aromatic structure tends to deprotonate. The same tendency should be present in the structural units, although they have an additional aliphatic chain attached to the phenolic structure. With reactive parts both at the β and the 4 positions, it is, therefore, reasonable that the most common linkage found in the lignin units also arise at those positions.

However, even if the interunit linkages have been cleaved, the structural units in lignin can repolymerize. This means that the lignin units that were separated, once again connect to each other through a condensation reaction. Repolymerization is problematic when the goal is to degrade and depolymerize the lignin as much as possible. While mainly the labile β-O-4 linkage is cleaved during degradation of lignin, more stable C-C bonds arise during repolymerization. The C-C bonds are much harder to cleave, which results in a lignin structure that is significantly more difficult to degrade.[32] It has, nevertheless, been shown that more methoxylated lignin, having a more S-like structure, is less prone to repolymerize. In addition, a certain type of pretreatment can transform H units to S units (described in section 2.4.3.2).[29] An S-like structure is, thus, beneficial both for cleaving interunit linkages between lignin structural units, to maintain them separate and for improving the subsequent biodegradation.

2.3.3 Wheat straw lignin in particular

The amounts of H, G and S units vary with type of biomass. S and G units are predominant in lignins from wood, whereas the lignins in herbaceous plants consist of all three units. What concerns wheat straw, its lignin consists of 5% H units, 49% G unit and 46% S units. Hence, the ratio of S and G units is roughly 1, meaning that the amount of those units are roughly the same.[23] An average structural unit in wheat straw lignin has the element composition $C_9H_{7.39}O_{3.00}(OCH_3)_{1.07}$. Compared to the element composition of an average unit in rice straw lignin, the wheat straw lignin has a slightly higher degree of methoxylation but lower content of hydrogen and oxygen.[12]

As mentioned in section 2.2.2, the lignin-carbohydrate complex has a somewhat different structure in herbaceous plants. In fact, in those types of plants it is rather a "lignin/phenolics-carbohydrate" complex. In these complexes, the lignin and the carbohydrates are not connected directly with each other. In stead, there is a phenolic compound in between the lignin and the carbohydrate, acting as a bridge between those two. In general, the phenolic compound links to the lignin through an ether bond (R-O-R') and through an ester bond (R-O-O-R') to the carbohydrate. In wheat straw, *p*-coumaric acid and ferulic acid have been identified as the phenolic compounds that connect the lignin with the carbohydrate. The lignin units are linked to *p*-coumaric acids mainly through ester bonds. Ferulic acid, on the hand, is linked to the lignin through ether bonds but to the carbohydrate units through ester bonds. The ester bonds are cleaved when the alkalinity increases, whereas the ether bonds are cleaved by acids.[23]

2.3.4 Methods to depolymerize lignin

To make use of H, G and S units present in lignin, the very complex lignin polymer first needs to be depolymerized. Some common thermochemical methods to break down the structure of lignin to its monomer units include pyrolysis, hydroprocessing, acid- or base catalyzed depolymerization and oxidation. Pyrolysis is rapid heating in the absence of oxygen and gives a mixture of liquid oil, gases and solid (mostly char).[33] Hydroprocessing, on the other hand, involves hydrogen gas to depolymerize

lignin to aromatic compounds such as phenols, benzene or toluene. In acid- or base catalyzed depolymerization, the C-O and C-C linkages between the lignin units are cleaved. Since the catalyzed depolymerization provides monomeric phenols and other smaller lignin fragments, these methods are often combined with hydroprocessing or oxidation processes. Lastly, oxidation is a process at lower temperatures where aromatic alcohols, aldehydes and acids can be obtained as oxygen becomes assimilated to the structural units of lignin.[5] In addition to these thermochemical methods, biodegradation of lignin for the production of valuable compounds has lately also gained more interest. It involves enzymes that fragment the lignin into smaller or larger pieces.[9] Even though biodegradation is a promising method to depolymerize lignin, it is a high-cost technology that still needs further development for being applicable to lignocellulosic residues.[34]

Among the depolymerization methods mentioned, oxidation have some clear advantages. For instance, pyrolysis is very unspecific and thus gives a multitude of different components that have no value without further separation and purification steps.[2] Hydroprocessing usually gives high yield of phenolic compounds, but the main drawbacks are high operational costs and harsh conditions that can saturate the aromatic compounds.[2, 35] In contrast, oxidation can be controlled so that a high selectivity of desired compounds is obtained.[2] The oxidation can be performed at low cost with oxygen, which also is an abundant and environmentally friendly compound.[33] In addition, oxidation processes can generally be performed at lower temperatures compared to other depolymerization methods.[5]

2.3.5 Characteristics of lignin in UV spectrophotometry

Lignin can be monitored through UV spectrophotometry due to its high content of conjugated structures. Several absorption maxima can be observed due to the variety of functional groups present. The maximum absorbance differs, however, depending on the degradation method used to obtain the lignin. In addition, the medium in which the UV measurements are performed also have an impact on the result. A method to determine phenolic hydroxy groups in lignin is to compare the absorption of phenolic units in neutral and alkaline solutions at the wavelengths 292 and 370 nm.[36]

Maziero et al. (2011) used UV absorption at 280 nm as a measure of lignin degradation through alkaline oxidation. 280 nm was applied since the maximum absorbance had been obtained at that wavelength. The absorbance peak at 280 nm is due to phenolic groups without conjugated groups on the sidechains and is characteristic for lignin with mostly G units.[37] In addition, Lu et al. (2017) highlight that absorption maxima at 282 nm is due to conjugated bonds/aromatic rings.[36]

Another important aspect, regarding UV spectrophotometry and lignin, is time. Maziero et al. (2011) showed that compounds that absorbed at 340 nm were especially unstable over time. Absorbance at 340 nm was present in lignin that had been oxidized at pH 12.5 with 12.1% H_2O_2 (m/v). The peak that had been detected around 340 nm decreased after 2 months, and was reduced even more after 4 months.[37]

2.4 Lignin depolymerization through oxidation

Lignin oxidation is an advantageous method to degrade the complex lignin structure. There are, however, different methods to perform the oxidation, which will be briefly presented in this section. Since alkaline oxidation is highlighted as a promising method, it is dealt with in more detail. The oxidation products that can arise through lignin oxidation, and especially through alkaline oxidation, are then presented as a summary of the section.

2.4.1 Methods to oxidize lignin

Oxidation of lignin can be performed in many ways. Li et al. (2015) thoroughly describe different methods for catalytic oxidation with the aim to degrade lignin. Some of the systems described include metal catalysts, electrochemical catalysis and photocatalytic oxidation. Although these kinds of systems are promising, some issues still need to be resolved before they can be used on a large scale.[5] In contrast, alkaline oxidation is a mature and widely applied process. Lignin depolymerization on an industrial scale has traditionally been conducted through oxidation in alkaline conditions.[2] For instance, the paper industry has successfully applied alkaline oxidation to depolymerize lignin for bleaching and pulping.[33] Bleaching has basically been used as a synonym for oxygen delignification.[38] In relation to biogas production, alkaline pretreatments have been recommended to optimize anaerobic digestion of lignocellulosic biomass.[17]

2.4.2 Introduction to alkaline oxidation

Alkaline oxidation require an oxidizing agent and a base. Some milder oxidants that can be employed for oxidation of lignin to aromatic aldehydes are oxygen, nitrobenzene or metal oxides. Hydrogen peroxide and permanganate can also be used for oxidation, but these compounds may not be optimal for keeping the aromatic structure of the lignin units.[5] Despite these different options, oxygen can be considered as the preferred oxidant, due to its significant environmental and economic advantages.[11, 33] Being a mild oxidant, however, implies that oxygen in its normal state is not strong enough to be able to oxidize lignin. Therefore, a strong base is required as a catalyst to make the lignin units more receptive to oxidation.[33, 39] Some bases that are commonly used in alkaline oxidation are sodium hydroxide (NaOH), potassium hydroxide (KOH) or sodium carbonate (Na_2CO_3).[5]

Several steps are involved in alkaline oxidation. The exact mechanism for the oxidation depends, however, on which place on the lignin structural unit that is considered, further described in section 2.4.4.1. In general, when the conditions are alkaline, free hydroxyl groups in the phenolic lignin units are deprotonated, thus making the units ionized.[39] Oxidation reactions can then start, which both depolymerize the lignin and add acidic groups to the lignin structural units. When acidic groups are introduced, the lignin can thus dissolve in water and be removed.[33, 40] This is the main idea in chemical pulping processes, where lignin is dissolved and cellulose is left as a solid fraction.[5, 33] However, oxidation can continue also after the lignin has solubilized.[40] Oxidized monomeric phenols can degrade even further to carboxylic acids, and eventually to carbon dioxide and water.[35] As the oxidation proceeds, producing an increasing amount of acidic molecules, there is a possibility that the base eventually becomes neutralized. When the base is neutralized, it can not any longer serve as a catalyst for further lignin depolymerization.[5] In mild conditions, the oxidation only occurs on the aliphatic parts of the structural units, thereby maintaining the aromatic structure.[2] The more severe the conditions are, such as high temperature or reaction time[13, 37], the more likely is it that the aromatic rings in the structural units are opened and thus become carboxylic acids.[2, 37]

One problem in alkaline oxidation, as well as in other lignin depolymerization methods, is the tendency of lignin units to repolymerize. Repolymerization is problematic because the presence of condensed units can limit the lignin oxidation.[39-41] According to Ma et al. (2015), it seems like repolymerization is inevitable in depolymerization methods involving oxygen, as α-O-4 linkages tend to be cleaved to a greater extent than β-O-4 linkages when oxygen is used.[39] Demesa et al. (2015) mention repolymerization and condensation in relation to an increased lignin loading. They also discuss that when there are more lignin fragments, there is also a greater possibility that these fragments will start to interact with each other instead of being further oxidized.[41] An increased oxygen

pressure could, however, reduce the formation of repolymerized fragments.[40] In relation to anaerobic digestion, another issue with alkaline oxidation is that pH problems and ammonia loss can occur due to the high alkalinity.[9]

2.4.3 Alkaline oxidation methods

Different variations of alkaline oxidation have been studied to valorize lignocellulosic materials. Usually, the high yield of phenolic compounds and organic acids have been the main focus.[41-43] The combination of alkaline and oxidative treatment, or in other words alkaline oxidation, has also gained more attention in the biogas field for the valorization of agricultural residues.[1] In these studies, the focus has, however, rather been to degrade the lignin to reduce its inhibitory effect and to increase the methane production.[1, 17] Nonetheless, all these studies show that lignin is degraded by alkaline oxidation, regardless of what the specific purpose has been to use it as a treatment for lignocellulosic biomass. Some of the variations of alkaline oxidation that have been studied include wet oxidation, partial wet oxidation and slightly alkaline wet explosion.

Wet oxidation, or wet air oxidation, is a process having a high temperature and pressure where organic compounds in an aqueous solution are degraded to carbon dioxide by oxygen or air.[2, 44-45] It has been widely used in the treatment of both simple and more complex types of wastewater, for instance originating from industrial processes.[45] Wet oxidation is often employed before biological treatment when compounds that resist biological oxidation are present. For instance, a 99% removal of phenol can be achieved if the wet oxidation is designed specifically for degradation of that compound.[46] The aqueous solution in wet oxidation can be alkaline[2], thereby making wet oxidation a type of alkaline oxidation. Alkaline wet oxidation partially degrades lignin by cleaving its β-O-4 linkages.[5]

Partial wet oxidation is basically the same as wet oxidation, but where the temperature and/or the reaction time are not sufficient to reach complete oxidation. In this incomplete oxidation, organic compounds with low molecular weight are formed.[41, 45] Wet explosion, on the other hand, is a process where wet oxidation and steam explosion are combined. It has been shown that the biodegradability of lignin increases when the wet explosion is performed in slightly alkaline conditions.[16] Some relevant findings from these three different variations of alkaline oxidation are summarized in the following paragraphs. Lastly, a short description of alkaline oxidation applied specifically for anaerobic digestion is given.

2.4.3.1 Wet oxidation and partial wet oxidation

Lignin degradation by alkaline wet oxidation has been studied in different ways. Schutyser et al. (2018) studied the outcomes from alkaline oxidation of native poplar lignin when varying the temperature, NaOH concentration, reaction time, and oxygen partial pressure. One of the findings was that high NaOH concentration and temperature, in combination with a short reaction time, favoured the formation of phenolic compounds.[42] This implies that the process, in fact, was rather a partial wet oxidation. The compounds were, however, degraded quickly when the reaction time was increased. At the optimal conditions (175˚C, 10 min, 2M NaOH, 5 bar O_2) the yield of phenolic monomers reached 30%. An increased oxygen partial pressure speeded up the formation of aromatic oxidation products, but also accelerated their degradation. In addition, too high NaOH concentrations can cause problems. For instance, fouling or salt deposition can arise with a 4 M NaOH concentration. Since the alkaline wet oxidation process was so sensitive to temperature and reaction time, it was suggested to employ a reaction set up where a heating time much less than 30 minutes could be achieved.[42]

In a study by Srinivas et al. (2016), the wet oxidation of biorefinery lignin from wet exploded and enzymatically hydrolyzed Douglas fir residues yielded both lignin and cellulose oxidation products. With a 10% solid content, the maximum yields were obtained with 11.7 wt% NaOH concentration and 15 min residence time. The maximum yield was obtained in a temperature range from 230˚C to 300˚C, depending on which oxidation product being considered (further discussed in section 2.4.4.2).[43]

Partial wet oxidation has been studied by Demesa et al. (2015) for the production of carboxylic acids from lignin. Different parameters, such as temperature, oxygen partial pressure, lignin loading and reaction time, were varied in order to optimize the oxidation to give as high yield of carboxylic acids as possible. Their result showed that temperature and reaction time were the most important factors affecting the lignin oxidation. The oxygen partial pressure also had a significant influence on the product yield. The oxygen pressure was, however, not as an important factor when it came to decomposition of the obtained products as the temperature. At higher temperatures (200 and 225˚C), carboxylic acids with lower molecular weight tended to decompose. Furthermore, it was shown that the pH decreased significantly already after 10 minutes or less at 175-225˚C, when the wet oxidation was performed with 40 g/L lignin, 0.1 M NaOH and 10 bar O_2. Lastly, too high lignin concentrations counteracted the oxidation, as lignin fragments repolymerized.[29]

2.4.3.2 Wet explosion

As mentioned, wet explosion involves wet oxidation immediately followed by steam explosion. When lignin undergoes wet explosion treatment, its structure is altered. Phenolic and aliphatic compounds are also formed. The structure changes so that the lignin becomes more methoxylated, meaning that more methoxy groups are included in the lignin structural units.[9] It has been shown that H units are transformed to S units by wet explosion.[29] As mentioned in section 2.3.2, a more methoxylated lignin structure brings several benefits, such as a higher degradability and reduced tendencies for repolymerization.[9, 43] Some other advantages with wet explosion as a pretreatment of lignin is that it has a low energy requirement and no other chemicals than oxygen and water are needed. This makes the process both environmentally friendly and less expensive.[9] The outcome of the pretreatment is, however, improved by the addition of alkaline compounds, such as NaOH.[16]

In a study by Khan and Ahring (2020), more biomethane was obtained when the NaOH concentration was increased from 0 to 2% during a secondary wet explosion. The conditions applied for the highest biomethane yield were 220˚C, 10 min reaction time, 15% DM content, 4% O_2 and 2% NaOH loading.[16] To obtain the desired outcome, there is, however, a limit for how high the NaOH concentration should be. Hidayaty et al. (2018) showed that even though lignin defragmentation can be increased when NaOH concentration increases to 30%, the content of methoxy groups is the highest at 5% NaOH.[47]

2.4.3.3 Alkaline oxidation for enhanced anaerobic digestion

Alkaline and oxidative treatments have been tested specifically for the optimization of anaerobic digestion. It has been shown that alkaline oxidation, as well as just alkaline pretreatment, are more efficient in removing lignin than acid pretreatment.[17] After alkaline oxidation at 50˚C for 60 min and with 5 wt% H_2O_2, 2 M NaOH and a solids:liquid ratio of 1:20, the solid fraction of pretreated and anaerobically digested wheat straw showed higher methane yield compared to untreated wheat straw. An acclimation period was, however, required before any gas was produced. This significant inhibition was likely due to residual chemicals from the treatment with H_2O_2 and NaOH.[1]

2.4.4 Oxidation products

The degree of oxidation can be evaluated based on oxidation products formed. Different oxidation products also have different influence on anaerobic digestion. Before some of the most common compounds that are formed during alkaline oxidation of lignin are presented, a short description of the oxidation mechanism is given.

2.4.4.1 General mechanism of oxidation

Exactly how the oxidation proceeds depend on which place on the lignin structural unit that is considered. Different oxidation products are also obtained, depending on which functional group of the lignin structural unit that is targeted.

There are three main places on the lignin structural unit where the oxidation can occur. Firstly, the oxidation can occur on the phenolic hydroxy group at position 4 or on the C_1-C_α bond at position 1 (compare with section 2.3.2). After oxidation at these positions, the lignin units are converted to benzoquinone-like compounds. Secondly, oxidation can occur on the side chain of the lignin structural unit. The side chain is removed during the oxidation, thereby leaving behind an aromatic aldehyde, ketone or acid. Lastly, oxidation can induce an opening of the aromatic ring in the lignin unit. The cleavage of the aromatic ring arises first after the lignin unit has been transformed to a quinone intermediate. The ring-opening oxidation produces dicarboxylic acids, such as fumaric acid, maleic acid or muconic acid. At more severe oxidation conditions, these dicarboxylic acids can further degrade to simple carboxylic acids such as formic acid or acetic acid.[2] All these oxidation pathways involve radical chain reactions.[5, 39]

The exact mechanisms of lignin oxidation with oxygen, involving oxidation on side chains of the lignin units, the cleavage of the aromatic structure and the repolymerization of two units, are described by Ma et al. (2015). Nonetheless, water is the main byproduct when oxygen is used as an oxidizing agent. For alkaline oxidation, in particular, it is believed that the depolymerization and oxidation stop when derivatives of aromatic aldehydes and benzoic acid are formed.[39]

2.4.4.2 Common oxidation products

The main products formed during alkaline oxidation of lignin are aromatic aldehydes, such as vanillin and syringaldehyde.[2, 39] In fact, alkaline oxidation is considered as the only lignin depolymerization method where high yields of aromatic aldehydes can be achieved.[48] However, many other compounds are also formed. Normally, the reaction solution after alkaline oxidation contains a multitude of phenolic compounds and aliphatic carboxylic acids.[42] Exactly which oxidation products that are formed depend on which oxidant is being used.[48] However, the higher the temperature and the longer reaction time are during the oxidation, the more is the formation of oxidation products favored.[5] Some of the most common oxidation products and their properties, divided in separate subsections for phenolic compounds and organic acids, are described below.

Phenolic compounds

The most common phenolic compound formed during alkaline oxidation include aromatic aldehydes, aromatic acids, oligomer fragments and acetophenone-like compounds.[42] These aromatic compounds can be derived from the H, G or S units in the lignin. Phenolic compounds originating from the different structural units, have different degree of methoxylation on the aromatic ring. Phenolic compounds derived from the same structural unit, differ only by the different functional groups being attached to the aromatic structure. Usually, the amount of products obtained after lignin oxidation correspond relatively well to proportions of the structural units in the material that is oxidized.[33]

Phenolic compounds can be problematic in anaerobic digestion. The reason is that the integrity of biological membranes can be affected by those types of compounds.[49] At phenol concentrations above 1.2 g/L, it has been shown that the biomethane production was significantly reduced.[17, 49] According to Deublien and Steinhauser (2011), phenol retards anerobic digestion, but will be completely degraded as soon as the microorganisms have adapted themselves to it.[8] Whether this is true for all other phenolic compounds needs to be investigated. What can be said about phenolic compounds, though, is that the toxic effect on methanogenic microorganisms increases the more hydrophobic the compounds are. The hydrophobicity of a phenolic compound decreases, the more hydroxyl groups that are attached to it.[17] Nonetheless, many aspects interact related to the toxicity of phenolic compounds to microorganisms in anaerobic digestion. In mesophilic conditions, it has been demonstrated that more phenolic compounds can be converted than in thermophilic conditions.[49] Due to the mixed, and sometimes contradicting, results, it is thus difficult to draw general conclusions concerning the potential inhibition of phenolic compounds. Nevertheless, any potential inhibition of the anaerobic digestion is important to consider.

The phenolic compound that are presented in this section include vanillin, vanillic acid, guaiacol, syringaldehyde and syringic acid. In the end, some other phenolic compounds are also presented.

Vanillin

One of the main oxidation products from lignin is vanillin, an aromatic aldehyde derived from the G unit.[43] It is a high value-added compound, as it is commercially produced from lignosulfonates for the food and cosmetic industries[2, 42]. In fact, vanillin is the only aromatic aldehyde where alkaline wet oxidation is employed in the commercial production. A typical process is performed at 160–170 °C with a pressure of air or O_2 at 10-12 bar. The yield is very dependent on the oxygen concentration and the pH.[2] As previously mentioned (section 2.4.3.1), higher oxygen pressures accelerate the degradation of aromatic oxidation products. Nevertheless, the oxidative degradation of vanillin is reduced when pH is held above 12. When vanillin degrades, a variety of smaller carboxylic acids can be formed. The main degradation product of vanillin is, however, formic acid.[42] The maximum absorbance of vanillin in slightly alkaline medium can be found at the wavelength 348 nm.[50]

Previous studies report varying yields of vanillin through alkaline wet oxidation. Klinke et al. (2002), used a concentration of 60 g/L raw wheat straw and obtained yields between 8-96 mg vanillin/100 g straw depending on the reaction conditions used. The highest yield was achieved at 195˚C, 15 min, 12 bar O_2 and 2 g/L Na_2CO_3. The lowest yield was achieved at a higher Na_2CO_3 concentration (6.5 g/L), but applying 185˚C, 10 min and 10 bar O_2 instead.[35] Schutyser et al. (2018) never attained vanillin yields higher than 5 wt% when subjecting poplar sawdust to alkaline wet oxidation in 2M NaOH aqueous solution and 5 bar O_2. Their results show highest yields at 175˚C and 10 minutes. At 150˚C, the yield increased when reaction time was increased from 10 minutes to 30 minutes. The opposite was, however, shown at 175˚C and 200˚C, where the highest yields were achieved after short or no reaction time.[42] Lastly, Srinivas et al. (2016) performed alkaline wet oxidation on biorefinery lignin from Douglas Fir residues. The highest vanillin yield of 3.85 mg/g dry biomass was obtained at 10% solids loading, 11.7 wt% NaOH, 230˚C and 15 min reaction time. The yield decreased as the temperature increased with 11.7 wt% NaOH loading. With a NaOH loading of 17.4 wt%, the effect of decreasing yield was even more pronounced as the temperature increased. Thus, if the aim is to increase the vanillin, it was suggested to perform the oxidation at lower temperatures but for longer time instead.[43] To summarize some of these findings, a higher alkali loading generally seem to reduce the vanillin yield. Moreover, the effects of temperature and reaction time are interconnected, so if one of these parameters is high, the other needs to be low to obtain vanillin in significant amounts.

A problem with vanillin in an anaerobic digestion context is that it can be inhibiting. Vanillin has not been considered as an inhibitory compound at concentrations below 2 g/L, but some studies show a decreased biogas production already at 1 g/L.[51] Vanillin is, nevertheless, deemed as recalcitrant to microbial degradation[17], since it inhibits production rate and yield of methane.[51] When used as the only carbon source and at a 2 g/L concentration, the obtained methane is only 17% of the theoretical value.[17]

Vanillic acid

Vanillic acid is an aromatic acid. The only difference between vanillic acid and vanillin is that the former contains one more oxygen atom. Despite the similar structure of these G-type compounds, some considerable differences exist between them. Compared to vanillin, no inhibitory effects on anaerobic digestion have been shown from vanillic acid in the concentration range 1-5 g/L.[51] Furthermore, vanillic acid has been monitored and detected at the wavelength 254 nm in aqueous medium.[52-53] It has been reported that some vanillic acid is formed through oxidation of vanillin. It is, however, believed that vanillic acid has a different formation route than vanillin, suggesting that the formation of these compounds not necessarily correlate.[42]

Klinke et al. (2002) report yields of vanillic acid between 4-122 mg/100 g straw after wet oxidation of 60 g/L raw wheat straw in different reaction conditions. The highest yield of 122 mg/100 g straw was obtained at 195˚C, 10 min, 12 bar O_2 and 6.5 g/L Na_2CO_3, whilst the lowest yield of 4 mg/100 g was obtained with the same Na_2CO_3 concentration and reaction time but employing 185˚C and 10 bar O_2 instead.[35] In alkaline wet oxidation of biorefinery lignin from Douglas fir, the yield of vanillic acid also only reached a few percent. Even though the yield increased with increased temperature in the range 125-200˚C, it decreased as the reaction time increased from 10 minutes to 30 minutes, no matter which temperature was employed.[42]

Guaiacol

Guaiacol can arise both when aromatic aldehydes and aromatic acids are formed during oxidation of lignin structural units.[2] Guaiacol is also derived from G units in the lignin, just as vanillin and vanillic acid. In alkaline wet oxidation of raw wheat straw, yields of guaiacol in the range 3-35 mg/100 g straw have been obtained depending on the conditions used. The highest yield was obtained at 195˚C, 15 min, 10 bar O_2 and 6.5 g/L Na_2CO_3. Generally, the obtained yield was higher when the alkali concentration was held at 6.5 g/L Na_2CO_3.[35]

Syringaldehyde

Just as vanillin, syringaldehyde is an aromatic aldehyde. The difference is that syringaldehyde is derived from S units in the lignin.[43] This means that syringaldehyde contains one more methoxy group than vanillin. This additional group has an important impact on the behavior of the compound. For instance, syringaldehyde is significantly more reactive than vanillin and, hence, more easily degraded.[42] It goes in line with the generally accepted fact that S-type compounds are more easily degraded than G- or H-type compounds, previously mentioned.[9] Syringaldehyde is mainly converted to formic acid and malonic acid during degradation.[42] The degradability of syringaldehyde has also been shown to be true during anaerobic digestion. At a concentration of 2 g/L and with no other carbon sources present, the obtained methane yield from syringaldehyde was 84% of the theoretical value.[17]

In a previous study of alkaline oxidation of wheat straw, the yield of syringaldehyde varied between 1 mg/100 g straw and 75 mg/100 g straw. The highest yield was obtained at 195˚C, 15 min, 12 bar O_2

and 2 g/L Na_2CO_3.[35] In a study by Shuyster et al. (2018), the yield of syringaldehyde was the highest at 175˚C and 200˚C and reaction times up to 10 minutes. At longer reaction times, the yield of the compound decreased at all the investigated temperatures except 125˚C.[42]

Syringic acid

In similarity with vanillic acid and vanillin, syringic acid is the corresponding acid to syringaldehyde. This means that it is an aromatic acid with an S-like structure. Just as vanillic acid, it has been proposed that syringic acid is formed through a different route than syringaldehyde. In fact, even less syringic acid has been shown to be derived from syringaldehyde, compared to vanillic acid from vanillin.[42] In a study by Schutyser et al. (2018), the yield of syringic acid was the highest at 175˚C and 200˚C and reaction times up to 10 minutes. At lower temperatures and longer reaction times, not as much syringic acid had been produced.[42]

Other phenolic products

Many other phenolic compounds that are common as lignin oxidation products can be worth to mention. Beside the previously mentioned compounds, 4-hydroxybenxaldehyde and acetosyringone have also been considered as the main products in alkaline wet oxidation of raw wheat straw.[35] 4-hydroxybenxaldehyde is an aromatic aldehyde derived from an H-type unit[43] and has a maximum UV absorbance at 329 nm in alkaline medium.[50] Acetosyringone, on the other hand, can be compared to syringic acid, but where the acidic hydroxy group on the sidechain is replaced by a methyl group.

In a study by Klinke et al (2002), other phenolic compounds were also formed during alkaline wet oxidation of wheat straw, but to a lesser extent than the above-mentioned compounds. Additional H-type compounds that were formed consisted of 4-hydroxyacetophenone and 4-hydroxybenzoic acid. The S-type compound syringol, corresponding to the G-type compound guiaiacol, was detected, as well as the G-type derivative acetovanillone. Phenol was detected as one of the oxidation products as well. Lastly, the hydroxycinnamic acids *p*-coumaric acid and ferulic acid were also detected.[35] As described in section 2.3.3, these acids connect the lignin with the other parts of the biomass through ester- or ether linkages.[23] The ester linkages can be hydrolyzed in alkaline conditions, meaning that the compounds can be extracted without requiring any oxidation.[33, 42] During oxidation, *p*-coumaric acid and ferulic acids can, however, be further converted to other H- or G-type compounds.[42]

Organic acids

Carbohydrates, as cellulose and hemicellulose, are also converted during alkaline oxidation. In alkaline conditions, these components are transformed to a mixture of aliphatic carboxylic acids.[42] However, carbohydrate-rich residues can still be left after oxidation in mild conditions. Schutyser et al. (2018) showed this during alkaline oxidation of poplar lignin in low temperature and short reaction time. When the severity increased, with an increased temperature and longer reaction time, the material was, however, almost completely converted.[42] In relation to anaerobic digestion, inhibition occurs when the total concentration of acids is higher that 3 g/L. Optimally, the total acidity value should be below 1 g/L.[8]

The main organic acids formed during alkaline wet oxidation are formic acid and acetic acid. A reported value for alkaline wet oxidation of wheat straw, involving maximum yields of acetic acid and formic acid, is 142 mg/g dry biomass.[43] Other acids, such as lactic acid, glycolic acid and trace amounts of compounds like oxalic acid, 3-hydroxypropionic acid, fumaric acid and malic acid can

also arise.[42] Since formic acid can be considered as a thermal degradation product, the main oxidation products from a cellulosic fraction in biorefinery lignin are lactic acid and acetic acid. Although acetic acid also can arise from lignin oxidation, the cellulose fraction in biorefinery lignin should be seen as the primary source of acetic acid.[43] More in-depth descriptions of formic acid and acetic acid are given below.

Formic acid

Formic acid can be considered as a thermal degradation product of glucose, which in turn also is a thermal degradation product of cellulose.[43] Formic acid is, however, also the main product during oxidative degradation of the phenolic compounds vanillin and syringaldehyde.[42] The boiling point of formic acid is 101.1˚C.[54]

Klinke et al. (2002) studied alkaline wet oxidation of raw wheat straw and obtained formic acid yields in the range 2.369-6.986 g/100 g straw.[35] In a study by Srinivas et al. (2016), the yields of formic acid ranged from 33.3-116 mg/g dry biomass with 11.7 wt% alkali loading and from 41.9-110 mg/g dry biomass with 17.4 wt% alkali loading. The highest yield of 116 mg/g dry biomass was obtained at 250˚C, 11.7 wt% alkali loading and 15 min reaction time.[43]

Acetic acid

Acetic acid can arise either from oxidation of lignin or cellulose. In biorefinery lignin, however, the cellulose fraction should be seen as the primary source for acetic acid.[43] Suzuki et al. (2006) reported that acetic acid was not produced from lignin model compounds to a great extent. Their explanation was that the wet oxidation of phenolic compounds rather produces unsaturated dicarboxylic acids with only 4 carbon atoms. The highest yields of acetic acid were, instead, due to oxidation of saturated dicarboxylic acids and unsaturated acids with 5 carbon atoms derived from glutatonic acid.[44] Some additional properties of acetic acid is that it boils at 117.9˚C [55]and inhibits anaerobic digestion already at 1 g/L when pH is below 7.[8]

During wet oxidation of wheat straw, Klinke et al. (2002) have reported acetic acid yields between 1.641 and 2.81 g/100 g straw.[35] In alkaline oxidation of biorefinery lignin from forests residues at a temperature range of 180-300˚C and 15 min reaction time, the acetic acid yields were in the range 23.6-107 mg/g dry biomass with 11.7 wt% alkali loading and 25.4-65.0 mg/g dry biomass with 17.4 wt% alkali loading. The highest yield of 107 mg/g dry biomass was obtained at 280˚C and 11.7 wt% alkali loading.[43]

2.5 From wheat straw to biorefinery lignin

Many changes arise in a material after undergoing processes from raw material to biorefinery lignin. Pretreatment of lignocellulosic feedstock is necessary as it makes it easier to access the desirable parts of the material.[9] For anaerobic digestion of wheat straw, in particular, the methane yield has been shown to increase by 39% after steam explosion pretreatment. Together with enzymatic hydrolysis, these pretreatments do not only increase the conversion rate but can also decrease the required reactor volume and therefore lower the processing costs.[18] Since the destiny of the lignin in the wheat straw is of specific interest in this study, a general description of how the lignin is affected by steam explosion and enzymatic hydrolysis is outlined in this section. It is, however, worth noting that it is essentially impossible to obtain purified lignin from lignocellulosic feedstock.[23]

Steam explosion is a physical pretreatment where most of the hemicellulose is removed as a water-soluble fraction.[11, 15] In wheat straw, the ether bonds linking the lignin and phenolics to the carbohydrates are cleaved. Some ester bonds with the lignin are also cleaved, meaning that the lignin also is slightly deformed. Free phenolic acids such as *p*-coumaric acid (an H-unit product), vanillic acid (a G-unit product) and ferulic acid (a G-unit product) can be detected after the steam explosion.[23] In general, lignin that has underwent steam explosion has a higher content of hydroxyl groups attached to its phenolic units compared to untreated lignin. Furthermore, there is a slightly lower content of methoxy groups and C-C linkages are more common between the lignin units. [39] A higher degree of methoxylation gives a more S-like structure, which more often are interlinked by the unstable β-O-4 linkages.[9] This does, therefore, imply that the lignin fraction can become slightly more recalcitrant after steam explosion. The cellulose, on the other hand, is essentially left unaffected after steam explosion.[11] After steam explosion, impurities like ash and residual carbohydrates that still bind covalently to the lignin units can be expected.[56]

In the enzymatic hydrolysis, both the amorphous and crystalline fractions of cellulose are broken down to glucose.[11] The glucose monomers solubilize in water, whereas the lignin fraction is left as a solid residue.[11, 39] Zhao et al. (2020) investigated the possible changes that occurred for corn stover lignin after ammonia fiber expansion and enzymatic hydrolysis. The elemental content was basically the same for untreated and treated lignin, but a significant change in molecular weight was, however, observed. They saw this as an indication for the lignin after enzymatic hydrolysis obtaining an uneven and widely distributed molecular weight.[57] According to Li et al. (2015), enzymatic hydrolysis decreases the content of hydroxyl groups on the phenolic structures in lignin. Furthermore, it increases both the molecular weight and the number of β-O-4 linkages.[5]

With this as a background, biorefinery lignin from wheat straw could thus be expected to have slightly modified lignin structures. Furthermore, significant amount of residual carbohydrates and ash are most likely left as impurities.

3 Materials and methods

The methodology in this study can be divided into three different parts. The first part deals with all the steps included in the pretreatment of wheat straw to obtain the desired biorefinery lignin. In the second part, the details concerning the alkaline oxidation experiments are outlined. Finally, all the analytical methods applied are described in the third, and last part.

3.1 Raw material and pretreatment

Several pretreatment steps were required to obtain the desired material for this study, essentially reproducing a current biorefinery process. The pretreatment steps included dilute acid-catalysed steam pretreatment and enzymatic hydrolysis, to make the different fractions of wheat straw more accessible. As a final step, the enzymatically hydrolyzed material was filtered to remove excessive liquid and thereby concentrate the solid fraction of the material. This concentrated solid fraction was the desired material for this study and is from now on referred to as biorefinery lignin. Further details for each of these steps, from wheat straw to biorefinery lignin, are described in the sections below.

3.1.1 Raw material

Mechanically pretreated wheat straw, harvested in February 2020, was provided by the Swedish University of Agricultural Sciences (SLU). The provided wheat straw had been milled with a 15 mm sieve and subsequently isolated through sieving, where the obtained material had constituted a sieved fraction of 2-10 mm.

3.1.2 Steam explosion

The wheat straw was impregnated with an aqueous solution of 0.2 wt% H_2SO_4 at room temperature for 1 hour, prior to the steam pretreatment. The ratio between the dry wheat straw and the total weight of the liquid was 1:20. The impregnated material was subsequently dewatered in a 25 L HP-25-M Tinkturenpresse automatic filter press (Fischer Maschinenfabrik GmbH, Germany) at a pressure of 200 bar, thereby reaching a DM content of approximately 50%. The filter-pressed material stood for 2 days in room temperature before undergoing the steam pretreatment. The steam pretreatment was performed continuously in MechaTron equipment (Schenck Process GmbH, Germany) at a temperature and pressure of 190˚C and 12 bar, respectively.

Total solids (TS) and water-soluble solids (WS) were determined according to standard procedures, to assess the required amounts of material for the enzymatic hydrolysis.[58-60] The amount of water insoluble solids (WIS) was calculated based on Equation 3.1 below.

$$WIS = \frac{TS - WS}{1 - WS} \qquad \textit{Equation 3.1}$$

3.1.3 Enzymatic hydrolysis

The enzymatic hydrolysis was performed in a 60 L batch reactor for 72 hours, using the enzyme Cellic CTec2 (Novozymes, China). The enzyme activity was estimated to 110 FPU/g. The loading of water insoluble (WIS) content was 12%, so the enzyme loading thus became 15 FPU/g WIS. The temperature was set to 50˚C and pH kept in the range of 4.5-5, which was maintained by manual addition of 50 wt% NaOH solution throughout the operation.

3.1.4 Filtration and storage

After the enzymatic hydrolysis had been performed, all the material was filtrated utilizing a Büchner funnel and a nylon filter mesh with 40µm pore size. The composition of the filter cake and filtrate was analysed, further described in section 3.3.2.

The material in the filter cake was the material of interest for this study, it was therefore collected and stored in a refrigerator. Some mold had started to grow on the uppermost layer of the material after approximately 4 months. The moldy layer was removed and the remaining material was then stored in a freezer instead. The frozen material was thawed in a refrigerator at least one day before being used. The material did not undergo any further treatment before the alkaline oxidation experiments.

3.2 Alkaline oxidation

The procedure for the alkaline oxidation experiments was developed throughout the study. The final procedure is described in this section, including the equipment used and the treatment of the oxidized material after the experiments had been performed. The experimental design is also presented in this section, as it was the basis both for the execution of all oxidation experiments as well as the subsequent analysis of their outcome. First, the equipment used is listed, followed by the experimental procedure. Then, details of the experimental design are described. Lastly, the treatment of the material after oxidation is outlined.

3.2.1 Equipment

The alkaline oxidation experiments were performed in a 2L Polyclave laboratory pressure reactor (Büchi AG, Uster, Switzerland), equipped with a Unistat T305 thermostat (Huber, Germany) and a Cyclone 300 magnetic stirring device (Büchi AG, Uster, Switzerland). In order to measure the oxygen consumption during the reaction, a PBA757-B60 precision bench platform model (Mettler Toledo, Greifensee, Switzerland) was used to record the weight of the oxygen gas tube, supplying the set-up with oxygen. The pH before and after the reaction was measured using a HI 8424 pH meter, equipped with a temperature probe (HANNA Instruments, USA). Lastly, an Adam PGL-3002 precision balance (Adam Equipment, Kingston, U.K.) was utilized to prepare the reaction mixture and to keep track of the mass balances throughout the experiment.

3.2.2 Procedure

For each experiment, the biorefinery lignin was mixed with a 50 wt% NaOH solution and additional deionized water so that a total mass of 750 g was obtained. The amount of biorefinery lignin and NaOH to add was based on an experimental design, presented in Table 3.1. The final concentrations of NaOH varied between 9.8 and 20.4 wt% and the DM content varied between 6.5 and 28.5%. In order to achieve the desired DM content in the mixtures, the amount of total solids (TS) in the untreated biorefinery lignin had to be determined, further described in section 3.3.1. With a value of TS and the determined total mass (m_{total}) of 750 g, the required amount of material ($m_{material}$) could be calculated as shown in Equation 3.2. After all components had been blended, the mixture was transferred to the reactor. To keep track of the losses throughout the procedure, the weight of the emptied container was recorded.

$$m_{material} = \frac{\frac{DM\%}{100}}{TS} * m_{total} \qquad \textit{Equation 3.2}$$

A constant mixing speed of 500 rpm and a set temperature program were used for all the experiments. In the temperature program, the reactor was first heated for 20 minutes to the desired reaction temperature, ranging from 150 to 210˚C. The temperature was then kept constant for additional

2 minutes, while the reactor was pressurized with 10 bar pure oxygen (HiQ 6.0). The inherent pressure of boiling water varies between 4.8 and 19 bar in the examined temperature range [61], which had to be taken into account when pressurizing the reactor with oxygen. The oxygen was, therefore, applied with a 10 bar higher pressure than the boiling pressure of water at each reaction temperature that was examined. As soon as the pressure had stabilized, the weight of the oxygen gas tube was recorded. Then, the reactor was held at the obtained operating conditions for the desired reaction time, varying between 10 and 35 minutes. When the reaction time had ended, the new weight of the oxygen gas tube was recorded, and the supply of gas was rapidly turned off. The oxygen pressure of 10 bar was, thus, only applied during the reaction time. Lastly, after the reactor had cooled down to room temperature for approximately 40-50 minutes, it was emptied and the weight of the oxidized material was recorded.

pH was also measured both before and after each experiment. The purpose of these measurements was mainly to estimate whether the pH had been held constant during the reaction or not, as a qualitative measure. The temperature of the mixture during the pH measurements was somewhat higher than room temperature. Before the oxidation experiments, this was due to heat that was dispersing from the mixture after NaOH had been added. After the oxidation experiments, on the other hand, it was mainly because the mixture could still be cooling after the oxidation reaction.

As the experiments proceeded, it was observed that the total pressure varied after the reactor vessel had cooled down depending on the conditions in the experiment. After it had been remarked, the total pressure was noted for all subsequent experiments and thus included as a part of the experimental procedure.

3.2.3 Experimental design

A chemometric approach was applied to design and analyse the result of the experiment. With this methodology, the influence of the four parameters temperature (T), dry matter content (DM), reaction time (t) and NaOH concentration (C_{NaOH}) was investigated. The experimental design was based on a hybrid design (416C) by Roquemore (1976), which is comparable to a central composite design but that has some additional features.[62] Response surface-modelling was applied to obtain models and a graphical overviews based on the experimental results. The general steps in the procedure, from establishing an experimental design to obtaining the final models and response surfaces, are outlined in the following paragraphs.

First, the ranges in which each parameter would be investigated in during the experiments were determined. These ranges were then converted in to coded units, so that all parameters would have comparable values and so that scaling effects could be avoided (all values presented in Table 3.1). The following conversion factors were used for the temperature, DM content, reaction time and NaOH concentration, respectively: 20˚C/coded unit, 7.5%/coded unit, 7.5 min/coded unit and 3.75 wt%/coded unit. When data from all 17 experiments had been collected, it was processed together with the coded units in MATLAB in line with the procedure described by Brereton (2003).[63]

Table 3.1: Experimental design for the alkaline oxidation experiments, where the influence of temperature (T), dry matter content (DM), reaction time (t) and NaOH concentration (C_{NaOH}) was examined. Both the real set of values as well as their corresponding coded values are presented for all the four investigated parameters.

Experiment no.	Temperature (°C)	Dry matter (DM) (%)	Reaction time (min)	NaOH concentration (wt%)	Coded units			
					T	DM	t	C_{NaOH}
1	180	17.5	23	20.4	0	0	0	1.7654
2	160	10	15	15.9	-1	-1	-1	0.5675
3	200	10	15	15.9	1	-1	-1	0.5675
4	160	25	15	15.9	-1	1	-1	0.5675
5	200	25	15	15.9	1	1	-1	0.5675
6	160	10	30	15.9	-1	-1	1	0.5675
7	200	10	30	15.9	1	-1	1	0.5675
8	160	25	30	15.9	-1	1	1	0.5675
9	200	25	30	15.9	1	1	1	0.5675
10	210	17.5	23	9.8	1.4697	0	0	-1.0509
11	150	17.5	23	9.8	-1.4697	0	0	-1.0509
12	180	28.5	23	9.8	0	1.4697	0	-1.0509
13	180	6.5	23	9.8	0	-1.4697	0	-1.0509
14	180	17.5	35	9.8	0	0	1.4697	-1.0509
15	180	17.5	10	9.8	0	0	-1.4697	-1.0509
16	180	17.5	23	13.8	0	0	0	0
17	180	17.5	23	13.8	0	0	0	0

The calculations were performed in two steps, where the first step was to attain a simplified mathematical model based on the full model shown in Equation 3.3. The second step was to make response surfaces based on the simplified model. The first step was thus a way to assess which terms that were the most important in the model. The second step, on the other hand, was a way to deduct which parameters (*T, DM, t* or C_{NaOH}) that were the most influential in the experiments.

Equation 3.3

$$
\begin{aligned}
Y = \quad & && \text{(Response)} \\
& b_0 + && \text{(Intercept)} \\
& b_1 T + b_2 DM + b_3 t + b_4 C_{NaOH} + && \text{(Linear terms)} \\
& b_5 T^2 + b_6 DM^2 + b_7 t^2 + b_8 C_{NaOH}^2 + && \text{(Quadratic terms)} \\
& b_9 T \cdot DM + b_{10} T \cdot t + b_{11} T \cdot C_{NaOH} + b_{12} DM \cdot t + b_{13} DM \cdot C_{NaOH} + b_{14} t \cdot C_{NaOH} && \text{(Interaction terms)}
\end{aligned}
$$

The mathematical model is a prediction of how the response (*Y*) is correlated to the effect of all the investigated parameters. The full model (Equation 3.3) includes an intercept term (b_0), linear terms, quadratic terms and interaction terms. To assess how well the values from the prediction model correlated to the experimental values, R^2 was calculated as shown by Equation 3.4-3.6.

$$R^2 = 1 - \frac{SS_{residual}}{SS_{replicate}} \qquad \textit{Equation 3.4}$$

$$SS_{residual} = \sum_i^n \left(y_{Experimental} - y_{Modelled}\right)^2 \qquad \textit{Equation 3.5}$$

$$SS_{replicate} = \sum_i^n \left(y_{Experimental} - y_{Mean}\right)^2 \qquad \textit{Equation 3.6}$$

The full model was simplified by removing one term at a time from the mathematical expression and calculating the value of R^2 for the new expression. R^2 was thus used as an indication for whether terms had a large influence in the model or not. If the difference of R^2 was large after a term had been removed from the model, the term was deemed to have an important effect and therefore it was part of the final model. On the other hand, when the difference of R^2 was small after a term had been removed, that term was not present in the final model. This procedure was repeated step-by-step for all 15 terms in the model, until as many terms as possible were removed without making the value of R^2 become lower than 0.90.

When a simplified model had been obtained, it was used to predict the response for all values within the investigated ranges previously mentioned. This was done by constructing response surfaces for two parameters at a time in MATLAB. The response-surface modelling was based on coded units from the experiment that had had the most desirable outcome, meaning the highest yield of oxidation products. In this study, two models, with two sets of response surfaces, were produced. One model was made for the ratio of TS in the material after and before the oxidation ($TS_{oxidized}/TS_{initial}$), whereas the other model was based on a ratio of UV absorbance at the wavelengths 348 nm and 254 nm ($Abs_{348\ nm}/Abs_{254\ nm}$).

3.2.4 Treatment after oxidation

After all the oxidation experiments had been conducted, the obtained materials underwent further treatment to separate its solid and liquid fractions. First, the original oxidized mixture was centrifuged so that a distinct sediment with a liquid phase on top appeared. The liquid phase was then decanted and filtrated, whereas the sediment in the bottom was washed and further centrifuged for a repeated number of times. In some trials before the actual oxidation experiments had begun, only filtration had been tested as a method for separating the solid fraction from the liquid. During these trials it appeared that the filtration of some samples was enormously tedious. It was thereby decided to include an initial centrifugation of all oxidized materials so that only the liquid fraction had to be filtrated. Slight exceptions to the combined centrifugation and filtration method occurred for some of the oxidized materials, but details for the general procedure are outlined in 3.2.4.1 and 3.2.4.2 below.

3.2.4.1 Centrifugation for separation of fractions and washing of the solid fraction

In total, material from all the oxidation experiments was centrifuged four times each. The aim of the first centrifugation was to separate the solid and the liquid fractions of the material. The purpose of the three last sets of centrifugation was to separate the solid fraction from the water that was used for washing. The material to be centrifuged had to be divided in to two centrifuge bottles, so that the maximum volume of 750 mL in the bottles was not reached. The difference in mass between two bottles never exceeded 0.3 g, in order to maintain a balance during centrifugation. When an appropriate mass balance had been obtained between each pair of centrifuge bottles, the centrifugation was executed for 20 minutes and with a speed of 4000 rpm, both for the separation and the three sets of washing. The centrifugation was conducted in a Jouan C412 centrifuge (Winchester, Virgina, United States).

As mentioned, the washing of the solid fraction was performed in three sets. The first step in each set, after the supernatant had been decanted, was to add 500mL of deionized water with a temperature around 50˚C. The water usage of the complete washing procedure was thus 1.5L, which roughly corresponded to the double volume of the original oxidized materials. After the warm deionized water had been added to the centrifuge bottles, they were shaken until the solid material dissolved in the water. When a homogenous mixture had been obtained, the centrifuge bottles were shaken for at least 10 more seconds to ensure good mixing. After the third, and final, washing set, the remaining solid at the bottom of the centrifuge bottle was transferred to a pre-weighted aluminium foil pan and dried

at 105˚C overnight. After cooling in a desiccator for 30 minutes, the dry weight of the washed sediment was then recorded. This weight was subsequently used for calculations of the WIS content in the original oxidized material.

As the initial DM content increased in the oxidized material, it got more difficult to get all material out from the centrifuge bottles. Emptied centrifuge bottles for experiments with an initial DM content of 17.5% or higher were therefore rinsed with 500 mL warm deionized water. The water, together with the residual particles, were then poured over the filter related to the same experiment after the initial filtration of the liquid fraction had ended. That volume of water was included as part of the filter cake washing, described below in 3.2.4.2. In cases where the liquid fraction after the initial separation was still filtrating, the centrifuge bottles were rinsed with only 100 mL. This smaller amount of warm water was taken just because its temperature would cool down to room temperature, due to the tedious filtration, before it could be poured over the filter. When the initial filtration eventually had finished, the 100mL room-tempered rinse water was poured over the filter together with 400 mL warm deionized water.

The same procedure was applied for the raw material, but with the difference that much smaller amounts were used. The total mass of material was 30 g and the washing was performed 3 times with 20 mL warm deionized water. The total volume of the wash water was, thus, 60 mL. A Sigma 3-16K centrifuge (Sigma, Osterode am Harz, Germany) was used for the separation of liquid and solid fractions and the subsequent washing of the solid fraction.

3.2.4.2 Filtration of the liquid fraction

The supernatant after the first centrifugation of the oxidized materials was decanted directly to a vacuum filtration setup. Büchner funnels and pre-weighted filter paper (Grade 5) were utilized. When the filtration of that decanted liquid fraction had finished, the filtrated liquid was transferred to a bucket and its total weight was recorded.

As in the washing of the solid fraction, the filter was then washed with in total 1.5 L deionized water with a temperature around 50˚C. When centrifuge bottles hade to be rinsed, that water volume was included in the total amount of 1.5L. This means that only 1L fresh deionized water was used to wash the filter cake and then additional 500 mL were added to gather the residual particles that had not been collected from the centrifuge bottles. After the washing of the filter cake had finished, the filter paper was dried at 105˚C over night. After cooling in a desiccator for 30 minutes, the dry weight of the washed filter paper was recorded. When a value of the remaining solids on the filter paper had been obtained, that weight was subsequently used for calculations of the WIS content in the original oxidized material.

3.3 Analytical methods

A multitude of analytical methods was applied in this study. The goal was to get as much information as possible about the biorefinery lignin and how it was affected by the oxidation experiments. The TS content was determined to measure the solid fraction in the untreated biorefinery lignin and to evaluate its degradation after the oxidation. The non-oxidized biorefinery lignin also underwent some compositional analyses to give more information about the starting material in the oxidation. In contrast, UV absorbance was measured to give an indication of how aromatic compounds in the lignin changed due to the oxidation. Lastly, three different chromatographic analyses were performed to evaluate the degradation of the material and the formation of oxidation products in the oxidized materials. Each one of the analytical methods has, step by step, brought a more refined understanding about the biorefinery lignin and its fate after the oxidation experiments. These methods are described

in the same order in the following sections, going from analyses on a more general level (as TS, compositional analysis, and UV) to more in-depth analyses where specific compounds could be detected (as the chromatographic analyses).

3.3.1 Total solids content

Total solids (TS) content was determined for the untreated biorefinery lignin, non-separated material after each oxidation experiment and for the filtrate of the liquid fraction in the oxidized material. The determination of the TS in untreated and non-oxidized was used to examine the impact of the oxidation on the material, by using a ratio of $TS_{oxidized}$ and $TS_{initial}$. Determination of TS in the filtrate was used to evaluate the overall mass balance in the experimental procedure.

TS content of the untreated biorefinery lignin was made using aluminium forms, whilst crucibles had to be used for the oxidized materials due to their high alkalinity. The general procedure for the oxidized materials were as follows. Firstly, the weight of the empty and pre-ashed crucibles was recorded using an AB304-S/Fact analytical balance (Mettler Toledo, Greifensee, Switzerland). Secondly, the crucibles were filled with material in triplicate samples from the different experiments and the new weight was noted. The crucibles were put in an oven at 105˚C, at minimum overnight but most often for a few days so that the samples were completely dried. When the water in all the samples had evaporated, the crucibles were weighed again, thereby giving the amount of residual lignin and NaOH. When aluminium forms were used for the determination of the untreated material, no pre-ashing was performed. After cooling in a desiccator for 1h, the dry weight of the different materials was then recorded. The average of the triplicate determinations was used in the subsequent calculations.

3.3.2 Compositional analyses

The composition of the non-oxidized, untreated biorefinery lignin was analysed by following National Renewable Energy Laboratory (NREL) standard procedures for sample preparation, determination of structural carbohydrates and lignin and determination of sugars in liquid samples.[64-66] The analysis of the liquid fraction was made roughly 6 months after the pretreatment of the raw wheat straw had been performed. A new compositional analysis was also performed on the solid fraction approximately 4 months after the biorefinery lignin had been obtained, since mold had started to grow on that part of the material. The most important details concerning the determination of lignin and carbohydrate content are presented in the following paragraphs.

3.3.2.1 Lignin content

The amount of ASL was determined using a UV-160 model UV-Visible spectrophotometer (Shimadzu, Kyoto, Japan). No samples were diluted prior to the measurement. The wavelength used for the measurements was 320 nm and each sample was analysed in triplicates.

The amount of AIL was, on the other hand, determined through drying, ashing and weighing. The drying was performed at 105˚C over night and the ashing was performed based on a temperature ramping program going up to 575˚C, described elsewhere by Sluiter et al. (2012).[65] A Nabertherm muffle furnace (Nabertherm, Lilienthal/Bremen, Germany) was used for the ashing and an AB304-S/Fact analytical balance (Mettler Toledo, Greifensee, Switzerland) was used for the weighing. Crucibles were ashed and weighted before being filled with material and were cooled for 1h in a desiccator after each step in the procedure. The determination of AIL was made for triplicate samples.

3.3.2.2 Carbohydrate content

The content of carbohydrates in both the solid and the liquid fraction of the material was analysed using an ICS-3000 system Dionex (Thermo Scientific, USA), equipped with a Carbo Pac PA1 column, GP50 gradient pump an AS50 autosampler. The flow rate was 1 mL/min and the injection volume was 10 µL. The calibration standards used were glucose, as indicator for the cellulose content, and xylose, mannose, arabinose and galactose, as indicators for the hemicellulose content. Before being analysed, all samples were filtered through a 0.2 µm filter and then diluted.

Samples from the liquid fraction were diluted 1:1000 in a three step dilution series, where an aliquot of 150 µL was taken at each step from a vortexed sample and then diluted with 1350 µL deionized water. For the analysis of the solid fraction, the samples were only diluted 1:2 by taking 750 µL of the sample and 750 µL of deionized water. The sugar recovery standards (SRSs) used in the NREL procedures were diluted 1:20. Material samples were analysed in triplicates and the final volume transferred to the Dionex vials was 1mL.

3.3.3 UV spectrophotometry

The filtrated liquid fractions from the different oxidation experiments were used in the UV measurements. The UV absorbance was measured using an UV-1800 UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Oxidized materials from the different experiments were diluted 1:10 000 with deionized water, to be within the optimal absorbance range 0.2-0.8. To obtain these dilutions, an 0.2 µL aliquot was taken from room-tempered oxidized material and was mixed with 199.98 mL deionized water. A manual EM PP dispenser in the range 10-60 mL (Hirschmann Laborgeräte, Eberstadt, Germany) and a pipette in the range 100-1000 µL were used to obtain the desired water volume. All measurements were performed in duplicates, meaning that two independent dilutions were analysed for each sample. Before each UV measurement, the cuvette was rinsed with the 1 mL sample solution at least twice. A volume of 1 mL was also used when each sample was measured in the spectrophotometer. The whole spectrum between 190 and 400 nm was analysed in each measurement. The subsequent calculations were based on a ratio of the absorbances at 348 nm and 254 nm.

During the initial UV measurements, the untreated biorefinery lignin was also analysed. It was prepared in a similar manner as the oxidized materials. However, 4 wt% NaOH, corresponding to a concentration of 1M, was added to the samples. The untreated biorefinery lignin was analysed at the DM contents 5%, 10%, 15% and 20%, where deionized water was used to dilute the samples to the desired contents. It was also tested to analyse the material at 25% DM content, but the mixture was too thick to be used. Furthermore, different dilutions were applied to be within the same absorbance range for all samples. The sample with 5% DM was diluted 1:5000, whilst the sample with 10% DM was diluted 1:7500. Both the samples for 15% and 20% DM were diluted 1:10 000, though. Measurements were only made for on one single, sample.

3.3.4 Chromatographic analyses

Three chromatographic analyses were used to investigate the oxidation products formed during the alkaline oxidation. The filtrated liquid fractions from the different oxidation experiments were used in these analyses. Size exclusion chromatography (SEC) was used as an initial analysis to assess whether any monomers had been formed due to the oxidation. Then, monomers were analyzed using two different types of High-Performance Liquid Chromatography (HPLC). Ultra Performance Liquid Chromatography (UPLC) was used to analyse monoaromatic compounds, whereas a more ordinary HPLC analysis was employed to assess the amount of organic acids. The overall procedure thus started from a coarser analysis, based on molecular weight, and ended with more sophisticated analyses, based on the concentration of specific compounds. The details for each one of these three analyses are outlined in the following paragraphs.

3.3.4.1 SEC – Molecular weight distribution

Prior to the SEC analysis, all samples of interest were filtrated through 0.2 µm syringe filters. The samples were then diluted with deionized water to attain a concentration of NaOH around 0.4 wt% (corresponding to 0.1 M). Samples with 9.8 wt%, 15.9 wt% and 20.4 wt% NaOH were thus diluted 1:25, 1:40 and 1:50, respectively. The dilutions were performed in series with two steps, with 1.5 mL as a total volume in each dilution. The first step for all dilutions involved 150 µL from the filtrated liquid fraction and 1350 µL deionized water. In the second dilution step, an aliquot in varying size was taken from the vortexed initial dilution. Aliquots of 600 µL, 375 µL or 300 µL were taken for samples with the initial NaOH concentrations 9.8 wt%, 15.9 wt% or 20.4 wt%, respectively. The new dilutions were vortexed after each dilution step. The diluted samples were frozen prior to analysis.

An Alliance 2695 HPLC system (Waters, Milford, MA, USA), equipped with an UV detector at 280 nm and an RI detector, was used for the SEC analysis. The system consisted of two room-tempered columns in series, one being a Superdex 200 Increase and the other a Superdex 30 Increase (Cytiva, Uppsala, Sweden). The mobile phase was 0.1 M NaOH, having a flow rate of 0.5 mL/min, and the injection volume was 20 µL. The standards used for calibration consisted of polyethylene glycol (PEG) ranging from 200 to 35 000 Da.

3.3.4.2 UPLC – Analysis of phenolic compounds

Samples from the oxidized materials needed to have a pH value lower than 4 before they could be analyzed in the UPLC. To achieve that, an aliquot of 5 mL was taken from the oxidized materials and then 5M HCl was added in amounts ranging from 1.75 to 4.25 mL. pH values between 2.7 and 3.8 were thereby obtained. The acidic mixtures were filtrated through 0.2 µm syringe filters and diluted according to the same procedure as described in section 3.3.4.1 above. The diluted samples were frozen prior to analysis.

An Aquity UPLC system (Waters, Milford, MA, USA) was used for the analysis of monoaromatic compounds. The system was equipped with an Agilent InfinityLab Poroshell 120 EC-C18 column, held at 50°C, and a PDA detector with detection at 280 nm. The mobile phase consisted of water, formic acid and acetonitrile in varying proportions. The fraction of formic acid fraction was held constant at 10% throughout the analysis, whereas the proportions of water and acetonitrile were modified according to a gradient method. This method, along with the fractions in the mobile phase at different time steps, are presented in Appendix A. Nonetheless, the flow rate of the mobile phase was 1 mL/min, and the injection volume was 3 µL. Vanillic acid, vanillin and guaiacol were used as standards.

The yield was calculated according to Equation 3.7, where the obtained concentration ($C_{oxidation\ product}$) was multiplied with total mass of the liquid fraction of the oxidized material ($m_{Liquid\ fraction}$), assuming that a 1 mL sample corresponded to 1 g. This was then divided by the initial mass of TS ($m_{TS,in}$).

$$Yield = \frac{C_{oxidation\ product} * \frac{V_{sample}}{m_{sample}} * m_{Liquid\ fraction}}{m_{TS,in}} \qquad \textit{Equation 3.7}$$

3.3.4.3 HPLC – Analysis of organic acids

All samples were prepared and diluted in the same manner as for the UPLC analysis (section 3.3.4.2). However, an Alliance HPLC system was utilized (Waters, Milford, MA, USA) for the analysis of organic acids. The system was equipped with an Aminex HPX-87H column (Bio-rad, Hercules, CA, USA), held at 60°C. The mobile phase was 5 mM H_2SO_4, having a flow rate of 0.6 mL/min, and the injection volume 20 µL. The standards used were fumaric acid, glutaconic acid, glutaric acid, glyceric acid, malic acid, malonic acid, methylmalonic acid, succinic acid, a mixture of oxalic and formic acid

and a standard mix consisting of xylose, glucose, glycerol and acetic acid. The yield was calculated according to Equation 3.7 above.

The concentrations of unknown organic acids were estimated by using the values of acetic acid, quantified by a refractive index (RI) detector. The estimation was thus made by multiplying the ratio between concentration and peak area of acetic acid with the peak area of the unknown compound, as shown in Equation 3.8.

$$C_{Unkown} = \frac{C_{Acetic\ acid}}{A_{Peak,Acetic\ acid}} * A_{Peak,Unknown} \qquad \textit{Equation 3.8}$$

4 Results and Discussion

First, the characteristics of the untreated biorefinery lignin are presented and discussed. Then, all the results related to the oxidation of the material are presented and analysed in the second part, followed by an evaluation of the experimental procedure in the third part.

4.1 Untreated biorefinery lignin

The results in this section are a description of the material used in the oxidation experiments. First of all, the composition and general characteristics of the biorefinery lignin are presented. Then, the UV spectra of the material is shown.

4.1.1 Composition of untreated biorefinery lignin

Biorefinery lignin was obtained from wheat straw after several pretreatment steps, involving steam explosion, enzymatic hydrolysis and filtration. The obtained biorefinery lignin could be described as a fine-grained, brown sludge, depicted in Figure 4.1. The TS content in the obtained material had an initial value of 39%. A new TS value of 37% was, however, determined when mold had started to grow on the material a few months later. The fraction of WIS in the biorefinery lignin was 22%, implying that slightly more than half of the solid fraction in the material was water insoluble.

Figure 4.1: Untreated biorefinery lignin from wheat straw, held on a spoon.

The proportions of lignin, cellulose, hemicellulose and ash in the material depend on which fraction of the biorefinery lignin that is considered. If the entire material is considered, it is evident that the obtained biorefinery lignin has significantly lower fractions of cellulose and hemicellulose compared to the raw wheat straw (compare with Table 2.1). As can be concluded from the TS determinations, the solid fraction barely constituted 40% of the biorefinery lignin. Slightly more than 60% of the material consisted thus of a liquid fraction. Nevertheless, lignin was still the main component when taking the whole material into account, see Table 4.1. In fact, the solid fraction was more than 75% made of lignin and just a few percent hemicellulose and cellulose.

Since the liquid fraction was such a large part of the material, the carbohydrates in the liquid fraction also constituted a more important part when taking the whole material in to consideration. As can be seen in Table 4.1, the amount of lignin and ash in the biorefinery lignin was roughly 28% and 4%, respectively. The content of cellulose and hemicellulose, on the other hand, was roughly 8% and 4%, respectively. With such high carbohydrate content, it can be expected that degradation and oxidation

products derived from cellulose and hemicellulose also will be found in the reaction solution after the alkaline oxidation (discussed in section 4.2.3.3).

Even though the compositional analyses did not show large differences before and after mold had started to grow on the material, it should be assumed that material has undergone some structural changes. It could be assumed that the lignin fraction has been kept relatively unaffected. but it should definitely be seen as a source of error with regard to the oxidation of cellulose and hemicellulose. It would, therefore, be desirable to perform a new study but where material has not been exposed to mold.

Table 4.1: Composition of untreated biorefinery lignin, where AIL corresponds to Acid Insoluble Lignin and ASL corresponds to Acid Soluble Lignin

Component		**Portion of solid fraction (%)**		**Portion of liquid fraction (%)**	**Total in material (%)**
		(Before mold)	**(After mold)**		**(After mold)**
Cellulose		2.2	2.5	7	**8**
Hemicellulose		1	1.1	3.4	**3.9**
Lignin	*AIL*	77.2	75.9	-	**27.3**
	ASL	0.8	0.3	-	**0.1**
Ash		11.8	11.5	-	**4.1**

Some noteworthy findings were made when the sugar content was analysed in both the solid and liquid fractions of the biorefinery lignin. In the solid fraction, a compound that could not be identified by the analysis equipment was discovered. As can be seen in the chromatogram in Figure 4.2, the peak of the unknown compound lies in between the peaks of glucose and xylose but is somewhat closer to glucose. This unidentified component is most likely some sort of glucose derivate, probably having an additional functional group attached somewhere to the glucose structure. Another aspect that supports this theory is that the unknown compound only was detected in the solid fraction, thereby suggesting that it originates from non-degraded cellulose in that fraction. Peaks for such an unknown compound were, furthermore, found in replicate samples from the biorefinery lignin both before and after mold had started to grow on the material. This implies that the unknown compound is inherent to the biorefinery lignin and has not appeared due to microbial activity or other types of degradation in the material.

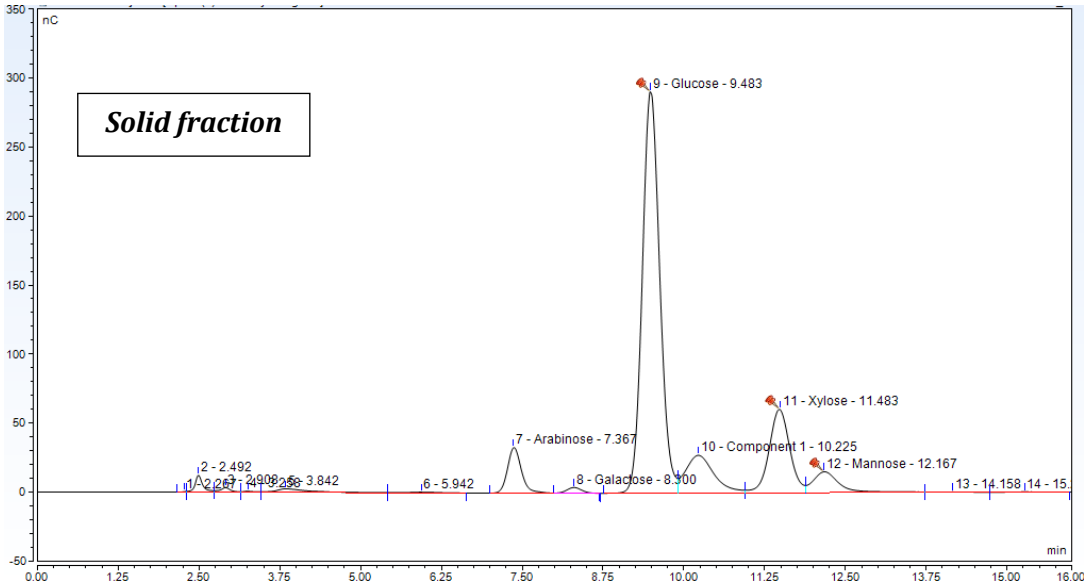


Figure 4.2: Dionex chromatogram for a replicate of the structural carbohydrates in the solid fraction of the untreated biorefinery lignin. The peak named "Component 1", in between the peaks for glucose and xylose, could not be identified in the analysis.

An approximate concentration of the unidentified sugar component and its fraction in the solid fraction were estimated. The approximate concentration was calculated by using the ratio between concentration and peak area for glucose and multiplying that with the peak area of the unknown component (compare with Equation 3.8). An averaged value for all analysed samples gave a mean concentration of the unknown component to 6 g/mL. An estimation of how much that would correspond to as a fraction in the material was then made. It was assumed that the values for calculating the glucose fraction in the material (namely the sugar recovered and the anhydro correction) also could be applied to the unknown component. The estimation showed that the unknown sugar compound, on average, roughly corresponded to 0.4% of the solid fraction. The corresponding values for xylose and mannose were 0.56% and 0.22%, respectively. Comparing these values with the peaks in the chromatograms in Figure 4.2, shows that it should be in the right order of magnitude.

In the liquid fraction, a mismatch between the material samples and the SRSs was observed in the analysis. As can be seen in Figure 4.3, mostly glucose and xylose are present in the liquid fraction of the biorefinery lignin. In contrast, the SRSs had a significantly higher content of xylose compared to glucose. In addition, the content of arabinose and galactose was much higher in the SRSs than in the material sample. The accuracy of the analysis is improved when the content of the different sugars in the SRS correspond to the one in the material samples. A recommendation for future analyses of the liquid fraction in biorefinery lignin from wheat straw is, therefore, to use SRSs where glucose has the highest concentration and xylose roughly half of the glucose concentration.

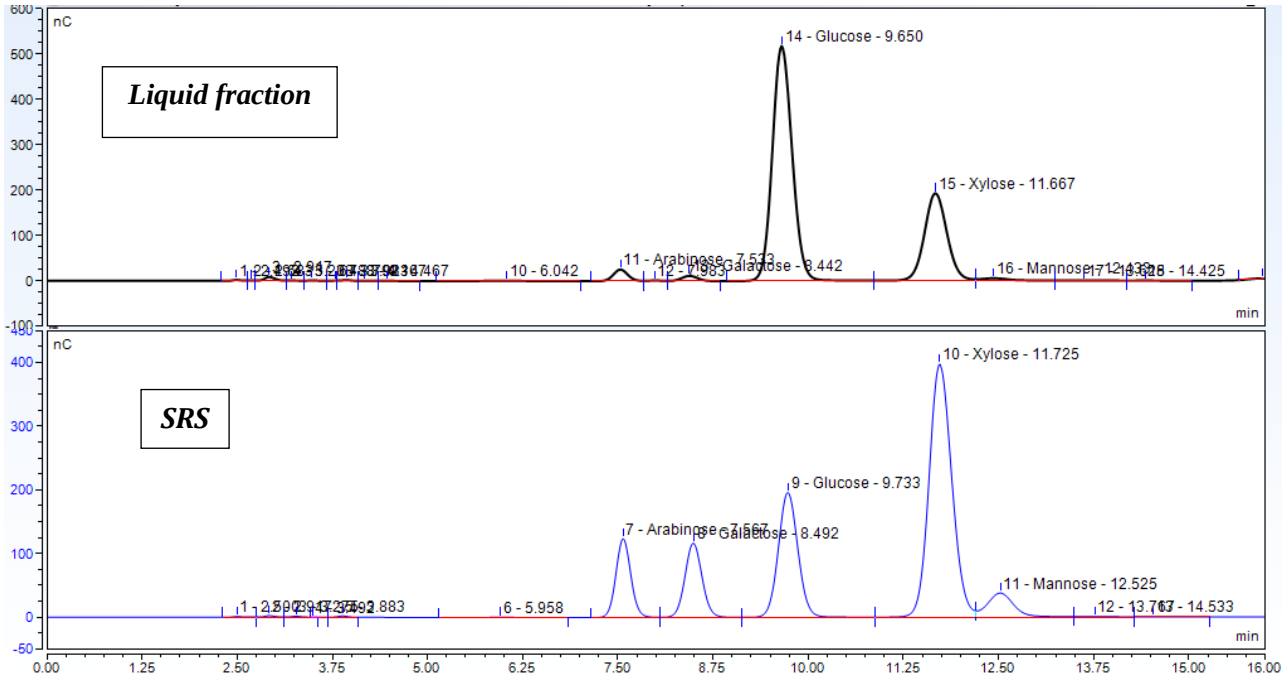


Figure 4.3: Dionex chromatograms for one replicate sample from the liquid fraction (above) and one of the sugar recovery standards (SRS) (below) used in the analysis of the liquid fraction.

4.1.2 UV spectrum of untreated biorefinery lignin

UV measurements have been used to evaluate the oxidation of the biorefinery lignin (described in more detail in section 4.2.2 and 4.2.4). To examine whether anything changed in the spectrum due to the oxidation, the UV spectrum of untreated biorefinery lignin was also determined in the initial measurements. UV absorbance was measured for untreated biorefinery lignin at different DM contents and dilutions, see Figure 4.4. As can be seen, a peak around 280 nm is indicated in all spectra. This correlates well with the maximum absorption found at 280 nm, as well as the shape of UV spectra of other types of lignin, in previous studies.[30, 37] This indicates that the untreated biorefinery lignin has similar properties to other lignin materials.

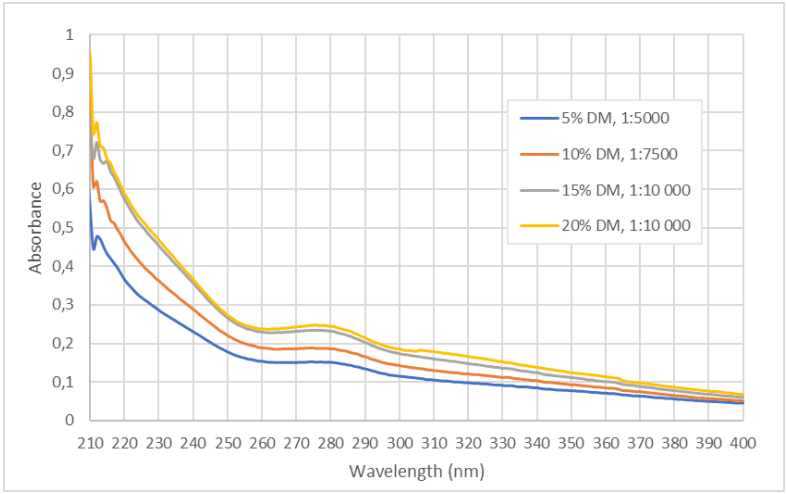


Figure 4.4: UV spectra of untreated biorefinery lignin at different DM contents and dilutions.

Table 4.2: Absorbance ratios of untreated biorefinery lignin with different DM contents.

DM (%)	Dilution	Abs_{348nm}/Abs_{254nm}
5	1:5000	0.476
10	1:7500	0.471
15	1:10 000	0.465
20	1:10 000	0.506

In general, the higher the DM content was in the measured sample, the higher was the absorbance. This occurred even though the samples with high DM content were diluted more than the samples

with low DM content. This suggests, thus, that even if samples are diluted, the amount of material has an impact on the results. In addition, the values of the absorbance ratio (Table 4.2) are generally higher compared to the values for the oxidized materials, presented in Table 4.4-4.7. The high values of the untreated material can be explained by a smaller difference between the absorbance at 348 nm and 254 nm.

4.2 Oxidation of biorefinery lignin

The materials after the 17 separate oxidation experiments were analysed in several different ways. In similarity to the analytical methods used, the results in this section are presented in an order where the understanding of the biorefinery lignin becomes more sophisticated for each subsection. First, the overall mass balances for each experiment are presented. The UV spectra of the oxidized materials are then shown, followed by chromatograms and values from the chromatographic analyses. The mathematical models that have been obtained based on values from the mass balances and UV measurements are subsequently presented, together with response surfaces based on the optimal experiment. Lastly, a qualitative description of the properties of the oxidized materials are given, just to provide a more hands-on picture of what has happened to these materials.

When processing the result, the experiments in Table 3.1 were reorganized so that it would be easier to detect overall trends. The primary parameter that experiments were reorganized after was the DM content, see Table 4.3 below. For all experiments with the same DM content, the experiments were then ordered according to an increased severity of the reaction conditions, starting with NaOH concentration, followed by temperature and, lastly, the reaction time. In other words, each new row in Table 4.3 means that the experiment on that row has a higher DM content, higher NaOH concentration, higher temperature or longer reaction time compared to the experiment on the row above. All following graphs and tables in this section follow the same order. To make them more readable, only the parameters that are deemed as most relevant for the discussion are indicated.

Table 4.3: All oxidation experiments performed in this study organized based on increasing DM content, NaOH concentration, temperature and lastly the reaction time. For some experiments, only the reaction time differed. These experiments have therefore been grouped together in pairs.

DM (%)	Experiment no.	NaOH concentration (wt%)	Temperature (C°)	Reaction time (min)
6.5	**13**	9.8	180	23
10	**2**	15.9	160	15
	6	15.9	160	30
	3	15.9	200	15
	7	15.9	200	30
17.5	**11**	9.8	150	23
	15	9.8	180	10
	14	9.8	180	35
	10	9.8	210	23
	16	13.8	180	23
	17	13.8	180	23
	1	20.4	180	23
25	**4**	9.8	160	15
	8	9.8	160	30
	5	9.8	200	15
	9	9.8	200	30
28.5	**12**	9.8	180	23

4.2.1 Mass balances

Mass balances were used as an initial assessment for the outcome of the oxidation. Important factors to consider are TS ratio, change in WIS and oxygen consumption during the experiments. During the experiments, it also appeared that pH also has a large influence on the outcome, as it decreased in many of the experiments. The outcomes of the experiments have, therefore, been put in relation to the stability of pH during the experiment.

pH has been defined as stable, slightly decreasing or significantly decreasing by comparing the pH values before and after the oxidation. Before all experiments, the pH was around 12. A stable pH value has, thus, been defined as a value around 12 also after the oxidation. A slightly decreasing pH, on the other hand, has been defined for experiments where pH value after oxidation was around 10. Lastly, a significantly decreasing pH value has been defined at pH values of 7 or 8 after the oxidation. In Table 4.4, experiments with a stable pH and a slightly decreasing pH have been marked with green and orange, respectively. All the remaining experiments, with no color, had a significant decrease in pH.

4.2.1.1 TS ratio and WIS

The overall mass balance was based on values for TS and WIS. The TS ratio of TS from the oxidized material over the initial TS in the material is a particularly relevant measure to analyse the oxidation.

If the TS ratio is above 1, it can be interpreted as if the material becomes heavier due to assimilation of oxygen and that the obtained oxidation products remain in the reaction solution. In contrast, if the TS ratio is below 1, oxygen has still been assimilated but with the difference that excessive oxidation has occurred. Excessive oxidation implies that the material has been converted to CO_2 and left the reaction solution. In comparison, WIS implies how large fraction of the material is soluble in the material. A percentage change of WIS before and after oxidation indicate how the amount of water-insoluble solids has decreased. A high value of the change in WIS shows that more solids in the material have become water-soluble in comparison to the untreated biorefinery lignin.

In general, the TS ratio is above 1 for almost all experiments where pH was stable throughout the experiment (experiments 13, 2, 6, 3 and 1). In addition, the WIS decreased with more than 90% for those experiments (Table 4.4). The TS ratio is also somewhat higher than 1 for the experiments where the pH decreased slightly (experiment 7 and 4), but the change in WIS is, however, not higher than 85%. The highest values for the TS ratios and change in WIS were obtained in experiment 3 (10% DM) with the values 1.07 and 100%, respectively.

Table 4.4: The TS ratio in the material after and before oxidation, the mass of oxygen consumed per mass of initial DM and the change in mass of the material mass of initial DM for all oxidation experiments. The total pressure after cooling of the reactor was also noted for most experiments. A dash (-) is indicated for the experiments were the final pressure had not been noted. Green implies a stable pH during the experiment, orange a slight decrease in pH and no color a signinficant decrease in pH.

DM (%)	Exp. no.	$TS_{oxidized}/TS_{initial}$ (g TS_{ox}/g TS_{in})	Decrease of WIS $(WIS_{in} - WIS_{ox})/WIS_{in}$ (%)	m_{O2}/m_{DM} (g/g TS_{in})	$\Delta m_{material}/m_{DM}$ (g/g TS_{in})	P_{Tot} after cooling (bar)
6.5	**13**	1.02	98.7	0.226	0.311	9
10	**2**	1.00	93.1	0.161	0.188	6.5
	6	1.00	97.5	0.268	0.315	7
	3	1.07	100	0.215	0.366	-
	7	1.05	85.6	0.376	0.463	9
17.5	**11**	0.97	83.4	0.184	0.133	7
	15	1.00	86.6	0.154	0.152	6
	14	0.85	86.9	0.177	0.092	-
	10	0.85	83.4	0.146	0.131	8
	16	0.93	83.1	0.231	0.242	-
	17	0.90	81.8	0.277	0.278	6
	1	0.94	91.9	0.252	0.163	4
25	**4**	1.02	82.4	0.120	0.039	3
	8	1.00	83.0	0.227	0.074	4
	5	0.78	86.1	0.199	0.080	6
	9	0.84	84.6	0.189	0.039	7
28.5	**12**	0.91	82.6	0.091	-0.060	8

A TS ratio below 1 suggests that degradation has occurred. In combination with the change in WIS, these measures can indicate what type of degradation that occurs. As mentioned, a low value of the

TS ratio implies excessive oxidation. This does, however, not necessarily mean that more of the insoluble part of the material, the lignin, is dissolved. The results from experiments 5 and 9 (both 25% DM) can serve as an example, as the lowest TS ratios of 0.78 and 0.84 were obtained in these experiments, respectively. The corresponding changes of WIS in these experiments were 86.1% and 84.6%, respectively. These values show that even if the TS ratio is low, some water insoluble particles are still present. Probably the significant decrease in pH during these experiments deactivated the NaOH, catalysing the oxidative degradation. This, in turn, stopped the dissolution of the insoluble fractions of the material and favored formation of CO_2 from the already solubilized fragments instead. In the case of experiments 5 and 9, this suggests that the low TS ratios were due to material that left the system as CO_2, rather than having more biorefinery lignin being solubilized. It can, thus, be established that a low TS ratio can not alone be used as measure of material degradation.

The occurrence of low TS ratios together with moderate changes in WIS could also be due to repolymerization of lignin fragments. As will be discussed in section 4.2.5, some gravel-like fragments were formed after experiments 8, 5 and 9 (all with 25% DM). Obviously, the formation of insoluble and repolymerized fragments will reduce the change in WIS. Regardless of how this should be interpreted in terms of dissolution, CO_2 formation, TS ratio and change in WIS, it can be concluded that repolymerized fragments are counteracting the initial aim and is a highly undesirable outcome.

4.2.1.2 pH stability

Based on the pH measurements made, it is possible to conclude that pH could be kept stable at lower DM contents. pH was stable for experiments 13 (6.5% DM), 2, 6, 3 (10% DM) and 1 (17.5% DM). A slightly decreasing pH was noted in experiments 7 and 4, with 10% and 25% DM content, respectively. All the remaining experiments, where the DM content was at least 17.5%, had a significant decrease in pH (Table 4.4).

The NaOH loading was sufficient to withstand an excessive oxidation in experiments with the low initial DM content. The outcome does, however, also depend on the severity of the reaction conditions. As can be seen when the reaction time was doubled from experiment 3 (10% DM, 200˚C, 15 min, 15.9 wt% NaOH) to experiment 7 (10% DM, 200˚C, 30 min, 15.9 wt% NaOH), a slight decrease in pH occurred. The present NaOH could, thus, not buffer the created acids any longer when the reaction continued for more than 15 min. The importance of an appropriate NaOH concentration is further confirmed by experiment 1 (17.5% DM, 20.4 wt% NaOH), having a higher DM content but also the highest NaOH concentration among all the experiments. It shows that a mixture with higher DM content can be oxidized having a stable pH, as long as the NaOH concentration is sufficiently high. A rough estimation of an appropriate NaOH amount per g lignin is proposed and further discussed in section 4.3.1.

The influence of the reaction severity can also be exemplified by experiment 4 (25% DM, 160˚C, 15 min, 9.8 wt% NaOH). Beside the already discussed experiment 1 (17.5% DM, 20.4 wt% NaOH), experiment 4 is the only experiment with DM above 17.5% that did not have a significant decrease in pH. What makes this experiment unique, compared to all the other ones above 17.5% DM content, is that it had the shortest reaction time at such a low temperature. In comparison, experiment 11 (17.5% DM, 150˚C, 23 min, 9.8 wt% NaOH) had the same NaOH concentration but lower DM content and temperature. The reaction time in experiment 11 was, however, 23 min instead of 15 min.

Another aspect that could be important to consider regarding the pH stability is the carbonate system and its buffering capacity. Since CO_2 is in equilibrium with H_2CO_3, HCO_3^- and CO_3^- in aqueous solutions, changes in pH can also affect the equilibrium between these species. Any CO_2 that arises

from excessive oxidation may be dissolved in the water and become H_2CO_3, which in turn can be deprotonated and become HCO_3^-. The HCO_3^- can both act as an acid as a base, which thus provide a buffering capacity to the system. The question is, however, how large effect the carbonate system has on the conditions in the reactor when relatively high temperatures and pressures are used. In general, CO_2 dissolves less in water the higher the temperature is. It is, however, hard to predict how this process becomes when a high pressure is applied. Perhaps the dissolution of CO_2 also changes throughout the experiment. For instance, if any CO_2 is formed during the reaction, it could leave the reaction solution as gas. During cooling of the reactor, more of the gaseous CO_2 could dissolve in the reaction solution again and impact the final pH in the system. Continuous measurements throughout the whole experiment would be required to examine this process. Since it is hard to assess the impact of the carbonate system, all further discussions related to pH and acidity are simplified so that only acids created from the oxidation of the biorefinery lignin are considered.

4.2.1.3 Oxygen consumption

Ratios have been used to assess the oxygen consumption and the change in mass of the material. The ratio of consumed oxygen (m_{O2}) over the initial DM content (m_{DM}) indicates the amount of oxygen assimilated by the material. The change in mass of the material (Δm) over the initial DM content (m_{DM}) is a ratio that indicates how much the mass has increased in the material due to the oxidation. As seen in Table 4.4, these two different ratios are roughly in the same magnitude for all experiments, with exception to the experiments with a DM content of 25% or above. Since the materials were still very thick and not very liquefied after oxidation in these experiments, it was rather difficult to transfer everything from the reactor without significant losses. In experiment 12 (28.5% DM), the material was still so thick after oxidation that the material losses in the reactor were so significant that the total mass before and after the reaction decreased. The ratio of consumed oxygen is, thus, a more reliable measure of what has happened during these experiments.

Among the experiments where pH was stable, a ratio of 0.268 g O_2/g TS in experiment 6 (10% DM, 160˚C, 30 min) appears to be the highest amount of oxygen consumed without having an impact on the pH. The lowest value of 0.161 g O_2/g TS was, in contrast, obtained in experiment 2 (10% DM, 160˚C, 15 min). As will be discussed in section 4.2.3, the obtained amount of acids per g TS was also the lowest for experiment 2, making it the experiment with stable pH yielding the lowest amount of oxidation products. Whether the recorded amount of consumed oxygen per g TS is a good measure to predict the outcome of the oxidation needs more investigation before any more general conclusions can be drawn, though.

The value of the total pressure after the reactor has cooled down is another interesting aspect of the oxygen consumption. An oxygen pressure of 10 bar was only applied during the reaction time. If the material would have stopped being reactive after the reaction time, it could be expected that the pressure gauge would indicate 10 bar when the reactor was cooling down after reaction. As can be seen in Table 4.4 though, this was not the case for almost any of the experiments where the final pressure had been noted. This suggests that the material in the reactor continued so assimilate oxygen, even after the reaction time, when heating and oxygen supply was turned off. The most remarkable about these results is that the pressure after the reactor had cooled down was, in general, lower for the experiments with higher DM content. The lowest pressures were recorded for experiments 1 (17.5% DM), 4 and 8 (both 25% DM), having values at 4, 3 and 4 bar, respectively. During experiment 4 and 8, large amounts of foam were also present when the reactor vessel was opened. It could be interpreted as if NaOH could not withstand the increased loading of acids so that they were further oxidized to carbon dioxide. The pressure after the reactor has cooled down is, thus, an additional aspect to examine the oxidation and should be noted with greater care in future studies.

4.2.2 UV spectra

The UV spectra of the oxidized materials can be compared to the spectrum of untreated biorefinery lignin (Figure 4.4). None of the oxidized materials have a peak around 280 nm in their UV spectrum, suggesting that degradation or modifications have occurred to the phenolic groups deemed as the reason to the absorbance peak at that wavelength. Otherwise, the UV spectra of all oxidized materials show more differences than similarities.

The main difference between the different oxidized materials is related to the pH of the material. Where pH was kept stable during the oxidation, the UV spectra have evident peaks around the wavelengths 250 nm and 340 nm (Figure 4.5). As mentioned in section 2.4.4.2, vanillic acid and vanillin have been monitored in previous studies at 254 nm and 348 nm, respectively. This could suggest that the peaks in Figure 4.5 are due to the presence of these phenolic compounds. In the experiments with a slight pH drop, the presence of peaks is only slightly hinted (Figure 4.6). Where the pH had decreased significantly during the experiment, no peaks can be seen (Figure 4.7).

The lack of obvious peaks in the experiments where pH decreased correlates well with the fact that the stability of aromatic compounds like vanillin is very pH dependent. As the pH decreased, so did the amount of that aromatic compound. This can be further strengthened by comparing the absorbance ratios presented in Table 4.5-4.7. The absorbance ratio could be interpreted as the absorbance of vanillin in relation to the absorbance of vanillic acid. For experiments where pH decreased, the meaning of the ratio is not as obvious since no peaks are present. What is clear, though, is that the ratios for experiments with stable pH (Table 4.5) are generally higher than the ratios in the experiments where pH decreased (Table 4.6-4.7). A higher value of the ratio could imply that there is more vanillin present, contributing to higher absorbance around 348 nm. However, the values of the absorbance ratio are rather high for the untreated biorefinery lignin as well (Table 4.2), which contradicts this interpretation as no peaks are present at the considered wavelengths. The absorbance ratio and its interpretation should, thus, only be limited to the evaluation of experiments with stable pH.

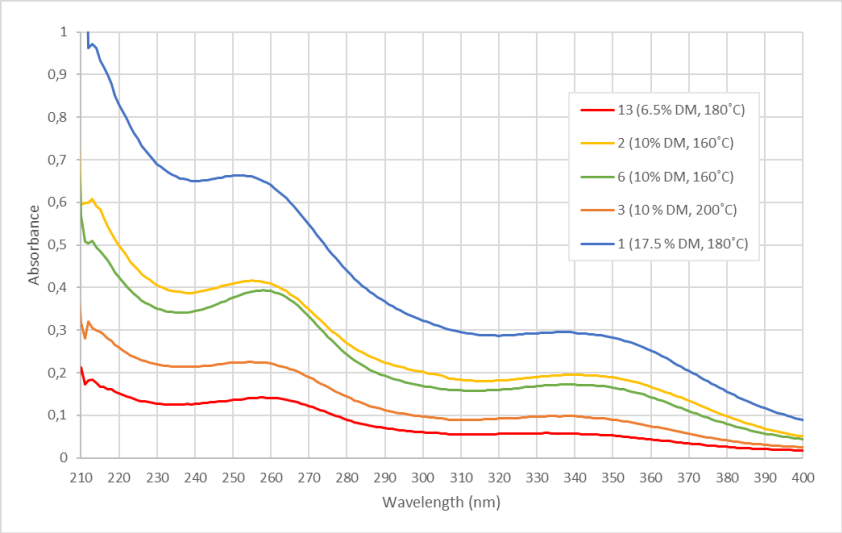


Figure 4.5: UV spectra for the experiments with stable pH.

Table 4.5: Absorbance ratio values for the experiments with stable pH.

DM (%)	Exp.no.	Abs_{348nm}/Abs_{254nm}
6.5	**13**	0.388
10	**2**	0.461
	6	0.432
	3	0.414
17.5	**1**	0.432

Another aspect that can be analysed in the UV spectra in Figure 4.5-4.7 relates to the severity of the oxidation. While the absorbance ratio can tell something about the oxidation products formed for the experiments with stable pH, the general shape of the spectrum can give an indication of the degradation of the material in all other experiments. Comparisons should, however, only be made between experiments with the same initial DM content. As an example, the spectra for experiments 5 and 9 (both conducted at 25% DM, 200˚C) in Figure 4.7 show absorbance at lower levels than the spectra for experiment 8 (25% DM, 160˚C). The difference between these experiments is that the temperature is higher in the two former. This indicates that a higher temperature will give lower UV absorbance. Comparing this result to the values of the TS ratios in Table 4.4, where both experiments 5 and 9 have lower values than experiment 8, it is possible to conclude that the lower absorbance is due to a higher degree of degradation. A similar tendency can be seen in Figure 4.5, where the absorbance of experiment 3 (10% DM, 200˚C) is much lower than for experiments 2 and 6 (both 10% DM, 160˚C). Experiment 10 (17.5% DM, 210˚C) is, however, an exception to this tendency as it shows a higher level of absorbance compared to all the other experiments with 17.5% DM content, even though it has the highest temperature of all experiments on the study. Temperature is thus not the only factor affecting the absorbance.

The level of absorbance is also correlated to the initial DM content. In general, a higher initial DM content gives higher absorbance, most clearly shown in Figure 4.6. In the cases with stable pH, the higher absorbance should relate to a higher concentration of phenolic compounds, further described in section 4.2.3.2. In the cases where pH was unstable, the higher absorbance in the material from some of the experiments could simply be due to amount of material. The more material that is present, the more it can absorb the light from the spectrophotometer. That is why all experiments with the highest DM content are on the upper parts in all graphs in Figure 4.5-4.7. The only exception, however, is the spectrum for experiment 12 (DM 28.5%, 180˚C), which have the lowest overall absorbance compared to the other experiments with a significant pH drop in Figure 4.7. The reason could be that the material from experiment 12 was prepared differently compared to the others prior to the UV measurements. Since no liquid could be decanted from the material after oxidation, the liquid used in the spectrophotometric analysis was composed of the water that the material had been washed in. Only the water-soluble compounds would solubilize in the washing water, so this might be why the absorbance is so much lower for experiment 12.

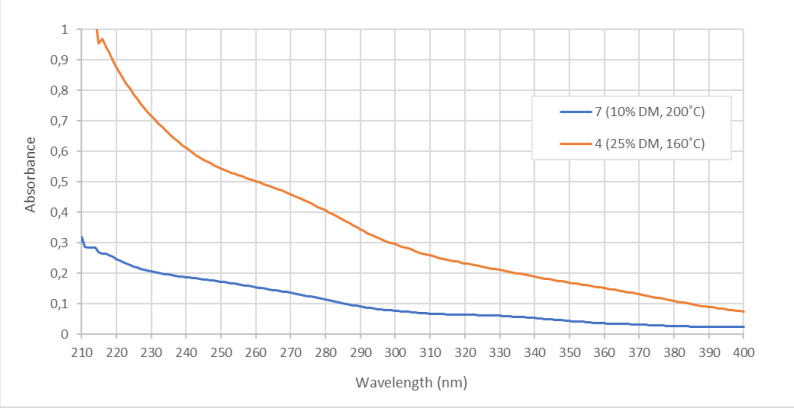


Figure 4.6: UV spectra for the experiments with slightly decreased pH.

Table 4.6: Absorbance ratio values for the experiments with slightly decreased pH.

DM (%)	Exp.no.	Abs_{348nm}/Abs_{254nm}
10	**7**	0.278
25	**4**	0.330

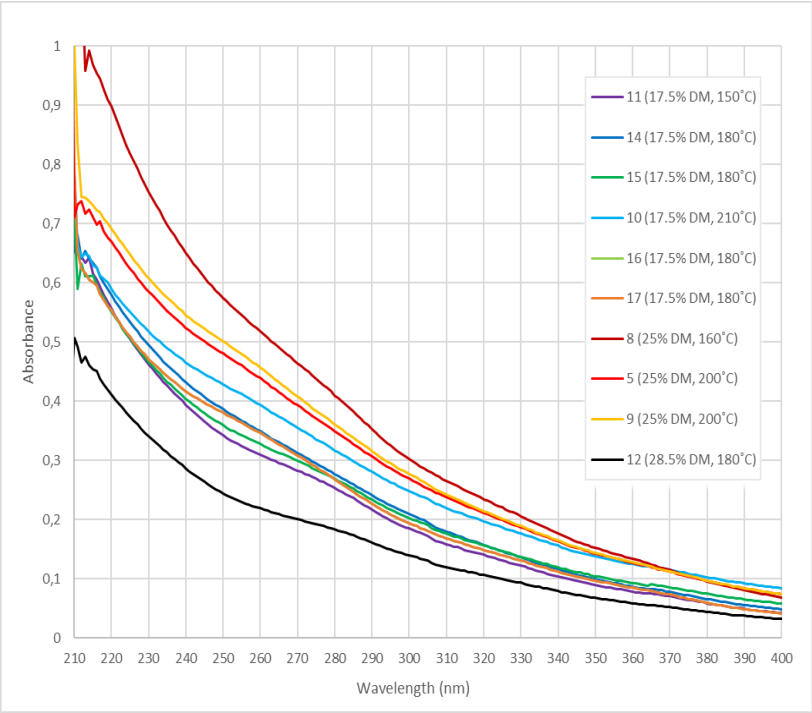


Figure 4.7: UV spectra for the experiments with significantly decreased pH.

Table 4.7: Absorbance ratio values for the experiments with significantly decreased pH.

DM (%)	Exp.no.	Abs_{348nm}/Abs_{254nm}
17.5	**11**	0.280
	14	0.278
	15	0.316
	10	0.341
	16	0.289
	17	0.272
25	**8**	0.285
	5	0.316
	9	0.305
28.5	**12**	0.301

4.2.3 Oxidation products

The oxidation products have been analyzed at several levels, starting from a coarse monomer analysis and ending with identification of those monomers. The analyzed monomers involved phenolic compounds and organic acids. All these analyses were, however, only performed for the experiments with stable pH (experiments 13, 2, 6, 3 and 1). Experiments without a decreased pH were chosen because stable systems are easier to optimize and make predictions from. To be coherent, the different experiments have been assigned the same colors in all graphs as in the chromatogram in Figure 4.8. Red has been used for experiment 13, yellow indicate experiment 2, green indicate experiment 6, experiments 3 has been assigned orange, whereas blue is used for experiment 1. The results show that all examined experiments have both phenolic compounds and organic acids present, but in different degrees.

4.2.3.1 Molecular weight distribution

The molecular weight distribution is a first step to see what has happened to the oxidized materials, based on the mass of its components. In Figure 4.8, it is possible to see that the chromatograms of all experiments have a similar shape. The magnitude of the response is roughly the same as well, except for experiment 1 (17.5% DM) where the signal is much stronger. The values for the weight average molar mass (M_w) and number average molar mass (M_n) are also the highest for experiment 1 (Table 4.8). This should relate to the fact that experiment 1 had the highest initial DM content and, therefore, cause a stronger response in the analysis, as previously discussed in relation to UV absorbance (section 4.2.2). Since a relatively large fraction of the chromatograms exceeds 1000 Da, this suggests that many large molecules are still left in the material that have not depolymerized. The analysis was,

however, performed on the filtrated liquid fraction of the oxidized materials, indicating that these large molecules at least are water-soluble.

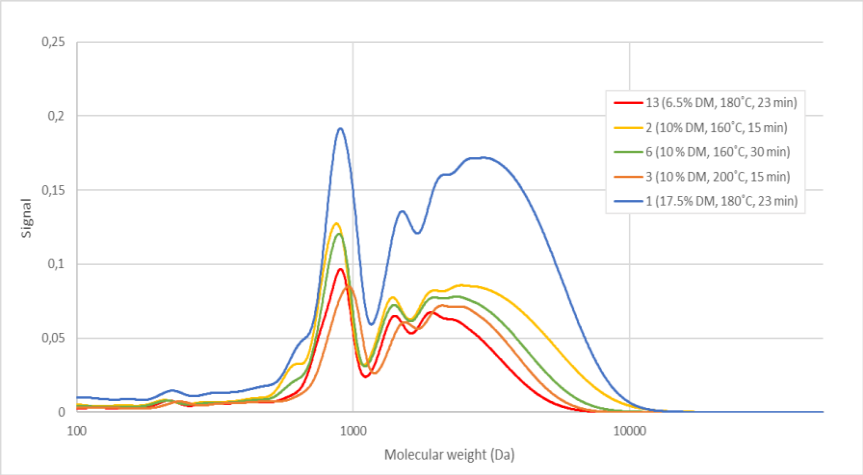


Figure 4.8: Molecular weight distribution for the experiments with stable pH.

Table 4.8: Weight average molar mass (M_w), number average molar mass (M_n) and the polydispersity index (PDI) for the experiments with stable pH.

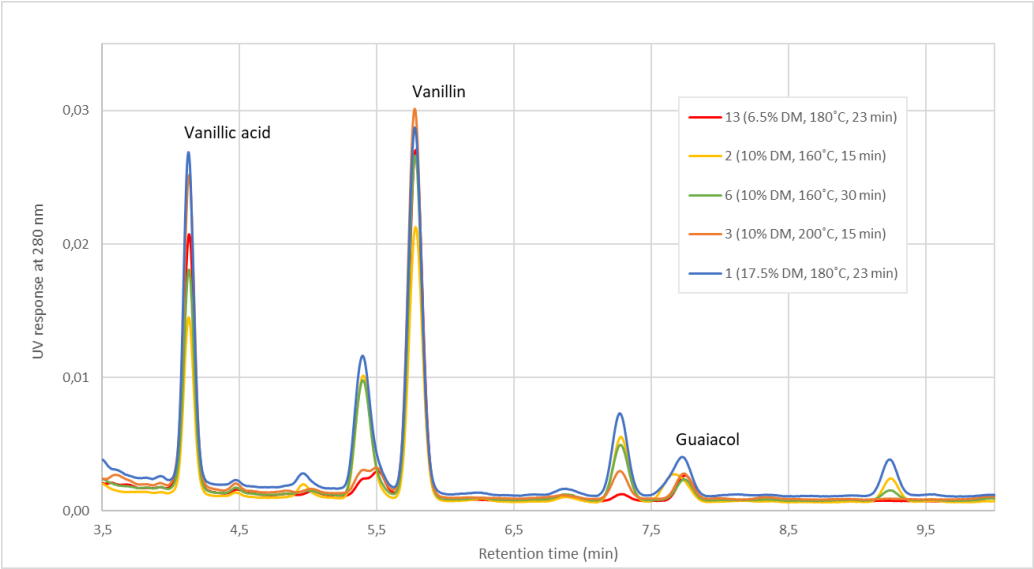
DM (%)	Exp. .no.	M_w (Da)	M_n (Da)	PDI
6.5	13	1740	1030	1.7
10	2	2370	1170	2.0
	6	2000	1100	1.8
	3	1980	1130	1.7
17.5	1	2570	1250	2.1

4.2.3.2 Phenolic compounds

Essentially the same phenolic compounds are present in all experiments with stable pH, see the chromatograms in Figure 4.9 a) and b). The chromatograms have been obtained at different dilutions, being 1:25 for experiment 13 (6.5% DM), 1:50 for experiment 1 (17.5% DM) and 1:40 for 2, 6 and 3 (10% DM). Only vanillic acid, vanillin and guaiacol could be quantified, but at least four more significant peaks from unidentified compounds can be seen. In general, small and/or hydrophilic molecules have shorter retention times, whereas larger and/or more hydrophobic compounds have longer retention times. This corresponds well to the chromatograms in Figure 4.9, as vanillic acid, with the shortest retention time, can be suspected to interact better with water compared to guaiacol, having a longer retention time and more of a nonpolar benzene structure.

Even though the height differs between the different peaks, it does not necessarily mean that the compound that causes the highest peak has the highest concentration. This is because UV response was used in the analysis. In such case, a strong signal only means that the UV absorbance is high for that specific compound. The UV absorbance increases when conjugated systems are present, such as in aromatic rings with adjacent double bonded carbon on the side chains. As an example, the peaks for vanillic acid and vanillin in Figure 4.9 a) can be compared with the obtained concentrations presented in Table 4.9. It is evident that the tabulated values of vanillic acid concentration are higher than the ones for vanillin for all experiments, even though the peaks for vanillic acid are slightly smaller compared to the peaks for vanillin. The only difference between these compounds is the additional hydroxy group in the vanillic acid, which thus seems to have a slight impact on the UV absorption.

a)



b)

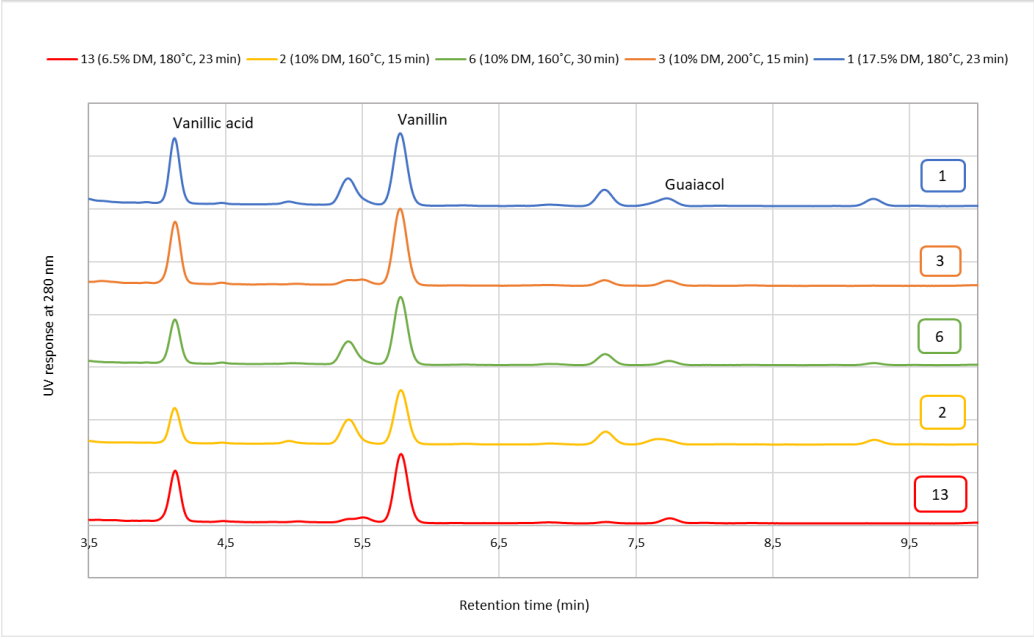


Figure 4.9: UPLC chromatograms for phenolic compounds formed during the experiments with stable pH. a) All chromatograms in the same scale but with different dilutions (1:25 for experiment 13, 1:40 for experiments 2, 6 and 3 and 1:50 for experiment 1). b) Stacked plot of the chromatograms in a).

Since not all peaks could be quantified, the actual amount of produced phenolic compounds is higher than what the tabulated values show. Further investigations are thus required to find appropriate calibration standards to relate the obtained peaks with. Some very likely phenolic compounds that should be present after alkaline wet oxidation of wheat straw lignin are syringaldehyde and syringic acid (see section 2.4.4.2). After all, wheat straw lignin generally contains almost equal proportions of G and S units (described in section 2.3.3), which suggests that some phenolic compounds with an S-type structure also should arise during the oxidation. Even though the lignin structure in biorefinery lignin can be expected to be slightly different than in the native lignin (section 2.5), it should still be probable to detect S-type compounds among the obtained oxidation products. In fact, the question is rather whether any H-type compounds can be obtained, due to the higher reactivity of the H unit. How large

the yield of the phenolic compounds *p*-coumaric acid and ferulic acid from degradation of the "lignin/phenolics-carbohydrate"-complex in the wheat straw is also an aspect to consider.

The concentrations of the phenolic compounds provide important information for a subsequent anaerobic digestion. The total concentration of the quantified phenolic compounds ranges between 1.36 g/L and 4.53 g/L in the analysed experiments (Table 4.9). None of the experiments have vanillin concentrations above the upper limit 2 g/L. However, since 1.2 g/L is the limit for phenol before methane production is inhibited, the question is what will happen to the anaerobic digestion when there is a mixture of phenolic compounds in total concentrations that are higher than that. Moreover, the obtained total concentrations should also be higher in reality, since not all phenolic compounds were quantified. This is yet another reason to why further investigations are needed.

Table 4.9: Concentrations and obtained amounts per g TS of the phenolic compounds vanillin, vanillic acid and guaiacol for the experiments with stable pH. To simplify comparisons between the very similar experiments 2,6 and 3, the temperatures and reaction times in these experiments are indicated.

DM (%)	Experiment no.	Vanillin (g/L)	Vanillic acid (g/L)	Guaiacol (g/L)	Total conc. (g/L)	Vanillin (mg/g TS)	Vanillic acid (mg/g TS)	Guaiacol (mg/g TS)	Total amount (mg/g TS)
6.5	13	0.40	0.70	0.26	1.36	5.80	10.11	3.81	19.72
10	2 (160˚C, 15 min)	0.48	0.80	0.63	1.90	4.16	6.95	5.48	16.59
	6 (160˚C, 30 min)	0.62	0.76	0.38	1.76	5.34	6.57	3.31	15.21
	3 (200˚C, 15min)	0.67	1.09	0.19	1.95	5.39	8.78	1.50	15.67
17.5	1	1.08	2.14	1.32	4.53	3.72	7.39	4.55	15.66

The values in Table 4.9 show that the concentration of the quantified phenolic compounds increase with an increased DM content, graphically presented in Figure 4.10 a). This is a very reasonable outcome since more material present in the oxidation also should bring more oxidation products. The most similar experiments can, furthermore, be compared in order to analyze the impact of the reaction conditions. The NaOH concentration are the same for the three experiments with 10% DM content. Hence, temperature and reaction time are the only parameters that differ between experiments 2 (160˚C, 15 min), 6 (160˚C, 30 min) and 3 (200˚C, 30 min). From Table 4.9 and Figure 4.10 a), it is thereby possible to deduce that more severe reaction conditions, with higher temperature and/or longer reaction time, increase the vanillin concentration and decrease the guaiacol concentration. However, this is only valid in the specific case when looking at experiments with the same content of DM and NaOH. For a more general analysis, see the response surfaces in section 4.2.4.

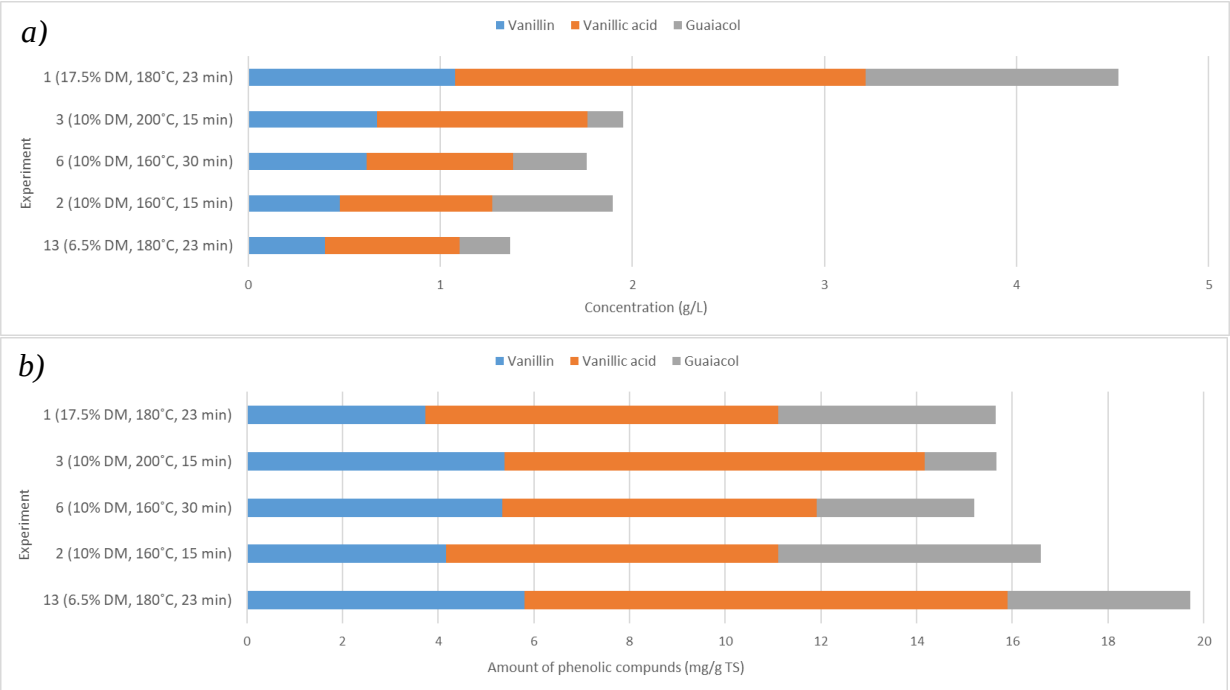


Figure 4.10: Graphical overviews of the values presented in Table 4.9 where a) Obtained concentrations of phenolic compounds, b) Amount of phenolic compounds per g TS.

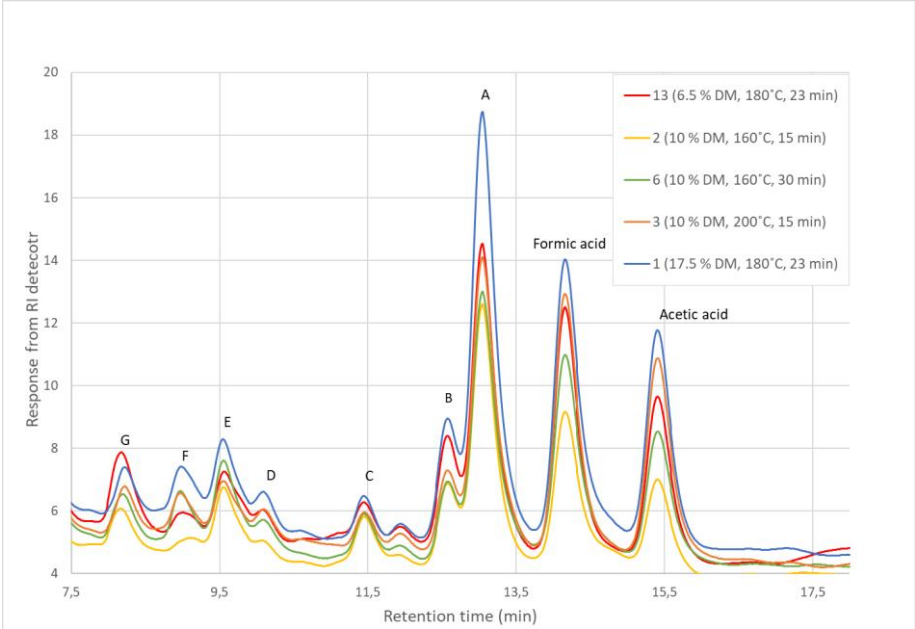
When the yield of the compounds is put in relation to the amount of TS instead, the results become rather different compared to when only the obtained concentrations are analyzed. Then, experiment 13 (6.5% DM) has the highest amount of phenolic oxidation products and experiment 6 (10% DM) has the lowest (Table 4.9 and Figure 4.10 b)). The same tendency as could be seen in the attained concentrations, where higher temperature and/or longer reaction time increased the amount of vanillin and decreased the guaiacol yield, can still be noted when comparing the obtained amount of compounds per g TS. In fact, the yields of vanillin and vanillic acid are the highest in experiment 13 (6.5% DM). The yield of guaiacol is, however, highest for experiment 2 (10% DM, 160˚C, 15 min), where the reaction conditions are the least severe among the five examined experiments. In all experiments, the highest yields are obtained for vanillic acid ranging from 5.57-10.11 mg/g TS. Vanillin has been produced in amounts in the range 3.72-5.80 mg/g TS. The yields of guaiacol are generally the lowest, with exception to experiments 2 (10% DM) and 1 (17.5% DM) where the amount of guaiacol exceeds the amount of vanillin.

The magnitude of the quantified phenolic compounds is in line with values reported in previous studies. For instance, in alkaline wet oxidation of biorefinery lignin from Douglas fir residues, a vanillin amount of 2.31 mg/g dry biomass was obtained at 210˚C, 10% solids loading, 15 min reaction time and 17.5% NaOH loading.[43] The most similar conditions present in this study were employed in experiment 3 (200˚C, DM 10%, 15 min, 15.9 wt% NaOH), where the obtained amount of vanillin was 5.39 mg/g TS. As presented in section 2.4.4.2, alkaline wet oxidation of raw wheat straw has given yields of vanillin, vanillic acid and guaiacol up to 96 mg/100 g straw, 122 mg/100 g straw and 35 mg/100 g straw, respectively. All these yields were, however, obtained at different conditions of the oxidation. Therefore, one of these oxidation products should be chosen as the most desirable, so that future experiments can be designed to optimize its formation.

4.2.3.3 Organic acids

The same organic acids are present in almost all analyzed samples (Figure 4.11 and Figure 4.12). Even though 7 out of 9 obtained acids could not be identified, an approximate quantification was made by relating the unknown peaks to acetic acid. The unidentified acids have been named Unknown A, B, C, D, E, F and G, respectively, to make it easier to differentiate between them. It is important to note that the chromatograms have been obtained at different dilution of the samples. The dilutions were 1:25 for experiment 13 (6.5% DM), 1:50 for experiment 1 (17.5% DM) and 1:40 for experiments 2, 6 and 3 (10% DM).

a)



b)

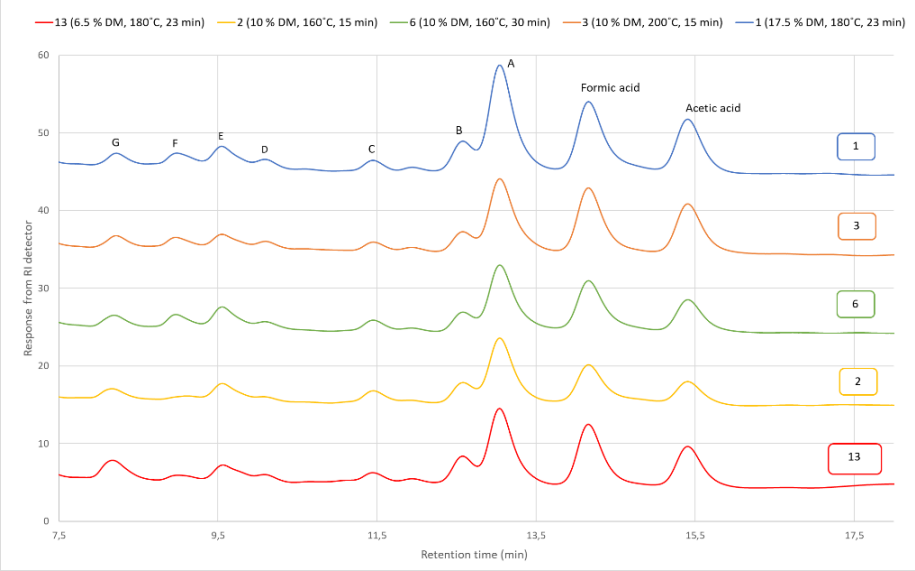


Figure 4.11: HPLC chromatograms for the organic acids formed during the experiments with stable pH. a) All chromatograms in the same scale but with different dilutions (1:25 for experiment 13, 1:40 for experiments 2, 6 and 3 and 1:50 for experiment 1), b) Stacked plots of the chromatograms from a).

As can be seen in the chromatograms in Figure 4.11 and the graphical overviews in Figure 4.12 a) and b), formic acid, acetic acid and Unknown A have been formed to the greatest extent in all ex-

periments with stable pH. Furthermore, Unknown F could not be detected in the sample from experiment 2, and neither could Unknown D for experiments 13 (6.5% DM), 2 and 6 (both 10% DM). The retention in the chromatograms in Figure 4.11 depends on pK_a and the polarity of the compound. Large molecules are retained early, whereas smaller compounds attain longer retention times. Even though 14 different compounds were used as calibration standards, only two acids were identified in the analysis. Most standards used in this study could, therefore, be excluded in future studies.

The concentrations and amounts of the obtained organic acids are significantly higher compared to the values of the phenolics compounds, see Table 4.9 and Table 4.10. The values of the total concentrations and the total amounts of acids are roughly 15-20 times larger than the corresponding values for the phenolic compounds. As previously mentioned, though, the quantities of the unknown acids are only estimated. All phenolic compounds present in the materials have not either been quantified. Despite these sources of error, the difference between the amounts of the different types of compounds is still very large. Such a large difference between the different oxidation products have been reported in previous studies as well (compare with section 2.4.4.2). Previous studies of alkaline wet oxidation of biorefinery lignin were in the ranges 33.3-116 mg/g dry biomass for formic acid and 23.6-107 mg/g dry biomass for acetic acid.[43] In this study, the yields of formic acid and acetic acid were in the ranges 62.4-118.9 mg/g TS and 26.2-57.2 mg/g TS, respectively. It is, thus, likely that organic acids are the main oxidation products formed during the experiments with stable pH. Whether these oxidation products come from oxidation of lignin, or oxidation of the other components in the biorefinery lignin, is, however, a crucial aspect to consider. Nonetheless, the obtained acid concentrations are above the acceptable limit of 3 g/L, which means that the concentrations in the oxidized materials are too high to be directly applied in anaerobic digestion.

Table 4.10: Concentrations and obtained amounts per g TS of organic acids for the experiments with stable pH. To simplify comparisons between the very similar experiments 2,6 and 3, the temperatures and reaction times in these experiments are indicated.

DM (%)	Experiment no.	Formic acid (g/L)	Acetic acid (g/L)	All unknown acids* (g/L)	Total conc. (g/L)	Formic acid (mg/g TS)	Acetic acid (mg/g TS)	All unknown acids* (mg/g TS)	Total amount (mg/g TS)
6.5	13	8.23	3.74	12.60	24.57	118.9	54.0	182.0	355.0
10	2 (160˚C, 15 min)	7.14	2.99	15.63	25.77	62.4	26.2	136.6	225.2
	6 (160˚C, 30 min)	12.08	4.49	18.46	35.03	104.3	38.8	159.4	302.5
	3 (200˚C, 15min)	13.95	7.13	20.03	41.12	111.9	57.2	160.7	329.8
17.5	1	21.33	10.18	36.03	67.53	73.7	35.2	124.5	233.4

** Estimated relative to peak area and obtained concentration of acetic acid.*

Overall, the tendencies of the organic acid formation are similar to the tendencies seen in the formation phenolic compounds. Firstly, the total concentration of organic acids also increases with increasing DM content (Table 4.10 and Figure 4.12 a)). Secondly, as the severity of the conditions increases, going from experiments 2 (10% DM, 160˚C, 15 min) and 6 (10% DM, 160˚C, 30 min) to 3 (10% DM, 200˚C, 15 min), the produced amount of organic acids increases. Lastly, experiment 13

(6.5% DM) appears to be the experiment where the highest amounts of organic acids per g TS have been formed.

One difference compared to the phenolic compounds is that experiment 2 (10% DM, 160˚C, 15 min) had the lowest amount of acids produced per g TS, only reaching 225.2 mg/g TS. In contrast, experiment 6 (10% DM, 160˚C, 30 min) was the experiment with lowest yield of phenolic compounds being 15.21 mg/g TS. These experiments had the exact same reaction conditions except the reaction time. This shows, thus, that lower temperature and shorter reaction time is more beneficial for the formation of phenolic compounds, whereas the formation of organic acids is enhanced when the reaction time is doubled. However, the difference in the yield of phenolic compounds between these two experiments is only 1.38 mg/g TS, compared to a difference of 77.3 mg/g TS for the organic acids. The variation of organic acid yield varies, hence, more than the yields of phenolic compounds, which also can be seen when comparing Figure 4.10 b) and 4.12 b). This makes optimization of the oxidation an even more important aspect, in order to obtain as high yields of oxidation products as possible. As previously stated, though, it is essential to consider the origin of the oxidation products, especially when it comes to biorefinery lignin with fractions that may respond differently to oxidation.

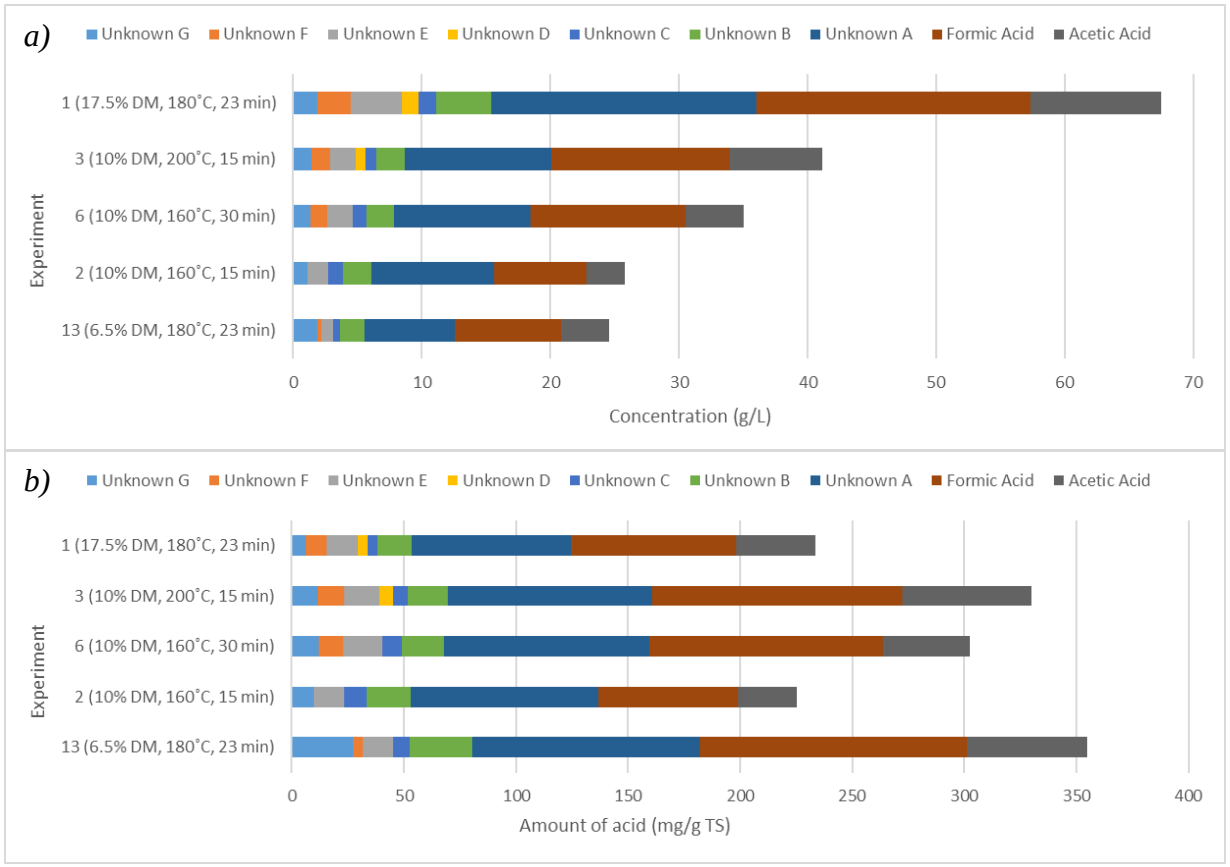


Figure 4.12: Graphical overviews of the values presented in Table 4.10 where a) Obtained organic acid concentrations, b) Amount of organic acids per g TS.

Phenolic compounds can only be derived from lignin oxidation, whereas organic acids can be obtained from oxidation of either lignin, cellulose, or hemicellulose. Since the untreated biorefinery lignin contained 11.9% carbohydrates (Table 4.1), at least the same share of the obtained organic acids should originate from that fraction. To assess whether the obtained organic acids originated from lignin or from the carbohydrate fractions, some approximate mass balance calculations were

performed. To simplify, it was assumed that the whole fractions of cellulose and hemicellulose in the entire material, including both the solid and liquid fractions of the biorefinery lignin, were converted to organic acids. It was also assumed that the carbohydrates were degraded more easily, and thereby were oxidized before the lignin. The theoretically maximal mass of acids that could have been formed from carbohydrates was subtracted from the obtained amount of organic acids in the measurements, see Table 4.11 for the result. A negative value could either be interpreted as if the oxidation of the carbohydrates was not complete, or as if the material had undergone excessive oxidation, so that mass was lost and had left as CO_2. A positive value, on the other hand, can be understood as if more organic acids have been formed than what could have been derived from the carbohydrate fractions only. The positive difference should, therefore, be the only amount of organic acids that with certainty has originated from lignin oxidation.

Only the experiments 13 (6.5% DM) and 3 (10% DM) have a positive value in Table 4.11, implying that these two experiments are the only ones where some organic acids have been formed through oxidation of lignin. It can also be seen graphically in Figure 4.12 b), where the bars for these experiments also reach the highest values. For experiment 13 (6.5% DM), at least 9.4% of the total amount of organics acids should be derived from lignin, corresponding to an amount of approximately 33 mg/g TS. The corresponding values for experiment 3 (10% DM) is 2.5% and 8.2 mg/g TS. It is difficult to draw any general conclusions for what the reason for this could be, based on the findings in this study. One aspect that could simplify and increase the understanding is, however, if the biorefinery lignin would be exposed to the same experiments but is washed before the oxidation. The washing should reduce the residual carbohydrates in the liquid fraction, which in turn should lower the yields of organic acids. That could, perhaps, make it more evident which conditions that promote oxidation of lignin to organic acids. In addition, compositional analyses of the carbohydrate content in the oxidized materials could also indicate how these fractions have change during the oxidation (further discussed in section 4.3.3)

Table 4.11: Mass balance of organic acids derived from cellulose and hemicellulose for the experiments with stable pH. The acid amount represents the difference between the amount of organic acids obtained and the theoretically maximum amount that could be derived from those carbohydrate fractions in the untreated biorefinery lignin.

DM (%)	Experiment no.	Acid amount derived from lignin ($m_{Obtained}$-$m_{Theorerical\ max.}$) (g)	Proportion derived from lignin (Acid amount/$m_{Obtained}$) (%)	Estimated amount of acids derived from lignin (mg/g TS)
6.5	**13**	1.6	9.4	33
10	**2**	-7.2	-	-
	6	-1.4	-	-
	3	0.6	2.5	8.2
17.5	**1**	-11.5	-	-

What can be concluded, based on both the amounts of phenolic compounds and organic acids obtained, is that experiment 13 (6.5% DM) has the highest amount of oxidation products per g TS compared to all other experiments with stable pH. In combination with the mass balance calculations, it

also becomes evident that experiment 13 has the highest proportion of organic acids that should originate from lignin. This means that the conditions in experiment 13 (180˚C, 6.5% DM, 23 min, 9.8 wt% NaOH) are the optimal to oxidize biorefinery lignin from wheat straw, at least within the ranges applied in this study and whilst a stable system is maintained.

4.2.4 Models and response surfaces

Response-surface modelling was applied to describe and predict the outcomes of the oxidation. The results from the modelling are analyzed from three different aspects: oxygen assimilation, UV absorbance ratio and degradation of material. Oxygen assimilation and the material degradation are, in fact, measures of the same thing, since both aspects are analyzed based on the TS ratio. The difference is what outcome from the oxidation that is desired. The desired outcome of oxygen assimilation is to produce as many oxidation products as possible without losing material, meaning that the goal is to obtain a TS ratio that is above 1 and as high as possible. The desired outcome of material degradation, on the other hand, is just to degrade the biorefinery lignin as much as possible, implying a TS ratio below 1 and as low as possible.

In this study, experiment 13 (180˚C, 6.5% DM, 23 min, 9.8 wt% NaOH) was deemed as the experiment with the most optimal conditions for high yield of oxidation products. Hence, the response surface-modelling was based on coded values from that experiment (Table 3.1). First, the results for oxygen assimilation are presented. After that, the results from the absorbance ratios are analyzed. Lastly, the degradation is analyzed.

4.2.4.1 Oxygen assimilation

The model has been based on the TS ratio values presented in Table 4.4. The final model contains 9 terms, where 2 of them are quadratic and 2 are interaction terms (Equation 4.1). A larger coefficient implies that the impact of that term has a greater impact on the response[67], in this case being the TS ratio. Although all coefficients are in the same order of magnitude in the presented model, the coefficients for terms including the parameter *T* or *DM* are slightly larger in general. The value of R^2 for the obtained model was 0.9120, which means that the model provides a good correlation between the predicted and the experimental values.

$$\frac{Y_{m_{TS,ox}}}{m_{TS,in}} = 0.915 - 0.037 \cdot T - 0.0521 \cdot DM - 0.0163 \cdot t + 0.0145 \cdot C_{NaOH} + 0.0303 \cdot DM^2 + 0.0118 \cdot t^2 - 0.065 \cdot T \cdot DM + 0.0331 \cdot t \cdot C_{NaOH} \quad \textit{Equation 4.1}$$

According to the response surface shown in Figure 4.13, the highest TS ratio can be obtained at 6.5% DM, 9.8 wt% NaOH and when lower temperatures are used. The highest TS ratio can be obtained at 160˚C, and increases the longer the reaction time is. This indicates that most oxidation products per g TS can be formed at 160˚C and 30 min.

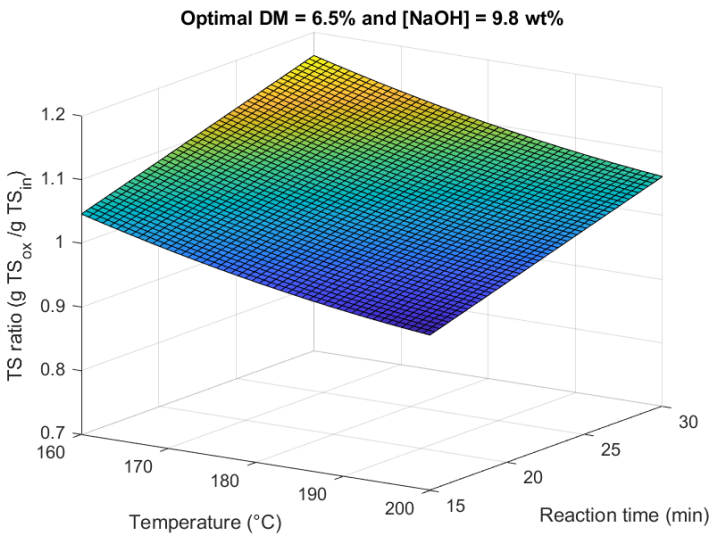
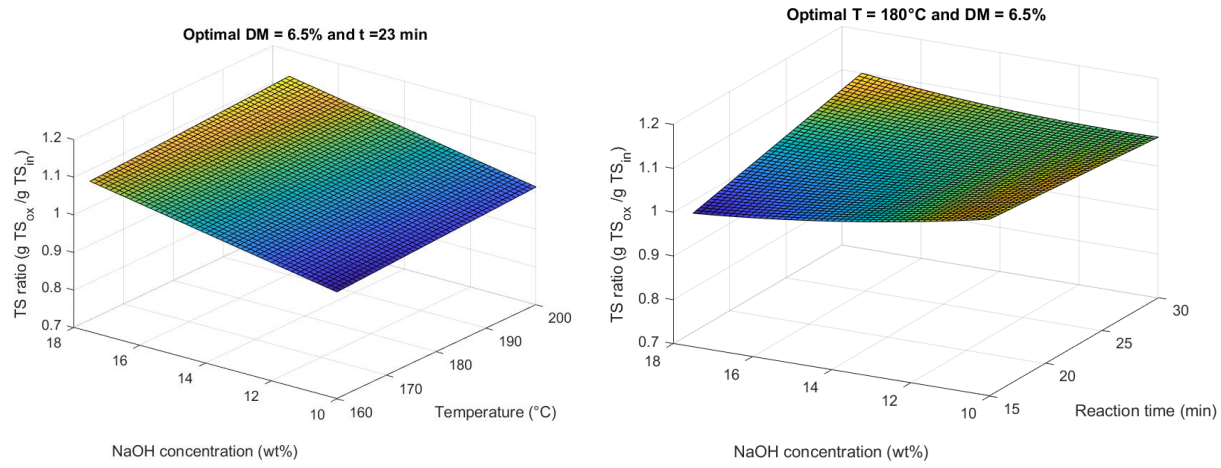


Figure 4.13: Response surface at optimal conditions for oxygen assimilation, 6.5% DM content and 9.8 wt% NaOH.

Since the TS ratio essentially just indicate that oxidation has occurred, it is a very unspecific measure of the oxidation products formed. One important source of error in the TS measurements is the loss of simple carboxylic acids during the drying at 105˚C. The boiling points of both formic acid and acetic acid are close to 105˚C (see section 2.4.4.2) It is, thus, very likely that these compounds, and formic acid in particular, evaporate during the drying and therefore are not included in the obtained TS values. Compounds that are not as easily evaporated at 105 ˚C, such as phenolic compounds or larger carboxylic acids, should therefore be the compounds that contribute to higher TS values. In fact, the general trends of oxidation product formation (section 4.2.3) and the overall mass balance (section 4.2.1) show that phenolic compounds are present to a larger extent at TS ratios above 1. Higher concentrations of organic acids are also produced for experiments with TS ratio below 1, but the yields are generally lower compared to the experiments with TS ratio above 1. The lower amount of acids is likely due to larger losses of the material as it has been converted to CO_2. To what extent a high TS ratio is caused by phenolic monomers or organic acids is something that can be investigated in more detail, though.

In comparison to previous studies (described in section 2.4.4.2), lower temperatures and longer reaction time have been suggested to increase the yield of vanillin. The fact that commercial production of vanillin also occurs at 160˚C and around 10 bar is a strong indication that those conditions are favorable for vanillin formation. In addition, the amount of vanillin obtained in this study also indicate that the vanillin yield increases when reaction time increases from 15 min to 30 min at 160˚C (compare experiments 2 and 6, Table 4.9). In comparison, previous studies had shown decreasing yields of vanillic acid as the reaction time increases from 10 minutes to 30 minutes, no matter which temperature was employed.[42] This tendency can also be noted in the results from this study (Table 4.9). What can benefit the production one oxidation product may, therefore, counteract the formation of another. Nevertheless, the tendencies of the response surface in Figure 4.13 seem to correspond well with the optimal conditions for vanillin formation. However, even if a high TS ratio could relate to a higher yield of vanillin, the highest amounts of oxidation products in this study were obtained for organic acids (compare Figure 4.10 b) and 4.12 b)). Further investigations are, therefore, needed to determine exactly which oxidation product that is favoured by oxidation at low temperature and with short reaction time.

When the DM content and reaction time are held constant, the obtained response surface is flatter (Figure 4.14 a)). The value of the TS ratio increases, however, when the NaOH concentration and the temperature increase simultaneously. Since all TS ratio values are above 1, no excessive oxidation should occur. It is a reasonable correlation, as the oxidation should accelerate at higher temperatures and the NaOH concentration thus needs to be higher in order to maintain the pH of the system stable. A stable pH in the system should, thus, make it possible for the TS ratio to become higher. Compared to Figure 4.13, though, the higher TS ratio is obtained at higher temperatures. This might suggest that the formation of vanillin is not favoured but that other compounds that are favored by higher temperatures, such as for vanillic acid or organic acids.



a) *b)*

Figure 4.14: Almost flat response surfaces at optimal conditions for oxygen assimilation. a) 6.5% DM content and 23 min, b) 6.5% DM content and 180˚C

On the other hand, when DM content and the temperature are held constant, a relatively flat response surface is also obtained (Figure 4.14 b)). When the NaOH concentration becomes higher than 14 wt%, the TS ratio is decreased to a value around 1. Otherwise, the TS ratio has a value closer to 1.1 at all other parts of the response surface. A flat surface indicates that the parameters that are held constant have a larger impact on the response compared to parameters that are varied in the response surface. Even though the response surfaces in Figure 4.14 a) and b) could be much flatter, together they could indicate which parameter that is more influential than the others when comparing to the response surfaces of all other parameter combinations (Figure 4.13, 4.15 and 4.17 a) and b)).

The DM content is constant in both response surfaces in Figure 4.14, whereas the reaction time is constant in Figure 4.14 a) and the temperature is constant in 4.14 b). This, together with the empirical experience from performing the experiments, could thus suggest that the DM content has been the most influential parameter during this study. In fact, it could already be seen by the mass balances and pH stability discussed in section 4.2.1. It is, however, also a matter of how the experimental design was constructed. If the NaOH loading would have been based on the DM content, maybe the pH would not have decreased as much as it did for many of the experiments in this study. Since pH stability is crucial for the outcome of the oxidation, the TS ratio would likely not go below 1 with appropriate NaOH loading. This could, in turn, have given different conclusions based on the appearances of the response surfaces.

Even though all the previously presented results correlate well, the response surface in Figure 4.15 contradicts it all. In short, it shows that the highest TS ratio can be obtained at a NaOH concentration around 10 wt%, no matter what the DM content is. All the empirical evidence, presented in Table 4.4,

has shown that this is not the case. The more the DM content increases, the harder it has it been to obtain a TS ratio above 1 during the oxidation experiments. To exemplify, experiment 12 has the same conditions as experiment 13 (180˚C, 23 min, 9.8wt% NaOH), except that the DM content is 28.5% instead of 6.5%. The pH decreased drastically in experiment 12, and the obtained TS ratio was 0.91, compared to the stable pH and TS ratio of 1.02 in experiment 13 (Table 4.4).

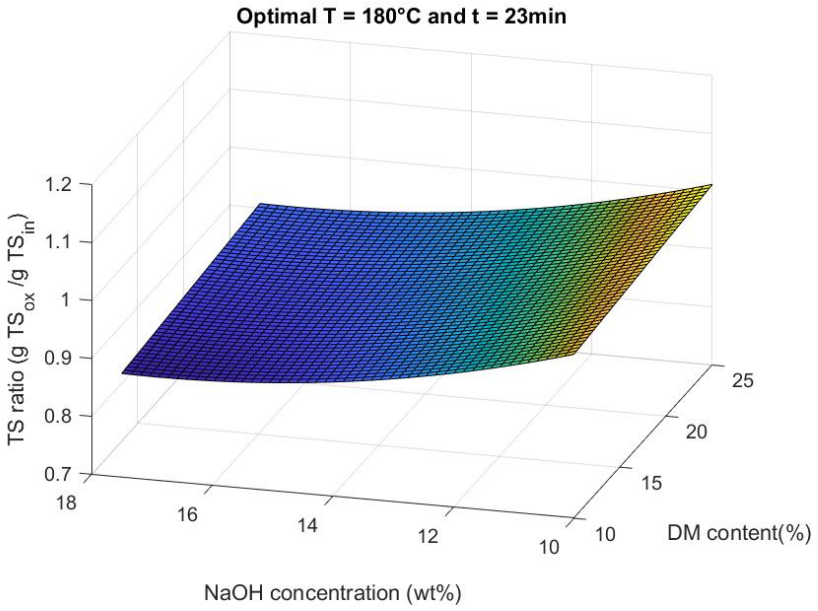


Figure 4.15: Response surface that contradicts all the previous findings in this study.

The contradictory response surface in Figure 4.15 indicates that the obtained model is not perfect. The question is whether there is something missing, as an important parameter, or if just small imperfections in the model can give rise to such an inaccurate response surface. Nonetheless, models are only simplifications of the complex processes in reality. Perhaps it is enough to conclude that the model has presented reasonable results in other ways, but is completely wrong regarding the increase in DM content at a NaOH concentration around 10 wt%.

4.2.4.2 Absorbance ratio

The model for absorbance ratio has been based on absorbance ratios presented in Table 4.5-4.7. The absorbance ratio can be seen as a measure of the formation of phenolic compounds, since it has been assumed that vanillic acid and vanillin are responsible for the absorbance at the wavelengths 254 nm and 348 nm, respectively. More specifically, the ratio of the absorbances at 348 nm and 254 nm (Abs_{348nm}/Abs_{254nm}) could be interpreted as the amount of vanillin in relation to the amount of vanillic acid. A low value of the ratio would imply less vanillin, whereas a high ratio would imply more vanillin. For anaerobic digestion applications, it would therefore be more desirable with a lower absorbance ratio due to the inhibitory effects of vanillin. As concluded in section 4.2.2, though, this interpretation should only be limited to the evaluation of experiments with stable pH. Values from all experiments were, nevertheless, used, which is a clear source of error in the response-surface modelling presented in this subsection.

The R^2 value of the full model based on the absorbance ratios only reached 0.5575, which is considerably lower than the acceptable limit of 0.9 that was applied in this study. No terms were thus removed from the model as shown in Equation 4.2, as that would have decreased the value of R^2 even more. Trials were made using the inverse of the employed absorbance ratio as well, but the obtained

values of R^2 became even lower then. These results show that the full model is not very good for predicting what absorbance ratios that could be achieved.

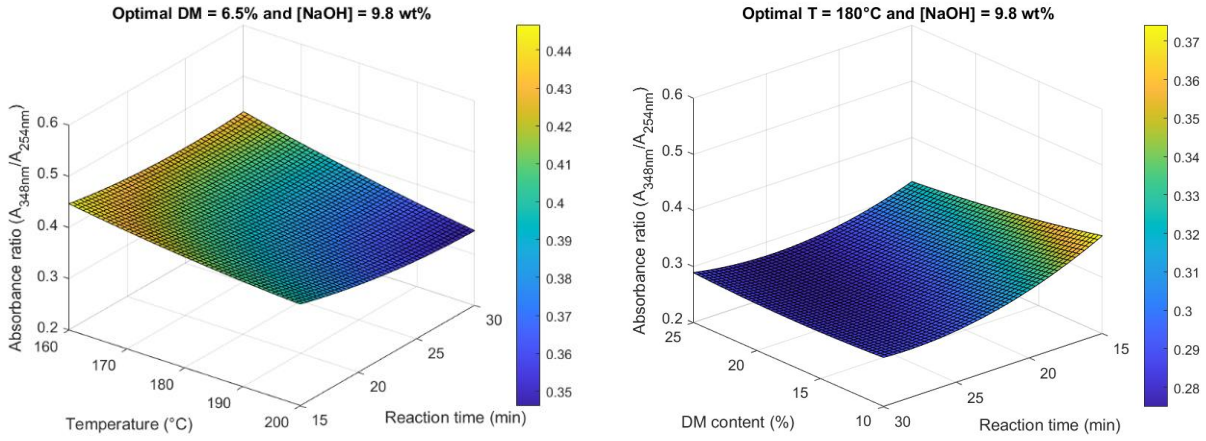
$$Y_{\frac{Abs_{348nm}}{Abs_{254nm}}} = 0.2805 - 0.0086 \cdot T - 0.0387 \cdot DM - 0.0225 \cdot t + 0.0294 \cdot C_{NaOH} + 0.0119 \cdot T^2 + 0.0276 \cdot DM^2 + 0.0056 \cdot t^2 + 0.0319 \cdot C_{NaOH}^2 + 0.0259 \cdot T \cdot DM - 0.0091 \cdot T \cdot t - 0.0279 \cdot T \cdot C_{NaOH} + 0.0136 \cdot DM \cdot t - 0.0087 \cdot DM \cdot C_{NaOH} - 0.0091 \cdot t \cdot C_{NaOH} \quad \textit{Equation 4.2}$$

The low R^2 indicate that another parameter, beside temperature, DM content, reaction time and NaOH concentration, might have an impact in the outcome. One such parameter, which is crucial in the interpretation of the absorbance ratio, is the pH. As was discussed concerning the UV spectra in section 4.2.2, the shape of the spectrum varies greatly depending on the pH of the sample (Figure 4.5-4.7). All experiments with a stable pH had significant peaks around 254 nm and 348 nm, which was not the case for the remaining experiments where pH also had decreased. In fact, the ratio of absorbance at 348 nm and 254 nm in the experiments with decreased pH does not say anything about vanillin and vanillic acid per se, since those compounds does not even seem to be present in sufficient amounts. A stable pH in all experiments is, therefore, essential to have the same interpretation of the absorbance ratio. In such case, where all experiments would have stable pH, the absorbance ratio model would perhaps obtain a higher value of R^2.

In addition, another problematic aspect of the model is the values used to calculate the absorbance ratios. As discussed in section 4.2.2, experiments with higher DM content generally have higher absorbance. As an example, the spectra and tabulated absorbance ratios of all the experiments with 25% DM content (experiments 8, 5 and 9) can be compared with the experiments with 17.5% DM content in Figure 4.7 and Table 4.7. This finding shows that an inherent error is present when the absorbance ratio is applied, especially for experiments with pH drop. It seems to be less problematic for experiments where significant peaks were identified, though. What this implies for the response-surface modelling, is that the absorbance ratio values used have been inconsistent in what they measure. The easiest solution would perhaps be to make sure that the pH is kept stable in all experiments, as previously mentioned. The DM content will probably still have an impact in some way, so maybe the dilution of the samples could also be optimized so that the spectra of all experiments are roughly on the same absorbance level.

Response surfaces were made based on the model in Equation 4.2, despite its inadequacy. The purpose was to compare it to the other results in this study. Based on that comparison, an assessment whether the chosen absorbance ratio is an appropriate measure to predict oxidation of biorefinery lignin could be made. Many combinations of parameters gave response surfaces that contradicted the empirical findings, similarly to the erroneous response surface in Figure 4.15. These results were therefore not further analysed. Nevertheless, two response surfaces showed tendencies in line with the previous findings.

In Figure 4.16 a), the absorbance ratio increases as the temperature decreases. The highest ratio is obtained at 160˚C. This goes in line with the ideal temperature for vanillin production, as previously discussed (section 4.2.4.1). A somewhat similar trend can be seen in the response surface in Figure 4.13, meaning that the TS ratio and the absorbance ratio could be connected in that case. This suggests that one of the main oxidation products could, in fact, be vanillin, as the high TS ratio is correlated with the high absorbance ratio.



a) *b)*

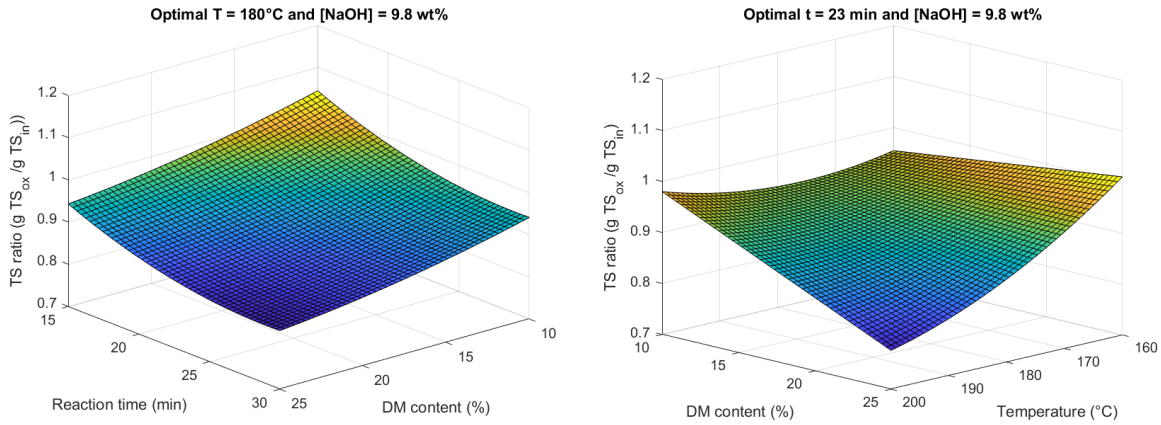
Figure 4.16: Response surfaces at optimal conditions for high UV absorbance ratios. a) 6.5% DM content and 9.8 wt% NaOH, b) 180˚C and 9.8 wt% NaOH.

In Figure 4.16 b), the response surface show that at 180˚C and 9.8 wt% NaOH, the absorbance ratio is the highest at 10% DM content and when the reaction time is no more than 15 min. This is also in accordance with the findings at higher temperatures, where the yield of vanillin increases the shorter the reaction time is.

Despite the poor predictability and inherent sources of error in the absorbance ratio model, it has provided some results that correspond to previous findings. The model has shown that with less severe conditions, at lower temperature or with shorter reaction time, the absorbance ratio becomes higher. The interpretation is that more vanillin can be formed when the conditions are less severe. The absorbance ratio model, together with the UV measurements that it was based on, are valuable as complement to the TS ratio. The peaks in the UV spectra for the experiments with stable pH (Figure 4.5) indicate the presence of conjugated systems, which for instance can be found in phenolic compounds. The absorbance ratio is, thereby, more specific about the oxidation products formed compared to the TS ratio.

4.2.4.3 Degradation

The same model as was used to evaluate the oxygen assimilation (Equation 4.1) can be applied to analyze degradation of the biorefinery lignin. The optimal conditions for degradation can be deducted from the two response surfaces in Figure 4.17. In the left graph (Figure 4.17 a)), where 180˚C and 9.8 wt% NaOH are constant, it is shown that the TS ratio only reaches values above 1 when the DM content is low and the reaction time is short. The same tendency can be seen in Figure 4.16 b), where absorbance ratio was applied in the modelling. In all other cases, excessive oxidation will occur, and the TS ratio will be lower than 1. The lowest TS ratio is obtained at 25% DM and 30 min reaction time.



a) *b)*

Figure 4.17: Response surfaces at optimal conditions for degradation of biorefinery lignin. a) 180˚C and 9.8 wt% NaOH, b) 23 min and 9.8 wt% NaOH.

In the right graph (Figure 4.17 b)), a response surface for when the reaction time and NaOH content are held constant at 23 min and 9.8 wt%, respectively, is shown. The TS ratio is very close to 1 at certain conditions and almost exceeds 1 at 25% and 160˚C. At the same temperature but with decreasing DM content, the TS ratio is also reduced. At 10% DM content, the TS ratio is also relatively close to 1, being somewhere in between 0.9 and 1 at all temperatures. Nonetheless, as both the DM content and the temperature increase, the TS ratio decreases. This goes in line with the previous findings, where excessive oxidation occurred when more material was present in the system.

The combination of the two response surfaces in Figure 4.17 show that the more severe the conditions are, the more likely will the value of the TS ratio decrease. The more this ratio decreases, the greater should the degradation of the material be. However, as discussed in section 4.2.1.1, a low TS is not necessarily coupled with good dissolution of the material. This means that the type of degradation that can be deduced from the response surfaces in Figure 4.17 should mainly arise from already solubilized fractions that are transformed to CO_2. If that kind of degradation is the desired outcome of the alkaline wet oxidation and if the NaOH concentration is held at 9.8 wt%, then high temperature (180-200˚C), longer reaction time (23-30 min) and a high DM content (most notably when it is higher than 20%) provide the best conditions. Probably, the same tendencies can also be seen at lower NaOH concentrations. More investigations are needed, though, to evaluate the minimum NaOH content required for an efficient process. Moreover, as will be presented in section 4.2.5, the occurrence of repolymerized fragments may also increase at higher DM contents. Many aspects are, thus, necessary to consider, even if the aim is only to degrade the biorefinery lignin as much as possible.

4.2.5 Properties of the oxidized materials

In addition to the various analyses performed, some significant differences could also be observed just by looking and handling the oxidized materials from the different experiments. These observations include changes in color and structure, noted in some of the oxidized materials from experiments with stable pH. Moreover, excessive foam formation and repolymerization could be observed for experiments with 25% DM content. These observations are presented in more detail in the following subsections, followed by a brief summary of the properties of the oxidized materials from all experiments in this study.

4.2.5.1 Changed color and crystallisation – experiments with stable pH

The first observation made was that oxidized material from experiments with a stable pH had obtained a more orange-reddish color compared to the other experiments. In contrast, the color of materials

from experiments where the pH had decreased significantly was dark brown or even black, see Figure 4.18 for an exemplification.

a) *b)*

Figure 4.18: Oxidized materials from different experiments.
a) Experiment 13 (6.5% DM) where pH was stable throughout the oxidation, b) Experiment 5 (25% DM) where the pH decreased during the oxidation.

Another observation, related to the first, was that some of the oxidized materials started to form crystalline structures after have being refrigerated for some time at a temperature around 8˚C (Figure 4.19). This observation relates to the previous one since the oxidized materials with a more reddish color showed this tendency to form crystalline structures. The effect was most pronounced for the oxidized materials from experiments 3 and 7 (both 10% DM), but could also be detected in the materials from the other experiments where the pH had been stable (experiments 2, 6 and 1).

a) *b)*

Figure 4.19: Crystalline structures after refrigeration at 8˚C.
a) Experiment 3 (10% DM, 200˚C, 15 min) after long storage time, b) Experiment 7 (10% DM, 200˚C, 30 min) after shorter storage time.

These crystals were present both in the original oxidized material as well as in the liquid fraction, obtained after centrifugation and filtration. This implies that with a stable pH, something is formed that alters the colour of the material, is water-soluble and starts to crystallize at lower temperatures.

4.2.5.2 Excessive foam formation and repolymerization – experiments with high DM content

As the initial DM content was increased to 25% or more, some new things started to happen in the oxidized materials. Firstly, the reactor vessel immediately after the oxidation was almost overfilled with foam, see Figure 4.20 below. As discussed in section 4.2.1, this effect is likely due to a greater capacity to absorb the supplied oxygen when the DM content increased. With higher DM content, more material can be oxidized and form acidic oxidation products. The formation of foam can, thus,

be interpreted as if NaOH could not withstand the increased loading of acids and thereby resulted in a decreased pH. With a decreased pH, the formation of carbon dioxide was favoured, causing the foam. Even though foam was present after all experiments, it is preferable to keep the amounts sufficiently small so that the reactor vessel is not overflown.

Figure 4.20: Excessive foam formation after oxidation, exemplified by experiment 8 (25% DM).

Secondly, some gravel-like pieces started to form at 25% DM content (Figure 4.21). This effect was present in the materials from experiments 5, 8 and 9 (all 25% DM). The formation of these gravel-like fragments should be related to the tendency of lignin units to repolymerize during alkaline oxidation. The strongest evidence for this is that no such fragments were present in the untreated biorefinery lignin. In addition, these gravel-like fragments did not seem to have been affected by the ongoing oxidation. As fragments like these did not occur after experiment 4 (25% DM, 160˚C, 15 min), is could imply that it is a consequence of the severity of the reaction conditions. Experiment 8 (25% DM, 160˚C, 30 min) was performed at the exact same reaction conditions as experiment 4, except for the reaction time that was twice as long for experiment 8. Formation of repolymerized fragments was noted in the oxidized materials from experiments 5 and 9 as well. These experiments had the same reaction conditions as experiments 4 and 8, respectively, but were conducted at 200˚C instead. Repolymerization was, hence, enhanced by longer reaction time or when temperature increased from 160˚C to 200˚C. However, experiment 4 was also the only experiment where the pH only decreased slightly. Whether the repolymerization is due to severe reaction conditions as such, or rather the result that severe conditions can have by significantly decreasing the pH, can be examined.

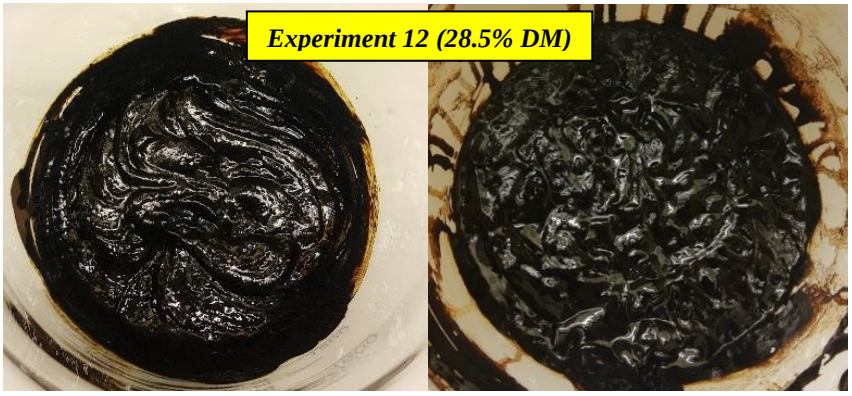
Figure 4.21: Gravel-like fragments from experiment 8 (25% DM). To the left, shown on the inside of a centrifuge bottle during the washing of the sediment. To the right, on a spoon after all washing steps had finished.

No matter what the exact cause for the repolymerization would be, the formation of such recalcitrant fragments is highly undesirable. To avoid this undesirable effect, a trade-off arises depending on the goal of the oxidation. An increased oxygen pressure could be applied to avoid repolymerization when DM content is high. However, a higher oxygen pressure also accelerates the degradation of aromatic oxidation products. Future scenarios in alkaline wet oxidation of biorefinery lignin could, therefore, be that the reaction conditions will need to be adjusted, depending on the aim of the oxidation. If the aim is to oxidize as much material as possible at the same time, and where it is less important which exact oxidation products that are formed, then high DM content and higher oxygen pressures could be applied. On the other hand, if high yields of monoaromatic compounds are desired, then lower DM contents and lower oxygen pressures might be needed. Further investigations are required, though, to examine the outcomes of oxidation at high DM contents and with a stable pH throughout the reaction.

4.2.5.3 Liquefaction and type of sediment

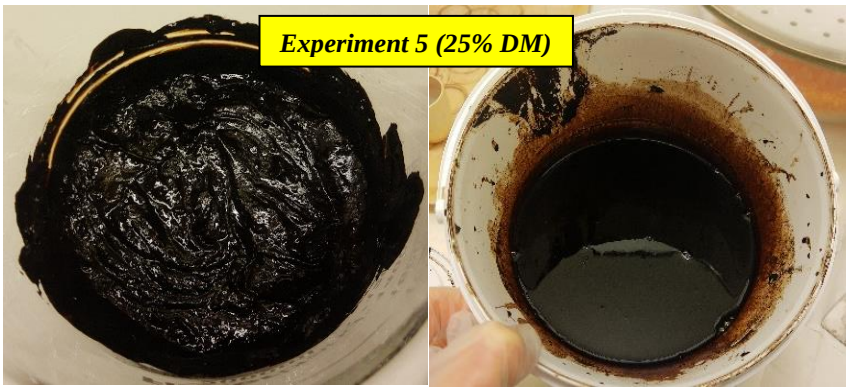
Beside evaluating the color and repolymerization of the oxidized materials, the liquefaction and the sediment properties can also be examined. These properties, together with the color, are summarized for all experiments in Appendix B.

Due to the different initial DM contents for the different experiments, there was a difference in the liquefaction of the oxidized materials as well. Nonetheless, basically all materials showed an increased liquefaction after the oxidation had been performed. The only exception was the material for experiment 12, the only experiment with a DM content as high as 28.5%. After that experiment, the material was essentially just as thick and non-liquified as before the oxidation, see photos of the material in experiment 12 compared to the material in experiment 5 shown in Figure 4.22 and Figure 4.23, respectively.



a) *b)*

Figure 4.22: The material for experiment 12 (28.5% DM), basically not liquefied at all after the oxidation. a) Before oxidation, b) After oxidation



a) *b)*

Figure 4.23: The material for experiment 5 (25% DM), showing a significant liquefication after the oxidation. a) Before oxidation, b) After oxidation

Furthermore, as the oxidized materials were stored in a refrigerator room for several weeks there was a good opportunity for sedimentation to arise. As previously mentioned, the sediment that arose for some of the oxidized materials had more of a crystalline structure (Figure 4.19). For the other experiments, the sediment that arose had more similarities to the untreated biorefinery lignin. What can be said, however, is that the sediment seemed somewhat coarser for the oxidized materials from experiments with a 25 or 28.5% DM content.

4.3 Evaluation of the experimental procedure

Several aspects in the experimental procedure of this study could be improved for future studies. These improvements include both minor modifications that easily can be implemented to the current procedure, as well as larger alterations that would require some new additions to the current setup. First, the improvements and general aspects specifically concerning the alkaline wet oxidation are presented. After that, the treatment of the oxidized material is evaluated. Lastly, some suggestions related to the applied analytical methods are provided.

4.3.1 Evaluation of the alkaline wet oxidation

The oxidation experiments are the very core of this study. It is, therefore, relevant to examine the possible improvements that could be made for similar studies in the future. Some minor modifications

to the employed procedure are first presented. Other aspects that could be developed, together with a more general outlook for alkaline wet oxidation, are then given.

4.3.1.1 Minor modifications to the procedure in this study

Firstly, a very simple improvement when performing alkaline oxidation experiments in the setup used in this study is to shorten the time for pressurizing the reactor. In this study, 2 minutes after heating the reactor were designated to obtain the desired oxygen pressure. The purpose of these 2 minutes was to ensure that all experiments would have the same amount of time to reach and stabilize at the pressure of interest. However, the desired pressure was usually achieved just after a few seconds. It would thus be sufficient to have a set time to pressurize the reactor for roughly 30-45 seconds instead. It is still important to pay attention to the reactor during the first minutes of the reaction, though, since the pressure might start to decrease. This occurred for several experiments after some minutes of reaction. The best is to counteract the decreasing pressure by increasing the flow from the oxygen tube as soon as possible. Significant effects on the reaction conditions can then be avoided. Such significant pressure drop occurred in four experiments in this study, so that they had to be remade. When results from the experiments with significant pressure drop are compared to the corresponding experiment without pressure drop, the values for TS ratio and absorbance ratios can differ from just a few percent up to 25%.

To make sure that the desired reaction conditions are maintained it is important that the appropriate oxygen pressure is kept constant. Some scales can be very sensitive to adjustments being made during the weighing. Fortunately, the scale on which the oxygen tube was standing in this study was rather stable. The scale returned to the same value even if it the pressure handle had been touched to increase the oxygen pressure. There is, thus, no conflict between maintaining the appropriate oxygen pressure in the reactor and an accurate measurement of the oxygen consumption.

Another aspect that could be examined in relation to the oxygen pressure, is to perform at least one experiment without any supplied oxygen. This could give valuable information about what happens to the biorefinery lignin when only high temperature and NaOH loading are applied, as a baseline. In an experimental design with different ranges for these parameters it could be difficult to decide for which ones to choose. Perhaps the parameters from the experiment with the most desirable outcome could be a reasonable experiment to start with.

Secondly, the amount NaOH has been shown to be a very important factor for the outcome of the oxidation. A rough estimation has shown that around 1.8 ± 0.3 g NaOH/g lignin should be an appropriate amount for oxidation experiments like the ones in this study, based on the amounts used in the experiments where pH had been stable. If only the optimal experiment of this study is considered, being experiment 13 (6.5%), then the value is approximately 2 g NaOH/g lignin. According to this estimation, most of the experiments where the pH decreased significantly (experiments 11,14,15, 10, 16, 17, 8, 5 and 9), would have needed roughly 1.8-2.5 times more NaOH to maintain a stable pH. In experiment 12, with 28.5% DM content, roughly 4 times more NaOH would have been needed compared to the amount used in this study. In a study where the material is washed and the amount of cellulose and hemicellulose is held as low as possible, maybe not as much NaOH will be needed to keep pH stable, though. As will be discussed in section 4.3.1.2, it is beneficial to keep the use of NaOH as low as possible.

Thirdly, handling of the biorefinery lignin could be improved to maintain a better control of the material. For instance, the biorefinery lignin should be frozen directly after is has been obtained, just to slow down any microbial activity or other kinds of degradation in the material. Furthermore, washing of the biorefinery lignin could be included as a pretreatment step. Both cellulose and hemicellulose

residues have been shown to be present in the biorefinery lignin. These components are also susceptible to oxidation, hence contributing to their own kinds of oxidation products. If the biorefinery lignin is washed prior to the oxidation experiments, it could be easier to deduce which products have been formed due to the oxidation of the lignin. A suggestion could be to redo the most successful experiments (experiments 13, 2, 6, 3, and 1) with washed biorefinery lignin, and then compare the resulting chromatograms for organic acids with the one presented in this study (Figure 4.11). If there are any differences, such as significantly lower concentrations or absence of certain peaks, then it could be possible to determine which organic acids that originate from the lignin.

Lastly, the aim of the lignin oxidation in this study has been to valorize the whole lignocellulosic structure of wheat straw for anaerobic digestion. To obtain a process that is more like the one sought for on large scale, it could be valuable to use residues from anaerobic digestion as raw material in the oxidation experiments instead of biorefinery lignin from steam exploded and enzymatically hydrolysed wheat straw. This adjustment could be applied relatively easily in the current setup, as it just is a matter of what material that is used.

4.3.1.2 General aspects in alkaline oxidation that could be developed

Other improvements that could be considered, but that are not implemented as easily, are related to the reactor and how the desired reaction conditions can be achieved in a better way. Some critical factors for the reaction conditions are heating of the reactor and the presence of oxygen. In addition, some crucial aspects relate to how well the oxidation method of biorefinery lignin is adapted to subsequent anaerobic digestion. First the adjustments to the current setup are discussed, followed by a discussion of an alternative method that could be more appropriate for applications in anaerobic digestion.

In the current setup, the reactor was heated for 20 minutes and cooled for about 40-50 minutes. As mentioned in section 2.4.3.1, a heating time less than 30 minutes would be desirable in experiments with alkaline wet oxidation as oxidation products can degrade. Equally, the cooling time should also be shortened, since little is known when the oxidation reactions stop during the cooling. Shorter time for heating and cooling would give more precision and control over the reaction conditions. It has, in fact, been achieved in previous studies. Wu et al.(1994) performed alkaline oxidation in a 500 mL autoclave that was immersed in a preheated salt bath. The heating rate was around 50˚C/min, which made it possible to obtain the reaction temperature of 170˚C after 3-5 minutes. The cooling was then performed by immersing the reactor in a cold water bath.[42, 68] This example shows that alternative methods to perform alkaline oxidation with short time for heating and cooling exist. It also means, though, that the setup used in this study can not really be used if shorter heating and cooling are desired.

What concerns oxygen, the problem is that oxidation reactions can start when oxygen is present during preheating of the reactor. Oxygen was present all time in this study, either as a part of the air or as the gaseous oxygen that was supplied when pressurizing the system. It would be more appropriate to keep the reactor in an inert atmosphere during heating, and not turn on any oxygen supply until the desired reaction temperature has been reached. Inert gases, such as helium of nitrogen gas, have been used in other studies to flush the reactor prior to the oxidation.[41-42] Just as was the case for long heating and cooling, more control of when oxygen is supplied to the system is also a matter of having more precision and control over the reaction conditions. In the setup used in this study, an additional gas tube could perhaps be connected in parallel with the tube that supplies the system with oxygen. In fact, if an inert atmosphere can be obtained prior the oxidation then maybe it is not as important to reduce the heating time.

The above-mentioned aspects are slightly harder to implement directly but are still related to the procedure applied in this study. Whether the alkaline wet oxidation procedure in this study is the most optimal for subsequent anaerobic digestion can, however, be discussed. The main drawback with this procedure is that it requires large amounts of NaOH. Not only can high NaOH loading have a negative impact on anaerobic digestion, on a large scale a considerable usage of NaOH could also require costly recycling and recovery processes. Perhaps the procedure of this study is more appropriate for raw materials that already are highly alkaline, such as Kraft lignins from black liquor.

A method that could be more appropriate to oxidize biorefinery lignin from wheat straw for subsequent anaerobic digestion is, instead, wet explosion (described in section 2.4.3.2). As slightly alkaline wet explosion of wheat straw has shown to improve the methane yield, it also confirms that it is suitable as a treatment before anaerobic digestion. The alkaline wet oxidation in this study has shown to produce oxidation products, put how well these compounds are digested by anaerobic microbes is still an aspect to examine. It is, thus, recommended to consider wet explosion as a method to employ in future studies. However, findings of the alkaline wet oxidation of this study can still be useful to obtain a general understanding for what happens to the wheat straw lignin during oxidation. Perhaps the experiments of this study could be reproduced but with some of the modifications proposed in this section, just to gain some more insight in the findings. Then, these results could be compared to findings from wet explosion of wheat straw, to see how much these methods differ. In addition, the costs of the processes, both energetically and financially, should be investigated in future studies.

4.3.2 Treatment of the oxidized materials - combined centrifugation and filtration

One of the main drawbacks with the combined centrifugation and filtration of the oxidized biorefinery lignin is that it is a very time-consuming process. It was mainly all the washing steps using a centrifuge that were tedious. It could, therefore, be worth testing other methods to separate the solid and liquid fraction from each other and to achieve appropriate washing. The filtration of the liquid fraction from the different experiments is first evaluated. Then, some drawbacks and improvements of the washing procedure are highlighted.

4.3.2.1 Evaluation of the filtration

Filtration of the liquid fraction was, in fact, rather fast for most experiments, with exception for the materials from experiments 11, 1, 4 and 12 (see Appendix B). These four experiments all had a high DM content (17.5% or more) and reaction temperatures below 180˚C. The outcome from the oxidation was, however, very different. The pH decreased significantly in both experiment 11 (17.5% DM) and 12 (28.5% DM), but where the viscosity of the reaction mixture in experiment 12 barely seemed to have changed after the oxidation (Figure 4.22). In experiment 4 (25% DM), the pH decreased slightly to 10, whereas the pH was stable in experiment 1 (17.5% DM). Furthermore, experiment 1 could be deemed as the experiment where the concentration of both phenolic compounds and organic acids was the highest (section 4.2.3). The slower filtration rate could be related to an insufficient depolymerization of the lignin, or even repolymerization of lignin units. It is, however, hard to draw any general conclusions regarding why the filtration in those experiments was so much slower compared to the other experiments. More analyses are, therefore, required.

What can be said about the slow filtration rate, however, is that it has something to do with the DM content. For instance, the material from experiment 13 (6.5% DM) could have been filtered from start, since almost no sediment was present after the first centrifugation step. Perhaps the amount of biorefinery lignin and NaOH was appropriate to obtain a good depolymerization of the material. In this study, the NaOH concentration was too low in most of the experiments to maintain a stable pH. If this would be adjusted for in a future study, the filtration could perhaps either go faster, due to a

more dissolved biorefinery lignin. The filtration could also go slower, though, if new interactions arise in material with high alkalinity, as in experiment 1 (17.5% DM, 20.4 wt% NaOH).

When the oxidized material has a low amount of sediment, such as in experiments 13 (6.5% DM), 2 and 6 (10% DM), the amount of particles left on the filter paper is also much smaller. In this study, some negative values were obtained after subtracting the weight of used filter papers after drying from the weight of unused filters. It could, thus, be worth to consider pre-drying the filter papers before using them in the filtration of materials from experiments where the DM content is rather low.

4.3.2.2 Evaluation of the washing procedure

The purpose of the washing procedure was to remove alkaline compounds so that a solid fraction, only consisting of the water insoluble particles from the oxidized biorefinery lignin, was obtained. One aspect of the washing is that other particles from the material, beside the NaOH, also are washed away. For instance, when the oxidized material from experiment 12 (28.5% DM) was washed, roughly 2/3 of its initial volume was lost after the first washing step. Even though these losses are an inevitable part of washing, one drawback with the washing method used in this study is that the same amount of water was used for all experiments. The problem with having the same amount of washing water for all experiments is that the ratio of washing water to the DM content changes as the amount of biorefinery lignin increases. To exemplify, the washed sediment for all experiments with an initial DM content of 17.5% or lower lost its colour during the washing procedure (Figure 4.24 a)). However, the color in the sediments with 25% or more as initial DM, were still dark brown after all three washing steps, as shown in Figure 4.24 b).

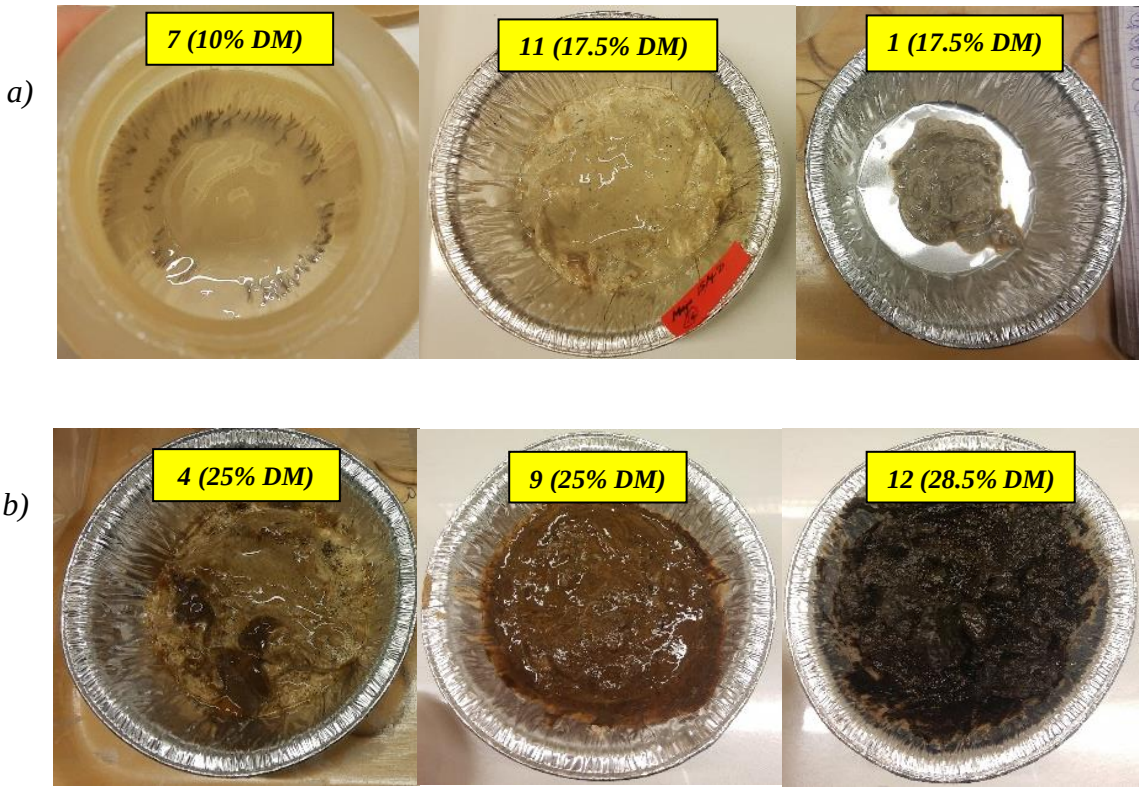


Figure 4.24: Sediments of oxidized materials from experiments 7, 11, 1, 4, 9 and 12 after three washing steps. a) Materials with 17.5 % DM or less that lost the color, b) Materials with 25% DM or more, not showing any significant loss in color.

Calculations have been performed to assess the washing procedure of this study. As the water volume was held constant, no matter what the initial DM content of the oxidized material was, the ratio of water to TS has varied roughly from 30:1 to 7:1 (g deionized water per g initial TS). With such a large difference between the different experiments, it should thus be expected that the oxidized materials have not been washed to the same extent. To make sure that the washing is as equal as possible for all oxidized materials, a suggestion is to employ a constant ratio of water to the initial DM content instead. Perhaps a ratio of 15:1 could be suitable, as the volume of deionized water would approximately range from 0.7 and 3L for initial DM contents of 6.5% to 28.5%, respectively.

Beside the total deionized water volume used for washing, another aspect is also how much of the water should be used in each washing step. It was investigated whether a larger water volume could be used in a single washing step, to speed up the washing procedure of the oxidized materials from experiments 2 and 6 (10% DM). A water volume of 750 mL was used instead of 500 mL. This investigation showed that 750 mL washing water (divided in to two centrifuge bottles) was a too large volume for efficient sedimentation through centrifugation. As a consequence, the solid fraction of these experiments had to be filtered and the filter cake was washed instead. If the same equipment is used as in this study, it is therefore recommended to not use more than 500 mL deionized water divided in to two centrifuge bottles during all the washing steps.

To conclude, if maximum 500 mL is used in each washing step, and the ratio of total amount of deionized water to initial DM content held constant at 15:1 for all experiments, at least 4, 5 or 6 washing steps would be required for material with initial DM content of 17.5%, 25% or 28.5%, respectively.

4.3.3 Evaluation of the analytical methods used

Improvements can always be made, also when it comes to analytical methods. Some minor improvements, mainly concerning the chromatographic analyses, are first presented. Since there were many aspects to consider in the UV spectrophotometry, a whole subsection is then dedicated to discuss them. Lastly, some additional analytical methods, that could be useful in future studies, are presented.

4.3.3.1 Minor improvements

Some minor improvements that could be made are related to reducing the sources of error. For instance, even greater care can be taken to ensure good mixing of materials to avoid gradients during sampling. Determination of the WIS fraction, which was based on the centrifuged and filtered material, could also be made more similar for the oxidized materials and the untreated biorefinery lignin. More specifically, larger amounts of untreated biorefinery lignin could be used, so that corresponding amounts as for the oxidized material were analysed.

What concerns the chromatographic analyses, at least duplicate samples could be employed. It could also be useful to perform chromatographic analyses on the oxidized materials from all experiments, to gain a broader understanding of the oxidation. Lastly, samples in the chromatographic analyses were frozen prior to the measurements. A small test was performed, where a duplicate of one sample was left at room temperature for two days, to evaluate whether any degradation would occur compared to its frozen equivalent. No significant changes were noted when the samples were analysed, which indicates that freezing of samples before the chromatographic analyses is not crucial if the measurements are relatively close in time. Nevertheless, it probably depends on how long time the samples need to be stored before being analysed.

4.3.3.2 Improvements for the UV spectrophotometry

Several things could be improved in the analysis using UV spectrophotometry. First of all, dilution of the samples to be analysed could be made in series to reduce the experimental error (compare with the dilutions made in the chromatographic analyses, described in section 3.3.4). Differences between samples in this study could be noted, though, even if a less accurate method was used. However, instead of using the same dilution for all experiments, even greater care could be taken to attain spectra in the optimal absorbance range 0.2-0.8. Secondly, and more importantly, the spectroscopic measurements should be performed closer in time to the oxidation reaction. For some samples in this study, the UV absorbance was not measured until after several weeks of storage in a refrigerator room. Since it has been shown that compounds that absorb at 340 nm decrease over time (described in section 2.3.5), it could be assumed that the same could have happened in the oxidized materials of this study. Even if the timespan could be rather long before degradation occurs, the best way to reduce this source of error is to measure all samples as soon as possible.

Thirdly, UV spectrophotometry has proved to be a useful analytical method in alkaline wet oxidation. The UV spectra were very useful to gain more understanding for what had happened during the oxidation experiments. The absorbance ratio, on the other hand, has served as a relatively fair measure of phenolic compounds but has its drawbacks. Many improvements are, thus, needed before values of absorbance ratios can be compared and used in modelling (as discussed in section 4.2.4.2). A stable pH in all experiments is the main improvement and is, in fact, a prerequisite for being able to compare the absorbance ratios. A stable pH does, however, imply the presence of phenolic compounds. Whether this is the most important measure for anaerobic digestion or not can be considered. In addition, it is essential to analyse which compounds in the material that cause the peaks. Even though vanillin and vanillic acid might be reasonable assumptions for the wavelengths 348 nm and 254 nm, respectively, other aromatic compounds might have maximum absorption in close proximity. With the UV spectra in this study, the wavelength at which the maximum absorbance could be found has not been studied in detail. With the maximum absorbance at 329 nm, 4-hydroxybenxaldehyde could, for instance, just as well have been part of the peaks in Figure 4.5.

4.3.3.3 Additional analytical methods to consider

The compositional analyses already applied in this study could be used to examine more aspects of the alkaline wet oxidation. Just as in a study by Khan and Ahring (2020), the solid fraction can be analyzed after treatment to evaluate if there has been a change in lignin content. Moreover, quantification of AIL can also give an indication for how well the material can be degraded by anaerobic microorganisms.[16] In addition, HPLC analysis of carbohydrates in both the solid and the liquid fractions of the oxidized material could be performed to detect if any changes have occurred. If a value can be obtained for how the amount of carbohydrates have changed due to oxidation, the mass balance calculations and the magnitude of the obtained acids that has been derived from lignin oxidation can become more precise.

Additional analytical methods could also be applied, such as liquid chromatography-mass spectrometry (LC-MS) and conductometric titration. The LC-MS could be used to detect compounds that are present after the oxidation, which could be useful for identifying the unknown peaks in Figure 4.9 and 4.11. Conductometric titration, on the other hand, could be a valuable complement to HPLC analysis for detection of organic acids. As an HPLC analysis would detect and quantify individual acids, the conductometric titration could quantify the total amount of acids and, thus, serve as a reference to the amount obtained through HPLC analysis.

Lastly, biochemical methane potential (BMP) tests could be used to assess the biodegradability of the oxidized materials through anaerobic digestion. It is, however, problematic if oxidized materials have

high pH. As in study by Bolado-Rodriquez et al., (2016), the highly alkaline samples can be neutralized prior to the BMP test, though.[1] The response in anaerobic digestion of oxidized biorefinery lignin has, after all, been of greatest interest in the alkaline wet oxidation experiments used in this study. It is, therefore, reasonable to use it as a method to analyse the oxidation as well.

5 Conclusions

Alkaline wet oxidation of biorefinery lignin from wheat straw has been examined during this study. This has provided answers to the research questions presented in section 1.2, thereby fulfilling the aim of the study. The research questions, together with the findings in this study, are summarized below.

1. How can an experimental procedure, with appropriate analytical methods, for alkaline wet oxidation of biorefinery lignin be constructed?

The experimental procedure in this study has successfully transformed biorefinery lignin to smaller compounds through alkaline wet oxidation, even though improvements to the procedure can be made. The outcomes from oxidation of biorefinery lignin can be monitored by measurements of pH, TS, WIS, oxygen consumption and UV absorbance. To quantify the obtained oxidation products, analyses using UPLC are HPLC are very useful. A TS ratio can be used to estimate the outcome of the oxidation, whereas a UV absorbance ratio can be useful to detect the formation of phenolic compounds. Future studies can be based on the same experimental procedure as in this study to increase the understanding for the findings, but development of the procedure is recommended. However, other oxidation methods that might be more suitable for anaerobic digestion could also be explored.

2. What products are formed during alkaline wet oxidation of biorefinery lignin from wheat straw?

Phenolic compounds such as vanillin, vanillic acid and guaiacol were formed during alkaline wet oxidation of biorefinery lignin of wheat straw. At least four more monoaromatic compounds were also formed in this study but could not be identified. The organic acids that were identified after the oxidation consisted of formic acid and acetic acid, but at least seven other compounds could also be detected. Organic acids were the main type of oxidation product in this study. Whether they were derived from lignin or from the carbohydrate fractions in the material can be studied more closely. Hence, more investigations are needed to identify the obtained oxidation products, to quantify them appropriately and to determine their origin.

3. How is the outcome of alkaline wet oxidation affected by varying the parameters temperature, dry matter (DM) content, reaction time and amount of NaOH?

The outcome of alkaline wet oxidation depends on all parameters studied. The DM content could, however, be seen as the most influential parameter in this study. This parameter, in connection with the NaOH loading, was closely related to the stability of pH in the system. In turn, the pH stability has been shown to be the factor that determines the outcome of the oxidation. It is possible that other conclusions would be drawn if the NaOH loading was adjusted based to the DM content, so that the pH could be kept stable in all experiments. In addition, high solids loading (25% DM content) can induce repolymerization of lignin fragments, which is a highly undesirable outcome.

The temperature and reaction time have been shown to be more influential for what kind of oxidation products that are formed. In systems with a stable pH, high temperature and longer reaction time favoured the formation of organic acids. Regarding the phenolic compounds, the influence differed depending on which phenolic compound that was considered. The parameters can, thus, be fine-tuned to enhance the formation of the most desirable oxidation products.

4. What reaction conditions give the highest yield of oxidation products?

The optimal reaction conditions in this study were found at 180˚C, 6.5% DM content, 23 min reaction time and 9.8 wt% NaOH, when applying 10 bar O_2. The total yield of phenolic compounds, including vanillin, vanillic acid and guaiacol, was at least 19.7 mg/g TS. The estimated yield of organic acids was 355 mg/g TS, including formic acid, acetic acid and seven other unidentified compounds. At least 9.4% of the obtained acids at these reaction conditions could be assumed to be due to lignin oxidation.

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Appendices

Appendix A – The gradient method in UPLC analysis

A gradient method was used in the UPLC analysis for evaluation of monoaromatic compounds. In the gradient method, the mobile phase can be considered as consisting of two fractions. One fraction was water with 10 mM formic acid, whereas the other fraction consisted of acetonitrile with 10 mM formic acid. The idea behind the gradient method has been to achieve appropriate washing of the column, basically increasing the amount of acetonitrile after 20 minutes from a few percent to 80%. The reason is to capture any residual non-polar compounds present that do not interact well with the water in the mobile phase.

Table A.1: The gradient method used in the analysis of monoaromatic compounds (described in section 3.3.4). The varying fractions of water and acetonitrile are shown for different time steps in the analytical procedure.

Time (min)	**Water (%)**	**Acetonitrile (%)**
0	97	3
12	85	15
15	85	15
20	20	80
20.1	10	90
25	10	90
25.1	97	3
35	97	3

Appendix B – Compilation of properties of the oxidized materials

In Table B.1, sediment described as fine-grained means that it was similar to the untreated biorefinery lignin. Just as in Table 4.4, the pH stability during the experiment has also been indicated through different colors in Table B.1. Green implies a stable pH during the experiment, orange a slight decrease in pH and no color a signinficant decrease in pH.

Table B.1: Compilation of properties of the oxidized materials. Green implies a stable pH during the experiment, orange a slight decrease in pH and no color a signinficant decrease in pH

DM (%)	Experiment	Color after oxidation	Type of sediment after oxidation	Other observations
6.5	**13**	Reddish with orange foam	No sediment	
10	**2**	Reddish	Barely any sediment	
	6	Reddish	Some sediment	
	3	Reddish	Coarse and crystalline (after cold storage)	
	7	Reddish	Coarse and crystalline (after cold storage)	
17.5	**11**	Dark brown	Fine-grained	Considerable amount of material left as a filter cake. Long time needed (>24h) for filtration of decanted liquid fraction.
	15	Dark brown	Fine-grained	
	14	Dark brown	Fine-grained	
	10	Dark bown	Fine-grained. not that large amount	
	16	Dark brown	Fine-grained	
	17	Dark brown	Fine-grained	
	1	Dark brown/Reddish	If any, coarse and crystalline fragments.	Material was non-viscous. Considerable amount of material left as a filter cake. Long time needed (>12h) for filtration of the decanted liquid fraction.
25	**4**	Dark brown/Black	Fine-grained but also somewhat coarse. Sediment not as viscous as in experiment 10 or 17.	Long time needed (>12h) for filtration of the very viscous, decanted liquid fraction.
	8	Dark brown/Black	Fine-grained but also somewhat coarse. Larger amount. Some gravel-like pieces.	Considerable amount of material left as a filter cake, with similar appearance as the filter cake from experiment 11.
	5	Dark brown/Black	Fine-grained but also somewhat coarse. Larger amount. Some gravel-like pieces.	Considerable amount of material left as a filter cake, with similar appearance as the filter cake from experiment 11.
	9	Dark brown/Black	Fine-grained but also somewhat coarse. Larger amount. Some gravel-like pieces.	Considerable amount of material left as a filter cake, with similar appearance as the filter cake from experiment 11.
28.5	**12**	Dark brown/Black	Very thick and fine-grained. Not liquid at all.	Long time needed for filtration of washing water