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Suppression of Lignin Repolymerisation to Enhance Cellulose Bioconversion and Lignin Valorisation – A Review

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Abstract: The recent discovery that the prevention of lignin repolymerisation/condensation in lignocellulosic biomass pretreatment can both enhance the bioconversion of cellulose and the quality of the obtained lignin, has brought a lignocellulose biorefinery closer to reality. In this work, the development of this approach and the latest advancements are reviewed. The review reveals the successful implementation for a wide range of lignocellulosic substrates including softwood, hardwood, and agricultural residues. In addition, it is shown that the approach can enhance various pretreatment technologies, including steam, acid and organosolv processes. Recent developments involve the discovery of new and greener additives which prevent lignin repolymerisation, the implementation of cellulose saccharification at industrially realistic conditions and high-yield fermentation. In addition, first applications of the lignin obtained in these processes are reviewed, showcasing its enhanced quality for functionalisation and use in polymers, as well as for its depolymerisation to aromatic monomers. The recent progresses bring the prospect of a biorefinery, that can valorise all fractions of lignocellulosic biomass, closer to reality.

Keywords: Biomass · Biochemicals · Carbocation · Condensation · Lignin · Lignocellulose · Repolymerisation

Dr. Thomas Pielhop holds a degree in Chemical Engineering from TU Munich (2009) and obtained a PhD in Process Engineering from ETH Zurich (2015). In his doctoral research, he pioneered a new technology which enables the enhanced valorisation of both cellulose and lignin, based on the prevention of lignin repolymerisation in lignocellulosic biomass pretreatment. After postdoctoral work at ETH Zurich, he joined Givaudan SA (Geneva) in 2017 as a Laboratory Head and Process Development Chemist/Engineer, developing and upscaling processes for the industrial manufacturing of novel and biobased fragrance and cosmetic ingredients. Since 2022, he is leading the Research Group of Biobased Resources at Zurich University of Applied Sciences ZHAW. His research interests include the conversion of lignocellulose to chemicals/materials and the sustainable production of fine chemicals.

1. Introduction

Lignocellulosic biomass such as wood, forest and agricultural residues are available at large scale and a potential source for the sustainable production of chemicals and materials. The biomass' carbohydrates, *i.e.* cellulose and hemicellulose, can be saccharified in order to produce various chemicals *via* fermentation of the resulting sugars. Lignin, an aromatic polymer in lignocellulose, is a potential source for the manufacturing of aromatic chemicals and polymers.

The use of lignocellulose is not in competition with nutrition production and its raw material cost is lower compared to food crops such as corn or sugarcane. However, the processing of this biomass is more complex and costly.[1] Due to the structure and entanglement of its major components cellulose, hemicellulose and lignin, the carbohydrates are not readily available for bioconversion.[1b] Enzymatic carbohydrate saccharification is generally preferred to chemical hydrolysis with concentrated acids at elevated temperatures, which suffers from high acid costs, by-product formation and sugar degradation.[1a,2] In particular, lignin hinders the enzymatic carbohydrate hydrolysis by acting as a physical barrier restricting the accessibility to cellulose, but also by the non-productive binding of cellulolytic enzymes.[3] A pretreatment capable of breaking down the lignocellulosic structure, or even removing the lignin, is necessary to render the enzymatic depolymerisation of its carbohydrates to soluble fermentable sugars possible.

Such pretreatments include, for example, autohydrolysis (steam) and acid processes, or lignin-removing organosolv approaches. In these processes, the biomass is treated under acidic conditions due to acid release from hemicellulose[4] or due to the addition of additional acid catalyst. These acidic conditions lead to the formation of carbocations in lignin, as shown in Scheme 1 (route a). Carbocations are intermediates in lignin depolymerisation reactions and help break down its structure, especially in the cleavage of β-arylether linkages (Scheme 1, route b). On the other hand however, the electrophilic carbocations can also form, through substitution, stable C–C bonds with the nucleophilic carbon atoms of the aromatic rings present in lignin[7,8] (Scheme 1, route c). High-molecular weight and repolymerised (or condensed) lignin structures are formed.

The author and coworkers hypothesised that these repolymerised lignin structures can hinder both the enzymatic cellulose conversion and the valorisation of the resulting lignin. In 2011, we introduced and patented a novel pretreatment concept targeting to obtain a cellulose fraction of improved digestibility and a high-quality lignin at the same time, by preventing lignin repolymerisation during biomass pretreatment.[9] This work, funded by the Swiss National Science Foundation[10] (2012–2014, 2014–2017), does not consider lignin solely as a by-product, like previous pulping or biorefinery approaches. Instead, the approach

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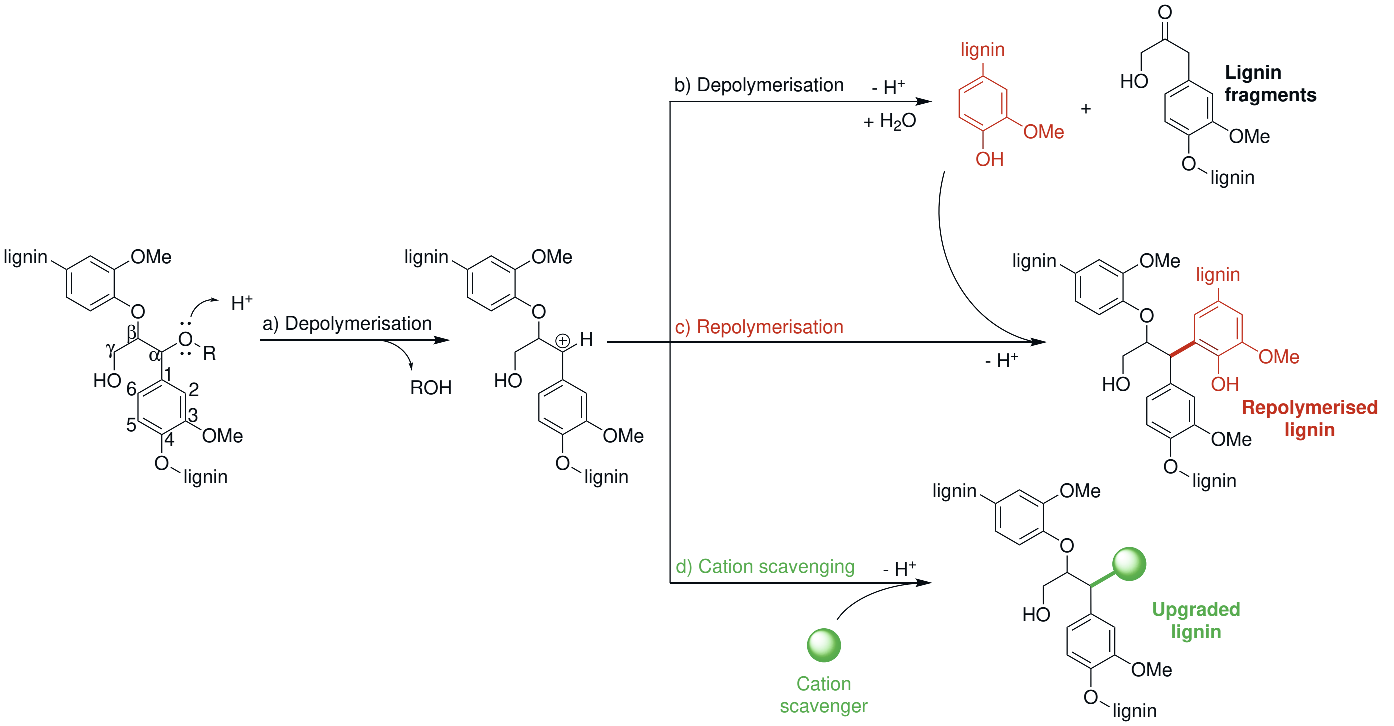


Scheme 1. Reactions of lignin in acidic media:[5] (a) acid catalysed depolymerisation and formation of carbocation;[6] (b) cleavage of β-O-4' bond and depolymerisation;[6] (c) lignin repolymerisation reaction;[7] (d) scavenging of carbocation.

takes the valorisation of both the cellulose and the lignin fraction into account from the very beginning of a biorefinery process, the pretreatment.[11]

A method for suppressing undesired lignin repolymerisation reactions is the use of blocking agents. Wayman and Lora[8a] first reported that certain aromatic compounds can act as scavengers in the autohydrolysis of wood, thus increasing the yield of organic solvent-extractable lignin and allowing to obtain a highly delignified pulp. The additives compete with the aromatic rings present in the lignin for the formed carbocations and prevent repolymerisation reactions (Scheme 1, route d). 2-Naphthol was found to be a very effective compound, yielding a lignin of lower molecular weight with enhanced extractability.[8,12]

We could reveal that the prevention of lignin repolymerisation with such scavengers also enhances the enzymatic cellulose hydrolysability.[9a,13] The enhanced digestibility is not based on an increased lignin removal during pretreatment, but on a different structure of the lignin, resulting in reduced enzyme adsorption[4b] and a better accessibility to the cellulose.[14]

Next to the enhanced cellulose saccharification, the prevention of lignin repolymerisation in pretreatment yields a lignin with less condensed C–C bonds.[9a] We also hypothesised that such types of lignin allow for higher yields of aromatic products in its depolymerisation.[9a] Technical lignins have already undergone several processes for their extraction/separation from cellulosic components and thus have already been subjected to repolymerisation, limiting the yield of aromatic monomers that can be obtained in their depolymerisation.[9a] Further, the obtained lignin fraction has a more homogeneous form with a lower and more defined molecular weight and features improved solubility, simplifying its further processing and enhancing its value for use as a polymer.[9a] We also described that the (tailor-made) scavengers can be used for the targeted functionalisation of the lignin structure for chemicals or polymer production.[9a,15]

The illustrated approach allowed for a breakthrough in the pretreatment of lignocellulose.[16] To the best of the author's knowledge, it is one of the first conceptual works which described the benefits of preventing lignin repolymerisation already in biomass pretreatment for simultaneously enhancing the (bio)chemicals production both from cellulose and lignin. In recent years, a series of works have been published which promote similar concepts for enhancing cellulose and lignin valorisation.

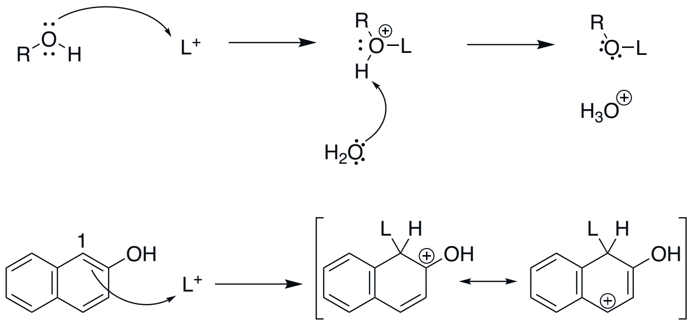
With regards to lignin, the term 'lignin-first' has been introduced to classify new biorefining methods which protect the lignin from degradation and repolymerisation already in biomass pretreatment, in order to enhance its use for the production of chemicals and materials.[17] These methods are often based on lignin extraction and the addition of compounds which prevent the formation of lignin carbocations,[18] directly react with the ions,[19] or react with their secondary products.[17a,19,20] Lignin-first is often interpreted as the disassembly of lignin prior to carbohydrate valorisation.[17a] It should be noted that these processes can lead to side-reactions such as the grafting of functional groups on carbohydrates,[17a] which can interfere with the binding of enzymes in cellulose bioconversion.[21,22]

The present manuscript focuses on research which can simultaneously enhance the valorisation of both the cellulose and the lignin fraction of lignocellulosic biomass. An emphasis is placed on biocatalytic cellulose degradation. The review aims at comparing the effectiveness of preventing lignin repolymerisation for different pretreatment technologies and biomass species. In addition, the various additives used in pretreatment are compared and the applications of the obtained lignin are presented.

2. Additives for Preventing Lignin Repolymerisation

Compounds that can react with lignin carbocations and block repolymerisation may also be referred to as carbocation scavengers.[5b] This class of compounds includes nucleophilic substances like aromatics, alcohols, amines or amino acids, which we have tested in an earlier screening study.[14] In order to act as blocking agents, the additives preferentially offer only one nucleophilic site.[8a,14] Additives which offer several reactive sites need to be added at higher concentrations, otherwise they may promote the crosslinking of lignin fragments.[14,23]

Alcohols may act as nucleophiles for attacking carbocations in lignocellulose pretreatment, forming new C–O bonds on the C-α of the lignin backbone[4a,14] (see Scheme 2, top). Aromatic compounds are also active towards nucleophilic substitution, usually forming new C–C bonds[8a,14] (see Scheme 2, bottom). Even though amines are stronger nucleophiles than alcohols, their addition was shown to negatively impact bioconversion.[14] This may be attributed to their potential to increase lignin crosslinking[14] and/or their integration into the lignin structure, leading to increased enzyme deactivation by hydrogen bonding with the amide groups in enzymes.



Scheme 2. Top: reaction of an alcohol with a lignin carbocation (L+). Bottom: nucleophilic attack of 2-naphthol on a lignin carbocation with the substitution occurring in C-1 position.[14]

Fig. 1 shows an overview of additives that have been successfully tested in pretreatment for enhancing bioconversion. Our first screening studies showed that in particular aromatic alcohols are effective for enhancing the bioconversion of lignocellulose.[14] 2-Naphthol has proven to be a very efficient additive. The two aromatic rings feature a high electron density and it undergoes a single electrophilic substitution with lignin.[5b,24] 2-Naphthol is easily substituted in position 1 (Scheme 2, bottom) but not in position 3, since substitution in position 3 would cause the loss of the molecule's aromaticity in the transition state.[8a,14]

Recently, further derivatives of 2-naphthol such as 3-hydroxy-2-naphthoic acid[25] or 2-naphthol-7-sulphonate[26] have been successfully tested in pretreatment. Lai *et al*. described that 2-naphthol-7-sulphonate offers the advantage of an enhanced scavenger solubility in aqueous pretreatments. Further, its integration into the lignin structure can increase the water solubility of lignin itself. This allows for a partial lignin removal during aqueous pretreatments, which further contributes to enhancing enzymatic conversion. In addition, the sulphonyl groups may enhance the hydrolysis by the reduction of hydrophobic enzyme-lignin interactions.[26]

Dimethylphloroglucinol is an example for another class of scavengers. It is an aromatic compound which is highly activated towards nucleophilic substitution by several hydroxy groups (in this case three), but only offering one single accessible reactive site for the substitution. In that way, it suppresses lignin repolymerisation and does not act as a crosslinker. We could show that it works as effectively as 2-naphthol for enhancing bioconversion.[14]

As already mentioned, compounds offering several free active positions may suppress lignin repolymerisation reactions as well. For instance, 4-hydroxybenzoic acid, vanillic acid and syringic acid have been tested successfully for enhancing bioconversion.[27] Such compounds should, however, be added in excess to prevent lignin crosslinking reactions.[5b,23]

It was recently shown that polyhydric alcohols are effective additives for preventing lignin condensation and enhancing bioconversion. Aliphatic alcohols such as ethylene glycol,[19,28] glycerol,[29] 1,4-butanediol,[21,30] and sugar alcohols like mannitol[31] have been tested. Noteworthy, those compounds feature several hydroxy groups, thereby also introducing hydrophilic functionality into the lignin backbone. This can further reduce lignin–enzyme interactions and enzyme deactivation.[31] Possibly, polyhydric alcohols need to be added at concentrations high enough to prevent lignin crosslinking, similarly to other compounds with several nucleophilic active sites.

In order to enhance sustainability, the additive should preferably be produced from renewable sources. Notably, some of the aromatic scavengers proposed above may be produced even from lignin itself.[9a,11,14,32] In addition, the scavengers 4-hydroxybenzoic acid, vanillic acid, syringic acid[27] and *p*-hydroxybenzyl alcohol[33] can be derived from lignin. The compounds ethylene glycol, glycerol and mannitol can be sourced from renewables as

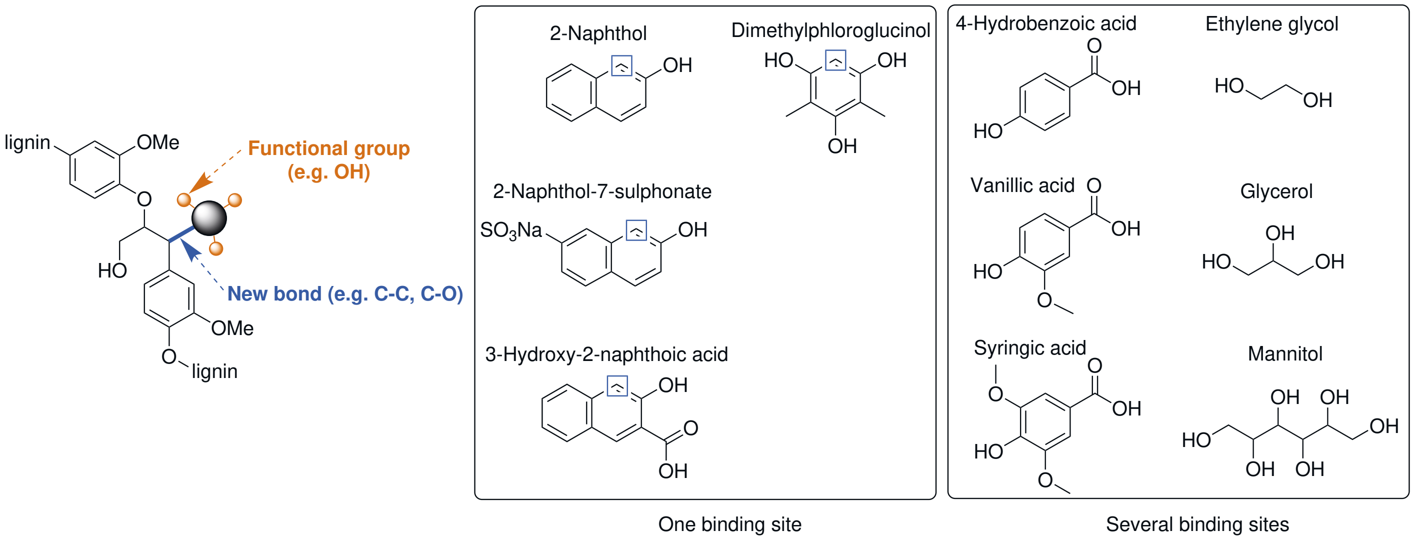


Fig. 1. Left: Illustration of a carbocation scavenger that has reacted with a lignin carbocation. A new bond (blue) and functional groups (orange) can be integrated into the lignin structure with the scavenger. Boxes: Carbocation scavengers that have been successfully tested in lignocellulose pretreatment for enhancing bioconversion. The binding site (blue) of aromatic compounds with only one position activated towards nucleophilic addition is highlighted.

well.[29,31,34] Such compounds may also be referred to as 'green' additives or scavengers.

Next to the discussed carbocation scavengers, aldehydes[18,35] or reductive reaction conditions can also prevent lignin repolymerisation. Aldehydes can prevent the formation of carbocations by active stabilisation of β–O–4′ bonds in lignin,[17a,18] while hydrogenation/hydrogenolysis of the biomass can stabilise depolymerised reactive intermediates.[17a,36]

3. Enhancing Enzymatic Cellulose Hydrolysis

3.1 *Autohydrolysis and Steam Pretreatment*

Autohydrolysis methods include hot-water or steam pretreatment and are attractive regarding their cost-savings potential. They do not require acid, base or solvent chemicals and result in simpler biorefinery processes.[4,37]

Table 1 shows an overview of the use of additives in such pretreatments for enhancing enzymatic hydrolysis. The addition of carbocation scavengers for enhancing cellulose bioconversion was introduced in our pioneering work.[5b,9a,13] We could further reveal that this processing can allow for almost complete cellulose conversions of softwood.[5b,38] Among lignocellulosic biomass, softwood has the highest recalcitrance to bioconversion, and autohydrolysis pretreatments are usually not efficient.[32,39] However, the addition of carbocation scavengers has opened up a new pretreatment route for softwood[40] and brought a softwood-based biorefinery closer to reality.[32]

We could successfully upscale and implement the process in a steam explosion pilot plant, using 2-naphthol as additive.[41] The synergistic effects of the scavenger (reduction of enzyme deactivation) and the explosion (increase of cellulose accessibility) allowed for almost complete cellulose conversion with an enzyme dose of 15 FPU (filter paper units) g^{-1} cellulose,[41] which is a remarkably low concentration for softwood and a pretreatment that does not remove lignin. Compared to a pretreatment without additive, the cellulose conversion was enhanced by 192%.

Recently, Hansen *et al*. also successfully upscaled the process in a steam explosion pilot plant, likewise using 2-naphthol for softwood pretreatment. Their work even achieved the complete enzymatic saccharification at industrially feasible conditions: hydrolysis was carried out with a high dry matter concentration of 10% w/w, a moderate enzyme dosage of ~20 FPU g^{-1} cellulose and a hydrolysis time of only 48 h (see Table 1).[32]

Seidel *et al.* could confirm the effectiveness of 2-naphthol in softwood steam explosion pretreatment and achieved complete cellulose conversion with an enzyme concentration of 30 FPU g^{-1} cellulose. In particular, their work successfully implemented 2-naphthol in a two-stage steam explosion pretreatment, allowing to increase the total sugar recovery from both hemicellulose and cellulose. 2-Naphthol was added to the harsher second stage of the pretreatment to prevent repolymerisation reactions, which allowed the enzymatic cellulose conversion to be enhanced by 52%.[42]

Borrega *et al.* compared the addition of 2-naphthol and of formaldehyde to minimise condensation reactions in pretreatment in the framework of the Lignin First Concept for High Value Applications (L4Value project).[35b] Pine, willow and birch wood were studied. In enzymatic saccharification, moderate enzyme

Table. 1. Examples of autohydrolysis and steam pretreatments with additive/carbocation scavenger for enhancing cellulose bioconversion.

Reference		Pretreatment				Enzymatic cellulose hydrolysis			
Year	**Author**	**Biomass**	**Method**	**Additive conc.**	**Additive/ scavenger**	**Digestibility increase**	**Digest-ibility**	**Enzyme dosage[c]**	**Conc.**
2011	Pielhop *et al.*[9a]	Spruce	Autohydrolysis	0.205 mol/mol lignin C9	2-Naphthol	+28%	65%	60 FPU g^{-1} cellulose[d]	1%w/w cellulose
2015	Pielhop *et al.*[5b]	Spruce	Autohydrolysis	0.205 mol/mol lignin C9	2-Naphthol	+64%	96%	60 FPU g^{-1} cellulose[d]	1%w/w cellulose
2016	Pielhop *et al.*[14]	Spruce	Autohydrolysis	0.205 mol/mol lignin C9	Dimethyl-phloroglucinol	+42%	93%	60 FPU g^{-1} cellulose[d]	1%w/w cellulose
2017a	Pielhop *et al.*[38]	Pine	Autohydrolysis	0.205 mol/mol lignin C9	2-Naphthol	+57%	93%	60 FPU g^{-1} cellulose[d]	1%w/w cellulose
2017b	Pielhop *et al.*[41]	Spruce	Steam explosion	0.205 mol/mol lignin C9	2-Naphthol	+192%	95%	15 FPU g^{-1} cellulose[d]	1%w/w cellulose
2019	Seidel *et al.*[42]	Spruce	2-stage steam explosion	0.205 mol/mol lignin C9	2-Naphthol	+50.9%	72%	60 FPU g^{-1} cellulose[d]	1%w/w cellulose
			1-stage steam explosion			N/A	100%	30 FPU g^{-1} cellulose[d]	1%w/w cellulose
2019	Chu *et al.*[43]	Poplar	Steam explosion	5% w/w dry biomass	2-Naphthol	+15%	73.3%	20 FPU g Cellulose[e]	2% w/v cellulose
2020	Wang *et al.*[29]	Corn stover	Steam explosion	50% w/w dry biomass	Glycerol	+28.7%	98.4%	20 FPU g^{-1} biomass[e]	2.5% w/v dry biomass
2021	Borrega *et al.*[35b]	Pine	Autohydrolysis	4.7% w/w dry biomass	2-Naphthol	+ 100%	52%	10.6 FPU g^{-1} biomass[e]	5% w/w dry biomass
		Willow		4.7% w/w dry biomass	2-Naphthol	+ 31%	88%		
				37% w/w dry biomass	Formaldehyde	+ 37%	92%		
2022	Hansen *et al.*[32]	Spruce	Steam explosion	0.205 mol/mol lignin C9	2-Naphthol	+62%	100%	~20 FPU g^{-1} cellulose[e]	10% w/v dry biomass
2022	Madadi *et al.*[44]	Pine	Autohydrolysis + green liquor extraction	5% w/w dry biomass	2-Naphthol	+64%	90%	~20 FPU g^{-1} cellulose[e]	5% w/v dry biomass
					2-Naphthol-7-sulphonate	+68.9%	92%		
					Mannitol	+60%	88%		

[a]Poly(ethylene glycol) diglycidyl ether; [b]Poly (ethylene glycol) diglycidyl ether; [c]FPU: Filter paper units; [d]Accellerase 1500, DuPont; [e]Cellic CTec 2, Novozymes;

concentrations of 10.6 FPU g^{-1} biomass were used. The use of 2-naphthol could double the yield in the saccharification of pretreated pine and increased the saccharification yield from willow by 31%. This study also revealed the enhancing effect of formaldehyde, which increased the cellulose saccharification yield of pretreated willow by 37%. As mentioned earlier, aldehydes do not directly react with lignin carbocations, but prevent their formation by creating an acetal with the β–O–4′ linkage's diol structure, thus stabilising the lignin.[18,35c] This means that also the prevention of lignin depolymerisation/repolymerisation can enhance enzymatic cellulose saccharification. 2-Naphthol and formaldehyde were reported to be particularly effective for biomass which included bark, possibly due to the specific lignin structure of bark and/or the included resins.[35b]

Madadi *et al.* implemented carbocation scavengers (2-naphthol, 2-naphthol-7-sulphonate, mannitol) in a process consisting of softwood autohydrolysis followed by green liquor extraction.[44] The process allowed for high cellulose digestibilities around 90% at moderate enzyme dosages of 20 FPU g^{-1} cellulose. The suppression of lignin repolymerisation and the integration of the additives (in particular 2-naphthol-7-sulphonate) enhanced lignin extraction from the biomass, but also reduced non-productive enzyme binding on lignin.[44]

Generally, the highest improvements of autohydrolysis/steam pretreatments with a carbocation scavenger were observed when softwood was used as substrate. This may be related to the exceptionally high recalcitrance of softwood to bioconversion and/or to its lignin structure, which may render it particularly susceptible to repolymerisation reactions.[5b] The additives could, however, also enhance the steam explosion pretreatments of poplar[43] and of agricultural residues.[29] Wang *et al.* used glycerol to enhance the steam explosion pretreatment of corn stover. Glycerol is supposed to prevent repolymerisation and preserve β–O–4′ linkages through α-etherification and γ-esterification of lignin and lignin derivatives. Notably, the used glycerol concentration of 50% w/w dry biomass did not increase lignin removal from the biomass, but still increased glucose yields in enzymatic saccharification by 29%.[29] The digestibility enhancement can therefore be attributed to changes in the lignin structure. Higher concentrations of glycerol further enhanced digestibility, but also led to some lignin removal.[29] Further studies with glycerol could also show the enhancement of sugarcane bagasse pretreatment[45] (reference not included in Table 1).

3.2 *Dilute Acid Pretreatment*

Dilute acid pretreatment is a suitable method for dealing with highly recalcitrant biomass, such as softwood.[46] The use of carbocation scavengers for enhancing dilute acid pretreatment was studied by the author and coworkers.[38] The pretreatment of spruce wood with 2-naphthol allowed to achieve a cellulose digestibility of 86%, which corresponded to a 60% enhancement compared to a pretreatment without additive (Table 2). At higher pretreatment severities, the cellulose digestibility could even be enhanced by 142%. However, this high enhancement was mostly related to the very low sugar yields obtained in the pretreatment without additive, and also hydrolysis yields in the experiments with additive decreased from 86% to 76%. Nevertheless, the study revealed that carbocation scavengers may enhance dilute acid pretreatment even more than autohydrolysis pretreatment (compare Table 1, Pielhop *et al.* 2015[5b]), most likely due to the increased rate of lignin repolymerisation in the more acidic environment.

Ever since, many studies have used carbocation scavengers in dilute acid pretreatment. The used additives were generally similar to the ones discussed in autohydrolysis/steam pretreatment (Section 3.1). The approach proved effective for a wide range of biomass, including several softwood species (spruce, pine, larch), hardwood (poplar), mixed wood, bamboo and different agricultural residues (wheat straw, corn stover, corncob, rice straw), as presented in Table 2.

Sheng *et al.* studied the addition of several naphthoic acids to the dilute acid pretreatment of poplar.[25] The additives were shown to suppress lignin repolymerisation and did also introduce new carboxylic acid groups into the lignin. The increased carboxylic acid content was supposed to decrease lignin hydrophobicity and thereby additionally reduce the non-productive adsorption of enzymes. 3-Hydroxy-2-naphthoic acid proved as the most effective additive, enhancing enzymatic cellulose saccharification by 72%. The additive even outperformed 2-naphthol (12% digestibility enhancement) and also decreased the molecular weight (Mw) of the obtained lignin more than 2-naphthol.[25] However, the sugar yields in this study were low to moderate, not exceeding 53%. In a follow-up study, 3-hydroxy-2-naphthoic was also implemented for different agricultural residues.[47]

Zhai *et al.* showed that 4-hydroxybenzoic acid, vanillic acid and syringic acid can serve as effective additives in the dilute acid pretreatment of pine.[27] The additives enabled the cellulose hydrolysis of the whole pretreated slurry without any post detoxification step. Very high digestibility enhancements of up to 277% were observed, compared to a treatment without additive. The researchers attributed the effect in particular to the suppression of lignin depolymerisation to smaller ketone-type phenolics (see also Scheme 1, route b), which may hinder enzymatic conversion.[27]

Sheng *et al.* introduced ascorbic acid as a carbocation scavenger in the dilute acid pretreatment of wheat straw, corn stover and corncob residues.[54] Next to enhancing the enzymatic cellulose conversion of all studied residues, the additive could also slightly enhance hemicellulose (xylan) removal. In a separate study, the compound could enhance the pretreatment of poplar as well.[56] Ascorbic acid is water soluble and unlikely to hinder enzymatic or fermentation processes, therefore having the potential to simplify a biorefinery process accordingly.[54]

Chiranjeevi *et al.* have tested a combination of glycerol and boric acid (*ca*. 5% and 10% w/w of dry biomass, respectively) in the dilute acid pretreatment of rice straw residues.[59] Boric acid has been shown to reduce condensation reactions by forming borate esters with the hydroxy groups of lignin.[57] In combination with glycerol as a carbocation scavenger, the digestibility could be enhanced by 83% compared to the control without additives. A delignification rate of 42% contributed to the digestibility enhancement. Notably, the enzymatic hydrolysis experiments were carried out at a high biomass loading of 10% w/w dry biomass.

In further studies, glycerol could also enhance the dilute acid pretreatment of sugarcane bagasse[45,58] (references not included in Table 2). The integration of glycerol into lignin was reported to increase its hydrophilicity, leading to reduced enzyme adsorption and deactivation.[45]

Moreover, carbocation scavengers were also reported to enhance acid sulphite (sulphuric acid and sodium bisulphite)[52] and acid-chlorite (acetic acid and sodium chlorite)[53] pretreatments. 2-Naphthol and 2-naphthol-7-sulphonate proved more effective in acid sulphite than in dilute acid pretreatment of poplar (88% vs 52% and 91% vs 58% yield enhancement, respectively).[52]

In addition, carbocation scavengers in acidic pretreatment could enhance several post-treatments. For example, post-treatments with alkaline hydrogen peroxide,[51] with poly(ethylene glycol) diglycidyl ether (PEE)[53] and with poly(ethylene glycol) diglycidyl ether (PEGDE)[49] could be enhanced, and led to higher digestibilities compared to the processes without additive. The post-treatments with alkaline peroxide and with PEGDE even allowed enhancing the effect of 2-naphthol on enzymatic hydrolysis. Thus, synergistic effects of the scavenger and the post-treatments were revealed.[49,51] In addition, high cellulose loadings of

Table. 2. Examples of dilute acid pretreatments with additive/carbocation scavenger for enhancing cellulose bioconversion.

Reference		**Pretreatment**				**Enzymatic cellulose hydrolysis**			
Year	**Author**	**Biomass**	**Method**	**Additive conc.**	**Additive/ scavenger**	**Digestibility increase**	**Digestibility**	**Enzyme dosage[c]**	**Conc.**
2017a	Pielhop *et al.*[38]	Spruce	Dilute acid	0.205 mol/mol lignin C9	2-Naphthol	+60%	86%	60 FPU g^{-1} cellulose[d]	1%w/w cellulose
2018	Chiranjeevi *et al.*[48]	Rice straw	Dilute acid	5% + 10% w/w dry biomass	Glycerol + boric acid	+83.3%	77%	20 FPU g^{-1} cellulose[f]	10% w/w dry biomass
2018	Lai *et al.*[26]	Mixed wood	Dilute acid	4% w/w dry biomass	2-Naphthol	+9.1%	39.7%	20 FPU g^{-1} cellulose[g]	2% w/v cellulose
					2-Naphthol-7-sulphonate	+47.8%	53.8%		
2018	Zhai *et al.*[27]	Pine	Dilute acid	5% w/w dry biomass	2-Naphthol	+72%	31%	40 mg protein g^{-1} cellulose[h]	~2%w/w cellulose (whole slurry)
					4-Hydroxybenzoic acid	+233%	60%		
					Vanillic acid	+178%	50%		
					Syringic acid	+277%	68%		
2019	Chu *et al.*[43]	Poplar	Dilute acid	5% w/w dry biomass	2-Naphthol	+48.7%	63.1%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
					2-Naphthol-7-sulphonate	+71.1%	72.5%		
2020	Lai *et al.*[49]	Larch	Dilute acid	4% w/w dry biomass	2-Naphthol	+14.4%	29.4%	60 FPU g^{-1} cellulose[e]	2% w/v cellulose
			Dilute acid + alkali/PEGDE[b]		2-Naphthol	+20.8%	52.3%		
2021a	Chu *et al.*[50]	Poplar	Dilute acid	5% w/w dry biomass	4-Hydroxybenzoic acid	+10%	39.6%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
					Vanillic acid	+20%	43.1%		
					Syringic acid	+40%	50.6%		
					2-Naphthol	+49%	55%		
					2-Naphthol-7-sulphonate	+77%	64%		
2021b	Chu *et al.*[31]	Poplar	Dilute acid	5% w/w dry biomass	2-Naphthol-7-sulphonate	+71%	72.3%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
					Syringic acid	+33%	56.6%		
					Mannitol	+24%	52.8%		
2021	Huang *et al.*[51]	Poplar	Dilute acid	5% w/w dry biomass	2-Naphthol	+16.8%	54.2%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
			Dilute acid + alkaline H_2O_2		2-Naphthol	+37%	90.2%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
2022a	Tong *et al.*[52]	Poplar	Dilute acid	5% w/w dry biomass	2-Naphthol	+7.7%	51.9%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
					2-Naphthol-7-sulphonate	+20.7%	57.8%		
			Acid sulphite		2-Naphthol	+24%	88%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
					2-Naphthol-7-sulphonate	+28%	90.6%		
2022b	Tong *et al.*[53]	Poplar	Dilute acid	5% w/w dry biomass	2-Naphthol	+33%	55.7%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
			Dilute acid + acid-chlorite		2-Naphthol	+8%	90.8%		
			Dilute acid + PEE[a]		2-Naphthol	+11%	100%		
2021a	Sheng *et al.*[25]	Poplar	Dilute acid	0.205 mol/mol lignin C9	2-Naphthol	+12.3%	34.7%	20 FPU g^{-1} cellulose	2% w/v cellulose
					6-Hydroxy-2-naphthoic acid	+23.9%	38.3%		
					2-Hydroxy-1-naphthoic acid	+16.5%	36%		
					6-Hydroxy-1-naphthoic acid	+16.5%	36%		
					3-Hydroxylic-2-naphthoic acid	+72.2%	53.2%		
2021b	Sheng *et al.*[54]	Wheat straw	Dilute acid	4% w/w dry biomass	Ascorbic acid	12.7%	70.7%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
		Corn stover				18.8%	50.3%		
		Corncob				13.6%	97%		
2022	He ***et al***.[55]	Bamboo	Dilute acid	8% w/w dry biomass	2-Naphthol	+40.8%	37.6%	60 FPU g^{-1} cellulose + 20 Ug^{-1} β-glucosidase[i]	2% w/v cellulose
				4% w/w dry biomass	2-Naphthol-7-sulphonate	+14%	47.3%		
2022	Manqing *et al.*[47]	Wheat straw	Dilute acid	4% w/w dry biomass	3-Hydroxylic-2-naphthoic acid	61.3%	33%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
		Corn stover				18.9%	36%		
		Corn cob				13.6%	85%		
2022	Sheng *et al.*[56]	Poplar	Dilute acid	4% w/w dry biomass	Ascorbic acid	20.2%	41%	20 FPU g^{-1} cellulose	2% w/v cellulose

[a–e]: See annotations Table 1; [f]SachariSEB C6 Plus, Advanced Enzymes; [g]UTA-8, Youtell Biochemical + BG188, Novozymes; [H]Cellic CTec 3, Novozymes; [i]L-100, Youtell Biochemicals

8% w/v were tested in enzymatic hydrolysis, resulting in sugar yields of 85% and sugar concentrations of more than 75 g L^{-1}, which enables high titre fermentation.[51]

3.3 *Lignin Extraction Pretreatments*

Lignin extracting pretreatments dissolve and remove lignin from the biomass, thereby greatly enhancing the enzymatic digestibility of the cellulose fraction. Organosolv pretreatments are often acid-catalysed, in order to break down the lignocellulosic structure. The acidic conditions, however, also lead to lignin repolymerisation.

In recent years, several studies about the use of carbocation scavengers (or other additives that prevent lignin repolymerisation) have been carried out in organosolv pretreatment. These works showed that organosolv pretreatments can be enhanced to a high extent in that way. The pretreatment of different biomass, including softwood,[19,59] hardwood[28,35a,59b,60] and bamboo[30] could be improved. Studies about the effect of such pretreatments on the digestibility of agricultural residues are still needed, however.

The used additives were mostly similar to the ones discussed in autohydrolysis/steam and dilute acid pretreatment (Sections 3.1 and 3.2). Several solvents such as ethanol,[59b,60b,c] 1,4-butanediol,[21,30,59a] *p*-toluenesulfonic acid/pentanol,[60a] dimethyl carbonate[19] or choline chloride/oxalic acid[28] have been tested in combination with the additives (Table 3).

Cellulose digestibility enhancements of up to 85% have been attained, compared to pretreatments without additive.[60b] In addition, moderate to high cellulose digestibilities were achieved with low enzyme dosages <10 FPU g^{-1} cellulose.[60a,c] Generally, the high digestibility of organosolv-pretreated biomass can be related in large part to its delignification. In addition, the prevention of lignin repolymerisation further enhances the lignin extractability,[8] thereby increasing this effect even more.[60a]

On the other hand, the additives in organosolv pretreatment also modify the lignin structure in a way that reduces enzyme deactivation. For example, Chu *et al.* achieved lignin removals of up to 76% in the ethanol organosolv process of poplar with carbocation scavengers.[60b] However, only 2-naphthol-7-sulphonate enhanced lignin removal from 56% (control) to 76%. Notably, the addition of 2-naphthol, mannitol and syringic acid resulted in similar or lower lignin removals (55.5–59.7%) as in the control experiment. Thus, the digestibility enhancement with those additives (up to +61%) was not related to lignin removal, but to a change of the lignin structure. In this context Wang *et al.* could also show that the addition of lignin, isolated after ethanol organosolv pretreatment with carbocation scavenger, to the hydrolysis of pure cellulose (Avicel) resulted in up to 78% higher yields, compared to control experiments without pretreatment additive. These experiments further confirmed the reduction of enzyme deactivation by carbocation scavengers in organosolv pretreatment.[59b]

Notably, the solvent in organosolv processes may also react itself with the carbocations formed in lignin, and thereby block repolymerisation. Alcohol solvents may react as nucleophiles,[5a,14] similarly as discussed earlier for *e.g.* glycol or mannitol additives. In organosolv processes with ethanol, alkylation of lignin has been observed, resulting in the etherification of the C_α position.[61] In that way, the solvent in organosolv processes can also functionalise the lignin. It has also been proposed that lignin alkylation by ethanol may reduce the affinity of enzymes for lignin binding, and thus enhance enzymatic hydrolysis.[61b]

Nevertheless, it was found that etherification reactions can also be reversible and lignin condensation reactions in organosolv pretreatment still take place, in particular at higher temperatures. [30,61b] Therefore, the addition of carbocation scavengers can further enhance such pretreatments. For example, the solvent 1,4-butanediol has been shown to block lignin repolymerisation and to be integrated into its structure during organosolv pretreatment.[21] Yet, organosolv pretreatment with 1,4-butanediol could be further enhanced by the addition of carbocation scavengers. The pretreatment of pine could be enhanced by the additions of 2-naphthol, 2-naphthol-7-sulphonate and 3-hydroxylic-2-naphthoic acid (+23%, +78%, +39% digestibility enhancement, re-

Table. 3. Examples of lignin extraction pretreatments with additive/carbocation scavenger for enhancing cellulose bioconversion.

Reference		Pretreatment				Enzymatic cellulose hydrolysis			
Year	**Author**	**Biomass**	**Method**	**Additive conc.**	**Additive/ scavenger**	**Digestibility increase**	**Digest-ibility**	**Enzyme dosage[c]**	**Conc.**
2018	Lan *et al.*[35a]	Poplar	1,4-Dioxane/H_2O, acid	77% m/m biomass	Proprionaldehyde	N/A	87%	30 FPU g^{-1} cellulose	~3% w/v cellulose
2019	Dong *et al.*[21]	Eucalyptus	1,4-Butanediol / H_2O, acid	65% v/v solvent	1,4-Butanediol (solvent)	N/A	> 90%	7.5 FPU g^{-1} cellulose[e]	2% w/v dry biomass
2020	De Santi *et al.*[19]	Pine	Dimethyl carbonate, acid	400% w/w wet biomass	Ethylene glycol	N/A	79%	50 FPU g^{-1} dry biomass[e]	2.9% w/w dry biomass
2021	Chu *et al.*[60b]	Poplar	Ethanol/H_2O, acid	5% w/w dry biomass	2-Naphthol	+61%	71.2%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
					2-Naphthol-7-sulphonate	+85%	81.3%		
					Mannitol	+46%	64.2%		
					Syringic acid	+35%	59.4%		
2021	Liu *et al.*[28]	Birch	Choline chloride, oxalic acid	720% w/w wet biomass	Ethylene glycol	+63.1%	95.9%	N/A[e]	4% w/v dry biomass
2022	Cheng *et al.*[30]	Bamboo	1,4-Butanediol, choline chloride, $AlCl_3$	33% m/m solvent	1,4-Butanediol (solvent)	N/A	100%	25 FPU g^{-1} cellulose[e]	2.5% w/w dry biomass
2022a	Wang *et al.*[60c]	Poplar	Ethanol/H_2O, acid	5.4% w/w dry biomass	2-Naphthol	+25.3%	62.9%	5 FPU g^{-1} cellulose[e]	2% w/v cellulose
2022b	Wang *et al.*[59b]	Pine	Ethanol/H_2O, acid	4.75% w/w dry biomass	2-Naphthol	+36%	N/A	20 FPU g^{-1} cellulose[e]	2% w/v Avicel
		Poplar				+77.5%			
2023	Fang *et al.*[59b]	Pine	1,4-Butanediol / H_2O, acid	5% w/w dry biomass	2-Naphthol	+23%	63%	20 FPU g^{-1} cellulose[e]	2% w/v cellulose
					2-Naphthol-7-sulphonate	+76.6%	98%		
					3-Hydroxylic-2-naphthoic acid	38.8%	77%		
2023	Madadi *et al.*[60a]	Poplar	p-Toluenesulfonic acid/pentanol	5% w/w dry biomass	Mannitol	+44.7%	95.5%	7.5 FPU g^{-1} cellulose	N/A

Annotations: see Table 1

spectively).[59a] This process also allowed to achieve high cellulose loadings of 8% w/v in enzymatic hydrolysis and high glucose concentrations of 74 g L^{-1}. Interestingly, the addition of mannitol and syringic acid was not effective in the study, and hardwood pretreatment could not be enhanced by any of the additives.[59a]

De Santi *et al.* have used ethylene glycol as a carbocation scavenger/stabilisation agent in a lignin-first acidolysis process for softwood.[19] Next to the production of aromatic monophenols, a digestible cellulose pulp was produced which allowed for glucose yields around 80%. It was reported that the incorporation of ethylene glycol into the carbohydrates had a negligible effect on the activity of cellulase enzymes. Still, a rather high enzyme concentration of 50 FPU g^{-1} dry biomass was used, also reflecting the high recalcitrance of softwood to bioconversion.

In another lignin-first approach, Lan *et al.* achieved high enzymatic cellulose conversion of transgenic hybrid poplar after aldehyde-assisted pretreatment, next to the production of aromatic monomers in high yields.[35a] The use of propionaldehyde was an improvement of previous work, where aldehyde-assisted pretreatment required harsh acid treatment to remove acetal groups from carbohydrates, which cause low digestibilities.[18,35a] The approach with propionaldehyde seems therefore more compatible with bioconversion routes.

As addressed earlier, lignin repolymerisation in organic solvents may also be prevented by early-stage catalytic conversion of lignin fragments, leading to their 'passivation' and protecting them from recondensation. This can increase the delignification of the biomass and in that way enhance its enzymatic cellulose conversion, compared to a standard organosolv pretreatment.[36]

Recently, 1,4-butanediol has also been introduced as a carbocation scavenger in deep eutectic solvent systems with choline chloride for biomass pretreatment, suppressing lignin condensation and forming α-etherified lignin.[30] Complete enzymatic conversion of bamboo cellulose was reached, which can also be attributed to the high lignin removal of 80%.

In another deep eutectic solvent pretreatment, Liu *et al.* have introduced glycol stabilisation, which could capture the formed carbocation species and prevent undesired lignin condensation.[28] Optimisation of the process allowed to achieve a high cellulose digestibility of 96% from birch, compared to 63% without glycol addition. No direct correlation between the lignin content and the hydrolysis performance was found, and therefore the enhanced digestibility was attributed to less condensed lignin structures in the pretreated biomass.[28]

Moreover, biphasic aqueous/solvent systems have been used to protect lignin from condensation reactions. The lignin is extracted into the organic phase and protected by etherification with the solvent, achieving high digestibilities of the extracted biomass[62] (reference not included in Table 3).

3.4 *Effects on Enzyme Activity*

The prevention of lignin repolymerisation/condensation in pretreatment has been shown to enhance the enzymatic cellulose conversion of different biomasses, with different pretreatments and for different cellulase cocktails (see Tables 1–3). The effect on cellulase enzymes can basically be ascribed to two mechanisms. On the one hand the prevention of lignin repolymerisation itself, and on the other hand the functionalisation of the lignin.

3.4.1 *Prevention of Lignin Repolymerisation*

In the first studies on this subject, the author and coworkers could reveal that the suppression of lignin repolymerisation can enhance the accessibility of cellulose,[14] but in particular decreases the adsorption and deactivation of enzymes on the lignin structure.[5b] This effect was mainly attributed to hydrophobic interactions between the carbohydrate binding module (CBM) of cellulases and lignin, which are a key driver in enzyme adsorption processes[63] (in addition, hydrophobic interactions may occur between the aromatic groups in lignin and aromatic amino acids in enzymes[64]). Repolymerised lignin structures were found to have an increased binding potential for cellulases.[5b] It was further shown that lignin repolymerisation leads to a higher specific surface area and thus to increased enzyme deactivation. The positive effect of a carbocation scavenger was therefore attributed in large part to decreasing the lignin's specific surface area.[5b] In a similar finding, recent studies have shown that the prevention of lignin repolymerisation leads to larger lignin particles, decreasing its surface area and reducing the contact area for enzymes.[59b]

Further, it has been observed that the prevention of repolymerisation can reduce the loss of acidic groups in lignin, thus decreasing hydrophobic lignin-enzyme interactions.[31] On the other hand, enzymes may also bind with lignin through hydrogen bonding and electrostatic interactions. Hydrogen bonding might take place between the amide groups in enzymes and the phenolic hydroxy groups in lignin.[64] In this context, it has been argued that lignin repolymerisation may lead to higher 'local' densities of aromatic rings,[64] which can trap and deactivate enzymes. It has also been hypothesised that condensed phenolic lignin units cause higher enzyme deactivation by the combined effect of hydrophobic interactions of aromatic rings and hydrogen bonding of phenolic hydroxy groups in lignin.[60b,64]

With regard to smaller lignin compounds, it has been suggested that carbocation scavengers may suppress lignin depolymerisation and the formation of inhibitory ketone-type phenolics, also known as Hibbert ketones (see also Scheme 1, route b). The improved cellulose hydrolysis was attributed to the mitigating effect of scavengers on pretreatment-derived phenolics.[27] Interestingly in that sense, it has recently been shown that the use of 2-naphthol reduced the formation of soluble phenolics in the water-soluble pretreatment fraction[51] and proved very effective in enhancing whole slurry hydrolysis.[27,50] Moreover, it has been found that the addition of 2-naphthol can strongly reduce the formation of Hibbert ketones in the steam explosion pretreatment of hardwood.[69]

3.4.2 *Lignin Functionalisation*

The introduction of the carbocation scavenger (or additive) into the lignin backbone can functionalise the lignin during pretreatment (Fig. 1). This functionalisation specifically depends on the used compound and can further influence lignin-enzyme interactions.

The introduction of hydroxy groups adds hydrophilic functionality, reducing the potential for hydrophobic enzyme deactivation. The use of dimethylphloroglucinol, diols, glycerol or mannitol may (further) enhance cellulose digestibility in this way.[14,21,31,45] With regard to alcohol additives, it therefore seems advantageous to use polyhydric alcohols with at least two hydroxy groups, since one group will be bound by lignin etherification.

Acid functionalities can be introduced as well, influencing the enzyme activity in a similar manner as hydroxy groups. For example, 2-naphthol-7-sulphonate,[43] syringic acid or naphthoic acid derivatives,[25,59a] can serve in that sense.

The introduction of functional groups can also alter the lignin's solubility properties. The sulphonate group of 2-naphthol-7-sulphonate increases the lignin solubility in aqueous media, thus influencing its redeposition or even removal in autohydrolysis, steam or acid pretreatments.[26,43,52] 2-Naphthol-7-sulphonate has been shown to increase the accessibility of cellulose in that way, due to partial lignin removal in dilute acid pretreatment.[26] The higher lignin solubility may, however, result in an increased inhibition of whole slurry hydrolysis, as a result of phenolic derivatives dissolved in the aqueous phase.[50]

The redeposition of lignin or the formation of (physically) condensed lignin droplets/agglomerates on the biomass surface can also negatively influence the biomass digestibility.[65]

Interestingly in that sense, 2-naphthol seemed to increase lignin migration to the biomass surface in aqueous/acid pretreatment, but actually reduced cellulase deactivation.[50] Moreover, 2-naphthol did not enhance the cellulose accessibility for enzymatic degradation in most pretreatments.[5b,26,51]

It has also been postulated that some additives may influence the enzyme activity more directly. For example, Hansen *et al.* suggested that 2-naphthol may boost the activity of polysaccharide monooxygenase (LPMO) in cellulase cocktails, thus enhancing the oxidative cellulose depolymerisation by LPMOs.[32] Notably in this regard, the addition of 2-naphthol-pretreated lignins to the hydrolysis of pure cellulose (Avicel) even enhanced the enzymatic hydrolysis performance.[59b]

4. Fermentation

4.1 *Potential Inhibition of Microorganisms*

The sugars obtained from cellulose and hemicellulose hydrolysis can be fermented to various chemicals, depending on the fermentation organism. In this respect, the effect of the different pretreatment additives on fermentation needs to be considered, since the compounds could inhibit fermentation organisms. Inhibiting compounds could potentially also cause environmental concerns in large scale application.

The author and coauthors have shown, for example, that 2-naphthol at a concentration of 0.4 g L^{-1} may inhibit the growth of *Saccharomyces cerevisiae (S. cerevisiae)* in the fermentation of pure glucose.[5b] However, 2-naphthol is largely consumed in pretreatment and integrated into the lignin structure. The remaining low concentration of free 2-naphthol was assumed to not inhibit fermentation anymore.[5b]

On the other hand, Seidel *et al.* have revealed that 2-naphthol can inhibit *S. cerevisiae* fermentation synergistically together with other inhibitors formed in pretreatment, such as hydroxymethylfurfural (HMF), furfural and acetic acid.[42] Due to this combinatory effect, the use of 2-naphthol in steam explosion pretreatment of spruce wood actually reduced ethanol yield in the simultaneous saccharification and fermentation (SSF) of whole slurry at high cellulose concentrations (5% w/w cellulose). At a reduced concentration of 1% w/w cellulose however, the use of 2-naphthol did increase the fermentation yield by 23%, due to the lower total inhibitor concentration (see Table 4). The study revealed that for the combination 2-naphthol/*S. cerevisiae*, a strategy for minimising inhibition should be provided for. It should also be noted that softwood pretreatment requires the highest severity compared to other lignocellulosic species, leading to a high formation of inhibitory compounds. Therefore, the observed effect may be less pronounced for other biomass types.

In another study with softwood, Madadi *et al.* successfully demonstrated ethanol fermentation with *S. cerevisiae*, after the pretreatments of pine with 2-naphthol, 2-naphthol-7-sulphonate and mannitol. All additives enhanced ethanol yields compared to a pretreatment without additive, and high yields between 90–95% were achieved[44] (Table 4). It must be pointed out though, that the biomass recovered after the pretreatments was washed with water, which can reduce or disguise potential inhibiting effects of the additives.

Tong *et al.* carried out fermentation experiments after the pretreatment of poplar with 2-naphthol-7-sulphonate.[52] The pre-hydrolysate (recovered liquor after biomass pretreatment) and the enzymatic hydrolysate were fermented separately. The pre-hydrolysate consisted mainly of xylose from hemicellulose degradation in pretreatment. It was fermented with *Gluconobacter oxydans*, which can convert C5 sugars, to xylonic acid with a good yield of 89%. It should be highlighted that this fermentation was carried out without any previous washing or detoxification step, meaning that potentially unreacted 2-naphthol-7-sulphonate did not inhibit *Gluconobacter oxydans* to a larger extent. The enzymatic hydrolysate consisted mainly of glucose and was fermented with *S. cerevisiae*, which converts C6 sugars, and a very high ethanol yield of 99% was reached. Notably, the biomass recovered after pretreatment was washed, and therefore no indication of *S. cerevisiae* inhibition by 2-naphthol-7-sulphonate can be given here.

4.2 *Alleviating Inhibition*

More studies are needed about the effects of pretreatment additives on fermentation. The reviewed studies could, however, already show that carbocation scavengers can inhibit microorganisms, though not in every case. The effect is individual for each combination of scavenger/microorganism and must be reviewed for each particular process.

Even if a compound shows inhibiting activity, this does not mean that a corresponding biorefinery process cannot be realised. There are several concepts for adapting a bioprocess accordingly. In the first place, the pretreatment process should be optimised to allow for a complete conversion of the additive. Since the additive is integrated into the solid lignin structure, it should not interfere with the metabolism of carbohydrate fermenting microorganisms anymore.

In addition, it is possible to remove remaining additive before fermentation, for example, by filtration of the pretreated biomass slurry and, if need be, by washing of the biomass. It may also be

Table. 4. Examples of pretreatments with additive/carbocation scavenger and fermentation of the lignocellulose-derived sugars.

Reference		**Pretreatment**				**Fermentation**					
Year	**Author**	**Biomass**	**Method**	**Additive conc.**	**Additive/ scavenger**	**Yield increase**[a]	**Yield**[b]	**Substrate**	**Preparation**	**Product**	**Organism**
2015	Pielhop *et al.*[5b]	-	-	-	-	N/A	82%	Glucose + 0.1 g L^{-1} 2-naphthol	N/A	Ethanol	*Saccharomyces cerevisiae*
							0%	Glucose + 0.4 g L^{-1} 2-naphthol			
2019	Seidel *et al.*[42]	Spruce	2-stage steam explosion	0.205 mol/mol lignin C9	2-Naphthol	+22.8%	90.4%	Whole slurry, 1% w/w cellulose	-	Ethanol	*Saccharomyces cerevisiae*
						-95%	3%	Whole slurry, 5% w/w cellulose	-		
2022	Madadi *et al.*[44]	Pine	Autohydrolysis + green liquor extraction	5% w/w dry biomass	2-Naphthol	+28.4%	95%	Enzymatic hydrolysate, ~25 g L^{-1} glucose	Biomass washed	Ethanol	*Saccharomyces cerevisiae*
					2-Naphthol-7-sulphonate	+23.0%	91%				
					Mannitol	+21.6%	90%				
2022	Tong *et al.*[52]	Poplar	Acid sulphite	5% w/w dry biomass	2-Naphthol-7-sulphonate	N/A	89.1%	Pre-hydrolysate, ~50 g L^{-1} xylose	-	Xylonic acid	*Gluconobacter oxydans*
							99.2%	Enzymatic hydrolysate, ~150 g L^{-1} glucose	Biomass washed	Ethanol	*Saccharomyces cerevisiae*

[a]Compared to pretreatement without additive; [b]Based on cellulose content added to fermentation

possible to recover unreacted scavenger in a two-stage pretreatment process and recycle it. The scavenger might be recovered by filtration of the pretreated biomass slurry and recycled with the liquid[38] (it should be noted that at the higher temperatures required for pretreatment, also aromatic compounds like 2-naphthol can be completely dissolved in the aqueous phase of a steam pretreatment[41,66]).

Chemical detoxification of the hydrolysates before fermentation is another option for alleviating inhibition. For pretreatments without additive, several methods for the detoxification of lignocellulosic hydrolysates have been developed already, which can highly improve their fermentability.[67] Those methods may also be efficient for a pretreatment with additives, and at least eliminate any synergistic inhibition effects of the additives and lignocellulose-derived inhibitors.

In addition to the discussed process optimisations, different combinations of pretreatment additives and fermentation organisms may be screened for their performance. This can allow a better understanding of potential inhibition by effective compounds, but also reveal promising combinations for avoiding inhibition in the first place.

5. Enhanced Lignin Valorisation

5.1 *Improved Lignin Properties*

Next to enhancing cellulose bioconversion, the suppression of lignin repolymerisation in pretreatment also enhances the quality of the obtained lignin fraction. In our work, we disclosed that the resulting lignin with fewer condensed C–C bonds and a lower and more defined Mw has a higher chemical value, both for the depolymerisation to aromatic chemicals in higher yields and for its application in polymers.[5b,9a,11] These considerations have been experimentally verified in the meantime. The advance of lignin-first approaches, which prevent lignin repolymerisation already in the fractionation of the raw biomass, has shown that higher yields of aromatic monomers can be obtained in that way.[18–20] Further, it has recently been confirmed in several studies, that pretreatments with carbocation scavenger enable an enhanced application of the resulting lignin in different polymers, compared to standard or technical lignins.[49,60c]

Referring to previous studies of the author and coworkers[5b,9a,11,15] and to recent studies,[49,55,60c] the enhanced lignin quality of a carbocation scavenger assisted process may be summarised as follows: i) lower Mw and polydispersity index (PDI), ii) tuneable Mw, iii) lower glass transition temperature (Tg), iv) increased solubility, v) fewer condensed C–C bonds, vi) integration of additional functionality by the scavenger (*e.g.* aromatic or hydroxy groups) v) enhanced functionalisation potential: higher content of accessible hydroxy groups and free phenolic ring positions, vi) high purity: sulphur-free, low carbohydrate content (<1%), low nitrogen content and vii) brighter colour.

5.2 *Improved Lignin Processability*

The lignin characteristics will enhance its processability in conversion or modification processes.[9a,15] Due to the lower Mw and reduced crosslinking, its solubility is improved and its functionalisability may be enhanced. This will facilitate chemical reactions and functionalisation. The reduced Mw and PDI can also prove beneficial for polymer applications.[5b,9a,15] The Tg can be reduced as well,[11,55] which enhances polymer processing like melting, extrusion or spinning. Less condensed lignin structures also increase the molecular mobility of the lignin polymer and positively affect its compatibility in blends/composites.[35b]

The lignin will feature a higher purity too. It is sulphur-free, in contrast to industrial pulping lignins,[9a,11] which suffer from odour problems or yellowing of final products. The lignin has a lower residual carbohydrates content compared to standard enzymatic hydrolysis lignin (EHL), due to the enhanced saccharification. Residual cellulose can be an obstacle for speciality chemicals applications such as plasticisers. In addition, the lignin will have a lower nitrogen content due to the reduced adsorption of cellulases.[5b]

5.3 *Application Studies: Polymer Use*

Table 5 shows an overview of lignins that were obtained after a pretreatment with carbocation scavengers or repolymerisation blockers. Only processes which combine the bioconversion of carbohydrates with the valorisation of lignin are shown. The Table highlights the improved lignin properties and potential applications.

In spruce autohydrolysis studies, the prevention of lignin repolymerisation distinctly reduced its Mw and PDI. For example, the use of 2-naphthol and dimethylphloroglucinol reduced the Mw by more than 15 times and the PDI by more than 3 times.[14]

Carbocation scavengers also allow for an enhanced functionalisation of the lignin, which can be achieved in two ways. On the one hand, the scavenger itself may functionalise the lignin by its integration into the lignin backbone.[9a,15] In this way additional functionality can be added, next to the functionalisation of the naturally present hydroxy groups or ring positions in lignin. For instance, the use of aromatic scavengers can increase the suitability of the lignin for aromatic polymers in that way.[5b,9a,15] In a broader sense, carbocation scavengers were already shown to functionalise lignin with aryl groups (2-naphthol), sulphonic groups (2-naphthol-7-sulphonate), or hydroxy groups (glycerol, mannitol), as presented in Table 5.

On the other hand, the prevention of repolymerisation allows for a higher functionalisation potential of the resulting lignin. That is, the accessibility of its hydroxy groups and (free) aromatic ring positions can be improved. In this context, Wang *et al.* have shown that the pretreatment with 2-naphthol enhanced the modification efficiency in a following esterification reaction by 51%, compared to a pretreatment without additive.[60c] Similarly, Huang *et al.* showed that 2-naphthol could enhance sulphonation by 38%[51] (estimation based on lignin acid groups content), and Lai *et al.* reported that 2-naphthol enhanced the functionalisation efficiency with PEGDE by 53%.[49] These highly functionalised lignins have been successfully used in the production of self-healing adhesives[60c] and adsorbents.[51,53]

The functionalisation of lignin usually takes place *via* the formation of C–O or C–C bonds (Fig. 1), depending on the used additive. Noteworthy, lignin functionalisation *via* ether bonds may not be stable at high temperatures.[61b] Functionalisation *via* stable C–C bonds may therefore prove beneficial for harsher post-processing conditions of the lignin polymer, such as in melting or in the additional modification/functionalisation at elevated temperatures with chemicals or acids.

5.4 *Application Studies: Depolymerisation*

As mentioned, preventing the formation of condensed C–C bonds in pretreatment may enable higher yields in the production of monoaromatics.[5b,9a,11,15] For example, the pretreatment with 1,4-butandiol has been proposed to enhance lignin depolymerisation to monolignols in this way.[21] Recently, Hansen *et al.* have also shown that preventing lignin repolymerisation with 2-naphthol allowed obtaining a lignin pyrolysis oil with a higher proportion of aromatic hydrocarbons.[32] The quality of the bio-oil was further enhanced due to the improved removal of polysaccharides, resulting in reduced levels of unstable sugar-derived furanics, anhydro sugars and acids. Such oils can be more suitable for the production of biobased resins and solvents.[32,68]

In a lignin-first depolymerisation approach with softwood, De Santi *et al.* could achieve up to 9% w/w aromatic monophenol

Table. 5. Examples of different pretreatments with additive/carbocation scavenger, which integrate cellulose bioconversion and lignin valorisation. The enhanced properties and applications of the obtained lignin are presented.

Reference		Pretreatment			Lignin quality	
Year	**Author**	**Biomass**	**Method**	**Additive/ scavenger**	**Improved properties**	**Proposed/studied lignin use**
2011, 2014	Pielhop *et al.*[9a,15]	Ligno-cellulose	Autohydrolysis	Aromatics, alcohols, amines and others	Fewer condensed C–C bonds, lower Mw, lower PDI, higher purity (less carbohydrates), enhanced solubility, functionalisation by scavenger	Enhanced depolymerisation to aromatic monomers; use in polymers (phenol-based)
2015, 2016	Pielhop *et al.*[5b,14]	Spruce	Autohydrolysis	2-Naphthol, dimethyl-phloroglucinol, alcohols	Fewer condensed C–C bonds, lower Mw, lower PDI enhanced solubility, higher purity (less carbohydrates and nitrogen), aryl functionalisation (2-naphthol), hydroxy functionalisation (dimethyl-phloroglucinol, alcohols), amino functionalisation (amines)	Enhanced depolymerisation to aromatic monomers; use in polymers (blend)
2018	Lan *et al.*[22,35a]	Poplar	1,4-Dioxane/H_2O, acid	Proprion-aldehyde	Fewer condensed C–C bonds	Enhanced depolymerisation to aromatic monomers (syringyl propanol)
2019	Dong *et al.*[21]	Eucalyptus	1,4-Butanediol / H_2O, acid	1,4-Butanediol (solvent)	Preserved β-O-4 linkages, increased reactivity, increased solubility, functionalisation (hydroxy groups)	Enhanced depolymerisation to aromatic monomers
2020	De Santi *et al.*[19,22]	Pine	Dimethyl carbonate, acid	Ethylene glycol	Fewer condensed C–C bonds	Enhanced depolymerisation to aromatic monophenols (C2-acetal)
2020	Hassanpour *et al.*[58]	Sugarcane bagasse	Dilute acid	Glycerol	Preserved β-O-4 linkages	Self-healing adhesives
2020	Lai *et al.*[49]	Larch	Dilute acid + alkali/ PEGDE[b]	2-Naphthol	Enhanced functionalisation: +51% modification efficiency with PEGDE, enhanced solubility/delignification	
2021	Chu ***et al.***[60b]	Poplar	Ethanol/H_2O, acid	Mannitol	Functionalisation (hydroxy groups)	
				2-Naphthol-7-sulphonate	Enhanced solubility, enhanced delignification, functionalisation (sulphonate groups)	Adsorbent (dye)
2021	Huang *et al.*[51]	Poplar	Dilute acid + alkaline H_2O_2	2-Naphthol	Enhanced functionalisation (sulphonation)	Adsorbent (lead ions)
2021	Liu *et al.*[28]	Birch	Choline chloride, oxalic acid	Ethylene glycol	Fewer condensed C–C bonds, preserved β-O-4 linkages, functionalisation (hydroxy groups),	Enhanced depolymerisation to aromatic monomers (C2-acetal)
2022	Hansen *et al.*[32]	Spruce	Steam explosion	2-Naphthol	High purity (less carbohydrates)	Pyrolysis oil: richer in monoaromatic phenolics; less sugar-derived compounds; reduced char
2022	He *et al.*[55]	Bamboo	Dilute acid	2-Naphthol	Fewer condensed C–C bonds, lower T_g (2-naphthol)	
				2-Naphthol-7-sulphonate		
2022	Madadi *et al.*[44]	Pine	Autohydrolysis + green liquor extraction	2-Naphthol	Preserved β-O-4 linkages, fewer condensed C–C bonds, sulphonate functionalisation (2-naphthol-7-sulphonate), hydroxy functionalisation (mannitol)	
				2-Naphthol-7-sulphonate		
				Mannitol		
2022	Tong *et al.*[53]	Poplar	Dilute acid + PEE[a]	2-Naphthol	Functionalisation (PEE)	Adsorbent (lead ions)
2022	Wang *et al.*[60c]	Poplar	Ethanol/H_2O, acidified	2-Naphthol	Enhanced functionalisation: +51% modification efficiency in esterification	Self-healing adhesives
2023	Fang *et al.*[59a]	Pine	1,4-butanediol / H_2O, acidified	2-Naphthol	Enhanced antioxidant capacity: +57%	Antioxidant
2023	Madadi *et al.*[60a]	Poplar	p-Toluenesulphonic acid/pentanol	Mannitol	Fewer condensed C–C bonds, preservation of β-O-4 linkages, lower PDI, high purity (less carbohydrates), enhanced solubility/ delignification, functionalisation (hydroxy groups)	

Annotations: see Table 1

yield by the addition of ethylene glycol.[19] The obtained yield is higher compared to a pretreatment without additive. Ethylene glycol can trap reactive depolymerisation intermediates (aldehydes) and/or directly react with lignin carbocations.[19,28]

Trapping of the lignin reactive intermediates and carbocations with ethylene glycol also enhanced the lignin quality obtained after deep eutectic solvent pretreatment.[28] A native-like lignin with largely preserved β–O–4′ linkages was recovered. The subsequent depolymerisation *via* hydrogenolysis resulted in six times more aromatic monomers than from the condensed analogues obtained in pretreatment without glycol addition.[28]

In the lignin-first approach of Lan *et al.*, lignin stabilisation with propionaldehyde prevented condensation reactions during pretreatment and extraction, thus allowing for very high yields and selectivities in the subsequent depolymerisation. Hydrogenolysis of the recovered lignin produced monomers at high selectivities from spruce (20%), birch (48%) and transgenic hybrid poplar (80%).[35a]

6. Integrated Production of Fermentable Sugar and Aromatic Lignin Monomers/Polymers

The prevention of lignin repolymerisation in lignocellulosic biomass pretreatment renders an improved valorisation of both cellulose and lignin possible,[5b,9a,11] as exemplified in detail in Sections 3 and 5. Fig. 2 shows a generalised flowsheet of this concept. The basis of this approach is laid by the additive used in pretreatment. The additive can reduce enzyme deactivation and enhance the access to cellulose in enzymatic hydrolysis. In addition, the additive determines the quality and fate of the lignin. The lignin may be depolymerised to aromatic compounds with enhanced yields, and/or it may be used as a substrate with improved properties for polymer production.

In lignin depolymerisation, the use of softwood lignin may result in lower yields compared to *e.g.* hardwood or herbaceous lignin, because of its lower content of β–O–4′ linkages and higher contents of β–5′and 5–5′ linkages.[19,35a] In addition, pretreatment additives that form C–O linkages with lignin may be preferred, so that the additive can be released again by hydrolytic cleavage.

As presented in Fig. 2, many applications for lignin as a polymer can be envisioned and have already been studied. The quality of these products can, however, be limited by the lignin feedstock. The use of technical lignins from *e.g.* pulping processes that have undergone severe degradation already, limit the quality of the final product in many cases. By contrast, the use of *e.g.* a carbocation scavenger can enhance the quality of the lignin starting material and its applicability in polymers. This approach could help to unlock the potential of lignin for a wide range of material applications. Most importantly, the additive used in pretreatment will impact the lignin functionalisation and thus its potential fields of application. Therefore, the pretreatment process should also be tailored to the later use of the lignin. Notably, the lignin functionalisation should both enhance the cellulose bioconversion and the potential lignin use in an integrative biorefinery concept.

7. Conclusions

Enhancing cellulose bioconversion and lignin valorisation by the suppression of lignin repolymerisation in pretreatment has gained increasing attention in recent years.

The related studies show that cellulose bioconversion can be enhanced in two ways, by the actual suppression of lignin repolymerisation and by the functionalisation of lignin with the additive used in pretreatment. The bioconversion of a wide range of biomass could be enhanced in this way, including softwood, hardwood, bamboo and agricultural residues. In addition, the approach has been successfully implemented in various different pretreatment methods, including hot water, steam, dilute acid, green liquor, organosolv and deep eutectic solvent methods.

Several new effective additives/carbocation scavengers have been discovered recently. The exact mechanism how these additives enhance bioconversion is not yet fully understood in each case, and more research is needed. Importantly, also green additives have been discovered which can render the approach even more sustainable. Ideally, an additive should be sourced sustainably from lignocellulose itself and also does not inhibit microorganisms.

The already discovered additives enable different functionalisation of the lignin, opening the door for new lignin applications. The protection of lignin in pretreatment together with its mild isolation *via* biocatalysis yields a high-quality lignin fraction, both for the depolymerisation to aromatic compounds and for its use in polymeric form. Several studies have already proven the higher chemical value of such a lignin and more applications are expected to be revealed in the future.

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Declaration of Competing Interests

The author and coworkers are inventors of granted patents on the use of carbocation scavengers in lignocellulosic biomass pretreatment for enhancing the saccharification of cellulose and for the preparation of a lignin fraction for improved polymers/monomers manufacturing.[9b,15]

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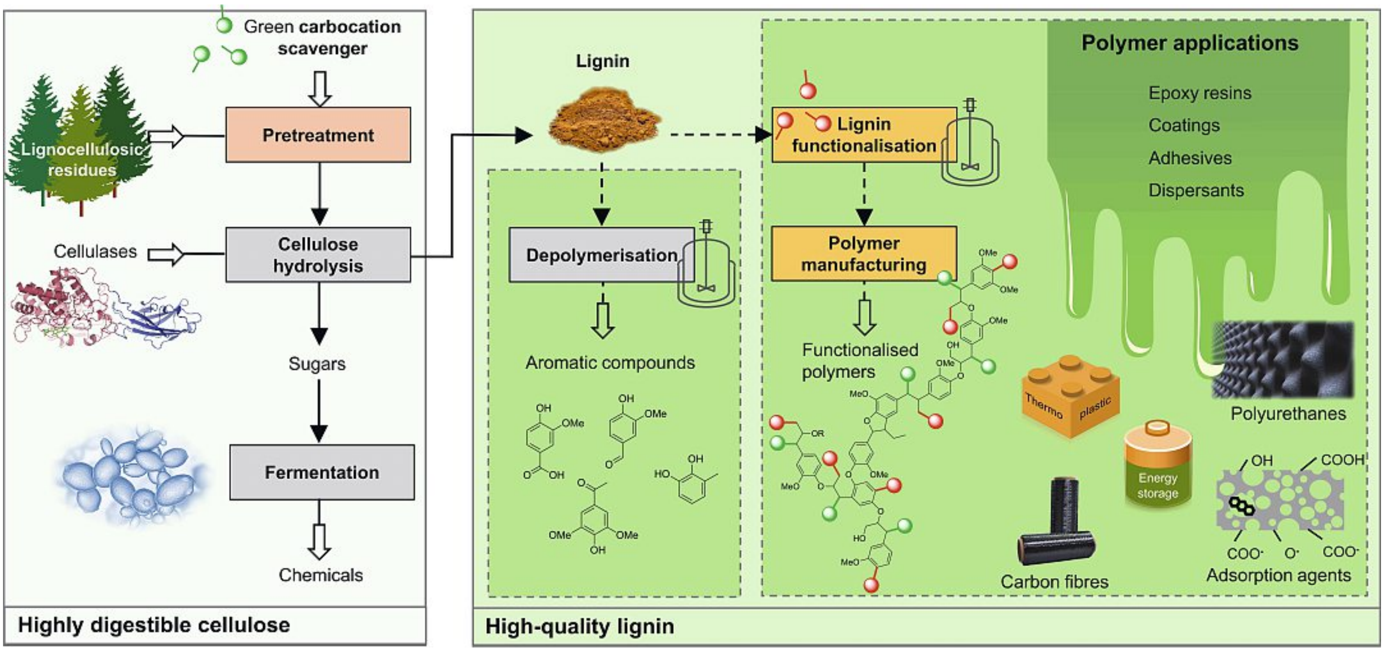

Fig. 2. Flowsheet of a biorefinery concept which prevents lignin repolymerisation in pretreatment by the addition of a carbocation scavenger. A highly digestible cellulose and a high-quality lignin can be obtained at the same time. The cellulose is saccharified enzymatically and serves as substrate for the fermentative production of chemicals. The obtained lignin has an enhanced quality both for the production of aromatic compounds and polymers. Lignin-derived polymers can be functionalised by the scavenger and by an additional functionalisation step.

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