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Review

Evaluation of hydrotropic pretreatment on lignocellulosic biomass

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HIGHLIGHTS

- Hydrotropic pretreatment leads to reduction in surface coverage by lignin.
- XPS and TOF-SIMS analysis provide important insights on surface chemical studies.
- Hydrotropic lignin can have potential application as bio based polymer.
- Enzymatic yield of glucose was enhanced by hydrotropic pretreatment.

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ABSTRACT

The production of cellulosic ethanol from biomass is considered as a promising alternative to fossil fuels, providing a sustainable option for fuels production in an environmentally compatible manner. The presence of lignin poses a significant challenge for obtaining biofuels and bioproducts from biomass. Part of that problem involves understanding fundamental aspects of lignin structure which can provide a pathway for the development of improved technologies for biomass conversion. Hydrotropic pretreatment has several attractive features that make it an attractive alternative for biofuel production. This review highlights the recent developments on hydrotropic pretreatment processes for lignocellulosic biomass on a molecular structure basis for recalcitrance, with emphasis on lignin concerning chemical structure, transformation and recalcitrance. The review also evaluates the hydrotropic delignification in comparison to alkaline delignification on lignin reduction and surface coverage by lignin. The effect of hydrotrope pretreatment on enzymatic saccharification has also been discussed.

Contents

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1. Introduction

Biofuels production from lignocellulosic biomass is one of the several paths toward alternative transportation fuels. Biofuels produced from biomass have taken a lead position as a viable option to petroleum-derived fuels (Pu et al., 2013). The production of cellulosic ethanol through biological route has garnered extensive interest over the past decade with one of its primary advantages being that it is based on non-food lignocellulosic (Saxena et al., 2009). Lignocellulosic biomass provides a sustainable source of sugars for biofuels and biomaterial production (Sangha et al., 2012). However, biomass resistance to degradation imposes difficulties for economical conversion of plant carbohydrates to fermentable sugars. Numerous pretreatment approaches including physical, chemical, physiochemical and biological techniques have been developed to reduce recalcitrance and improve sugar yields of cellulosic biomass. Although there are several methodologies readily available for the pre-treatment of biomass every technique has its advantages and limitations in regarding process cost, energy consumption and inventories. Hence, there is a critical need to improve pre-treatment processes or develop processes involving modification of the already well-established techniques in such a way that limitations of the selected methods are suppressed or addressed in an alternate way. Hydrotropic treatment has several attractive features that make it an interesting alternative for biomass refineries. Hydrotrope pretreatment methods are known or suspected to affect the biomass lignin and thus presumably facilitate enzymatic hydrolysis (Mou et al., 2013a). However, it is not entirely understood, how these pretreatments affect the topochemistry of biomass and what is the mechanism to improve enzymatic hydrolysis. This review highlights the recent understanding of the surface chemistry of biomass regarding surface coverage of lignin after hydrotrope pretreatment. The review also summarizes the effect of hydrotrope pretreatment on different lignocellulosic materials with the focus on lignin regarding chemical structure and recalcitrance. Effect of enzyme hydrolysis of hydro tropically treated material has been compared and evaluated for different lignocellulosic material.

2. Structure of lignocellulosic biomass and its recalcitrance

Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (Jørgensen et al., 2007). Cellulose is the chief structural constituent of plant cell walls and is found in an organized fibrous structure. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils. Hemicelluloses and lignin cover the microfibrils. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains constitute amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous form (Béguin and Aubert, 1994).

The backbone of hemicellulose is either a homopolymer or a heteropolymer with short branches linked by β-(1,4)-glycosidic bonds and occasionally β-(1,3)-glycosidic bonds (Kuhad et al., 1997). In contrast to cellulose, the polymers present in hemicelluloses are readily hydrolyzable. These polymers do not aggregate, even when they co-crystallize with cellulose chains.

Lignin is the most complex natural polymer and is considered as the most recalcitrant component of the lignocellulosic material. It is an amorphous three-dimensional polymer with phenylpropane units as the primary building blocks. More specifically, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol are the ones most commonly encountered. Lignin in softwoods are mainly composed of guaiacyl units with small amounts of *p*-hydroxyphenyl units existed while lignin in hardwoods primarily consists of both guaiacyl and sinapyl units including a minor amount of *p*-hydroxyphenyl units. Guaiacyl Lignin is chemically more resistant than sinapyl lignin. It is commonly assumed that the presence of lignin in biomass restricts enzymatic hydrolysis primarily by physically impeding the accessibility of enzyme cellulase to the substrate cellulose. Understanding the properties of lignin macromolecules in the cell wall matrix is useful for manipulating the biomass structure to generate more readily degradable biomass. In addition, to lignin as physical barrier, the surface distribution of xylan on biomass materials has been reported to have negative influence on the enzymatic hydrolysis efficiency (Leu and Zhu, 2013; Mooney et al., 1998).

3. Potential of hydrotrope for pretreatment

The application of hydrotrope for biomass processing was initiated by McKee (1946) who patented the pulping of different lignocellulosic raw materials with a concentrated solution of benzene derived hydrotrope salts. Small molecular weight amphiphilic molecules such as sodium and potassium salts of an alkyl group substituted benzoic and aryl sulfonic acids are known as hydrotropes because of their remarkable ability to dissolve other sparingly soluble organic compounds in aqueous solutions (Devendra and Gaikar, 2012). Neuberg (1916) described these phenomena giving a variety of molecules that exhibit this property. The structure of hydrotropic compounds is similar to those of surfactants, *i.e.* they are amphiphilic substances composed of both a hydrophilic and a hydrophobic functional group. Short-chain hydrotropic compounds have, however, a weaker hydrotropic character than surfactants (Mata et al., 2004). The main advantage of hydrotrope is the smooth recovery of the solute from hydrotrope solutions by simple dilution with water.

Lignin, a phenolic polymer is hydrophobic and essentially insoluble in water. Thus the use of aqueous hydrotrope solutions to solubilize lignin is potentially attractive. Being water based the hydrotrope solutions is greener solvent that is safe to handle. Further, the application of aqueous hydrotrope solution makes the pretreatment process environment-friendly as it avoids the inclusion of organic solvents. It is believed that hydrotropic pretreatment can be a potential alternative to the conventional acid and alkaline pretreatment process where recovery of acid/alkali and treatment of effluent are major problems. The recovered lignin can also be a potential source in several applications and for phenolic materials. Spent aqueous solution of hydrotrope can be reused till it gets saturated with lignin.

4. Effect of hydrotrope pretreatment on lignin removal

Hydrotropic pretreatment has been demonstrated to be useful for lignin removal from hardwood, and no sulfur bond was found in the isolated lignin (Korpinen, 2010; Mou et al., 2013a). The most commonly used hydrotropic compound for the recovery of lignin from the lignocellulosic material is sodium xylene sulfonate (SXS) because it is an effective solvent to remove lignin. Hydrotropic pulping of Norway spruce chips, birch chip and spruce sawdust was carried out by Korpinen (2010) using SXS at 150 °C for 12 h. After SXS pretreatment, up to 70.1% of lignin was extracted from Nordic birch. They hypothesize that the lignin structure of hardwood (Sy/Gu) is the reason for the difference in the extraction of lignin between Norway spruce and Nordic birch chips. Softwoods have highly lignified middle lamella containing mainly guaiacyl

lignin and less lignified secondary wall containing guaiacyl lignin. The distribution of lignin in secondary wall and middle lamella of hardwood fibers is similar to that in softwood fibers but the secondary wall is less lignified, and it contains a mixture of guaiacyl and sinapyl lignin (Donaldson, 2001). In another study carried out by Yasuda et al. (2001), it was found that acid-soluble lignin content was higher with sinapyl lignin-rich wood than with guaiacyl lignin-rich wood. The extraction of lignin was more efficient with Norway spruce sawdust than with Norway spruce chips. Both Norway spruce chips and sawdust contained mainly guaiacyl lignin, and therefore, the lignin structure cannot solely explain the difference in the degree of lignin extraction. It is believed that hydrotropic SXS molecules adsorb on the cell wall and disorganize its structure. After that SXS and water molecules penetrate the cell wall and access the lignin and solubilize it (Raman and Gaikar, 2003).

Hydrotropic fractionation of Birchwood into cellulose and lignin have been carried out by Gabov et al. (2013), they have modified the hydrotropic process by addition of hydrogen peroxide or formic acid or both. The pulps obtained by modified treatments had lower lignin contents than the reference. In another study, they have characterized the lignin extracted from birch wood by the modified hydrotrope process. The treatments resulted in significant changes in the structure of the lignins, a decrease in aliphatic hydroxyls and an increase in phenolic ones. The lignin isolated by the modified treatment underwent more substantial change than the reference one. Mou et al. (2013a) have compared hydrotropic pretreatment of wood biomass with an ionic liquid and hydrothermal pretreatment. They studied the surface chemistry of milled birch and pine wood pretreated by an ionic liquid, hydrothermal and hydrotropic method, followed by enzymatic hydrolysis. Hydrotropic pretreatment was made using SXS with the concentration of 30% as an aqueous extraction at a temperature of 150^{O}C. Surface coverage by lignin was measured by X-ray photoelectron spectroscopy (XPS). Time-of-flight secondary ion mass spectrometry (TOF-SIMS) was used to describe the surface chemical composition after pretreatment, in detail, and the morphology after pretreatment was investigated by FE-SEM. The O/C ratios were used for the estimation of surface coverage by lignin. The O/C ratio of the wood samples before and after acetone extraction and the surface coverage by lignin of birch and pine wood from hydrothermal, ionic liquid and hydrotropic pretreatment are summarized in Table 1. The O/C ratio of all the extracted samples was higher than that in the samples before extraction because of the removal of carbon-rich wood extractives. The difference in the O/C ratios was remarkable in the reference wood. During the pretreatment, particularly hydrothermal and ionic liquid treatments, some of the extractives were already removed before exposed to acetone extraction, which can be roughly estimated on the O/C ratios. Hydrotropic treatment did not increase the O/C as such, but prepared the samples for significant carbon removal during the following acetone extraction. Hydrotropic extraction seemed to be an efficient method to remove lignin from lignocellulosic biomass materials, as seen in Table 1. For birch, lignin was reduced to 16.6% after 30 min and to 13.9% after 120 min pretreatment. For pine, the reduction in the surface coverage of lignin was not that evident compared to the pine reference. The total content of lignin in pine was slightly decreased during hydrotropic pretreatment. Pine has more chemically resistant guaiacyl lignin compared to birch, which is speculated to be the reason for the lesser lignin removal from pine compared to birch wood (Andelin et al., 1989). It was assumed that SXS was not able to extract lignin from pine as well as from birch, and with longer treatment time lignin redeposit on the surface of pine.

Hydrotropic pretreatments of common Reed was studied by Mou et al. (2013b) and they evaluated the topochemistry of alkaline, alkaline-peroxide and hydrotropic pretreatments of common Reed to enhance enzymatic hydrolysis efficiency (Mou et al., 2013b). The low-temperature alkaline, alkaline-peroxide and hydrotropic pretreatments were employed to overcome the recalcitrance of Reed before enzymatic hydrolysis. The surface chemical compositions of Reed before and after pretreatments were investigated by X-ray spectroscopy (XPS) and time of flight secondary ion mass spectrometry (TOF-SIMS). The O/C ratios were calculated from the low resolution XPS spectra. The spectra were determined for both acetone extracted samples (Soxhlet, acetone–water mixture 9:1 (v/v), overnight) and un-extracted samples. The surface coverage by lignin (S_{lig}), the surface coverage by extractives (S_{ext}) and carbohydrates (S_{carb}) were calculated by the average O/C values as per literature (Laine et al., 1996; Ström and Carlsson, 1992). Table 2 shows the chemical compositions of untreated and pretreated Reed. As it can be seen, after alkaline, alkaline-peroxide and hydrotropic pretreatments, the lignin content of Reed decreased from the original 19.7% to 7.3%, 7.9%, and 12.0% respectively. Alkaline and alkaline peroxide pretreatment could degrade lignin into smaller fragments through cleavage of the β-ether bond that contributed to the lignin removal (Banerjee et al., 2011; Cao et al., 2012). The mechanism of hydrotropic pretreatment involves autohydrolysis and improving the lignin solubility to the hydrotropic solvent (Korpinen and Fardim, 2009). Table 3 exhibits the surface coverage by lignin, carbohydrates and extractives of Reed after pretreatments as detected by XPS. The samples O/C ratios

Table 1
The O/C ratios, surface coverage by lignin (S_{lig}) by XPS and total lignin of birch (B) and pine (P) with different pretreatment. Standard deviations are in parentheses, (source: Mou et al., 2013a,b).

	O/C	O/C extracted	S lig%	Total lignin%
Untreated reference				
B	34(7)	54(1)	59	23.3(0.5)
P	31(2)	47(2)	72	27.9(3)
Hydrothermal pretreatment[a]				
B 30 min	41(1)	44(7)	78	23.8(1)
B 120 min	40(0)	44(1)	78	22.8(2)
P 30 min	46(1)	54(2)	59	22.8(1)
P 120 min	42(1)	59(6)	48	21.8(1)
Hydrotropic pretreatment[b]				
B 30 min	34(5)	66(1)	34	16.6(0.8)
B 120 min	40(5)	65(1)	36	13.9(0.1)
P 30 min	27(5)	71(11)	25	27.7(2)
P 120 min	26(5)	38(3)	41	25.5(2)
Ionic liquid pretreatment[c]				
B 20 mmol, 1 h[c]	46(2)	47(3)	43	24.6(1)
B 50 mmol, 3 h[c]	37(6)	56(6)	45	24.5(0)
P 20 mmol, 1 h[c]	46(4)	57(6)	52	24.9(2)
P50 mmol, 3 h[c]	46(1)	60(3)	46	27.1(2)
B 20 mmol,1 h[d]	44(0)	49(3)	69	23.5(1)
B 50 mmol, 3 h[d]	38(6)	56(5)	55	24.0(2)
P 20 mmol, 1 h[d]	48(2)	59(7)	49	28.9(1)
P50 mmol, 3 h[d]	46(4)	60(6)	46	27.0(1)

[a] 165 °C, liquor to wood ratio (w/w) 5:1,
[b] 30% SXS, 150 °C, liquor to wood ratio (w/w) 8:1,
[c] Emim AC pretreatment at room temperature,
[d] Bmim Cl pretreatment at room temperature.

Table 2
Chemical composition of original Reed (R_{ref}) and alkaline (RN), alkaline- hydroxide (RH) and hydrotropic pretreated (R_{sxs}) samples. (Source: Mou et al. (2013b)).

	Glucan %	Xylan %	Arabinan %	Total lignin %
R_{ref}	37.7(1.1)	22.2(0.1)	2.2(0.1)	19.7(0.1)
RN	49.8(0)	23.8(0.1)	3.3(0.1)	7.3(0)
RH	50.7(0.4)	24.8(0.1)	2.8(0)	7.9(0.5)
RSXS	70.9(0.1)	9.2(0.2)	–	12.0(0.6)

Table 3
Oxygen-to-carbon ratios and surface coverage by lignin (S_{lig}), carbohydrates (S_{carb}) and extractives (S_{ext}) of Reed by XPS (source: Mou et al. (2013b)).

Samples	O/C ext	O/C	S_{ext} %	S_{lig} %	S_{carb} %
R_{ref}	0.32(0.11)	0.14(0.06)	80.5	~100	~0
RN	0.30(0.11)	0.33(0.14)	~0	~100	~0
RH	0.37(0.09)	0.41(0.15)	~0	91.5	8.5
R_{sxs}	0.75(0.03)	0.43(0.19)	48.7	16.5	83.5

with extraction and without extraction were both given in Table 3. After alkaline (RN) and alkaline peroxide (RH) pretreatments, the O/C ratio values of extracted and unextracted samples were close to the theoretical value for lignin (0.33) as seen in Table 3. The surface coverage by lignin of the alkali pretreated sample was approximately 100%, and that for the alkaline peroxide (RH) pretreated sample was 91.5%. Compared with reference (R_{ref}), the fiber surface was still mainly covered by lignin after alkaline and alkaline peroxide pretreatment, despite that the total lignin amount was clearly reduced, as presented in Table 2. During alkaline delignification process, lignin is easily precipitated on fiber (Sixta, 2006). The high surface coverage by lignin was probably caused by the lignin redeposition on the surface during pretreatment and washing processes. But this phenomenon did not occur on hydrotropic pretreated fiber because of the diluted alkali solution which was used for washing after hydrotropic pretreatment and, in turn, prevented the reprecipitation of lignin (Mou et al., 2013a). The surface coverage by lignin of R_{sxs} was reduced to 16.5%, and 83.5% carbohydrates exposure on Reed fiber surface was measured after treatment. Thus, the decrease of surface lignin amount has been found to be necessary for the enhancement of enzymatic hydrolysis of woody biomass (Mou et al., 2013a).

Mou et al. (2014) carried out pretreatment of corn stover with the modified hydrotropic method (MHP) to enhance enzymatic hydrolysis. In this study, the hydrotropic method at low concentrations of SXS was first employed to pretreat corn stover. To improve the pretreatment effectiveness, per acetic acid (PAA) treatment was conducted before hydrotropic pretreatment and post-mechanical refining (PFI) was also carried out for comparison. The chemical composition changes of corn stover after pretreatments are given in Table 4 which apparently shows that, after pretreatment, the glucan content increased significantly. For instance, the glucan content was about 64% after the MHP as compared to 39.5% and 48.8% for H_2O and SXS pretreatment, respectively. It may be due to alteration of the structure of lignin after PAA treatment, thus facilitating the lignin removal in the subsequent hydrotropic pretreatment. There have been reports on PAA promoting delignification (Abe et al., 2013; Kumar et al., 2013) also, it is observed that, after hydrotropic pretreatment, more than 33% lignin was removed from the feedstock, while the delignification was doubled after MHP (Table 4). PAA treatment of corn stover before hydrotropic pretreatment could significantly promote the delignification and MHP showed better selectivity upon reducing lignin and hemicelluloses than hydrotropic pretreatment.

Ansari and Gaikar (2014) have studied the delignification of sugarcane bagasse by using alkyl benzene sulfonates as hydrotropes. In this study, they have optimized the conditions for the delignification of sugarcane bagasse such as time, temperature, type of hydrotrope, hydrotrope concentration, and suspension loading in the extraction process. The delignification process was modeled as an extraction of an active compound (lignin) from the solid matrix (bagasse). The optimum conditions for the maximum delignification (85%) of bagasse comprised of temperature 115 °C, hydrotrope concentration (30% w/w) and bagasse loading (5% w/v) in 240 min. Sodium xylene sulfonate was most favorable amongst different hydrotropes for the delignification. Water dilution method was efficient over toluene extraction method for the recovery of lignin. Reusability of hydrotrope was around 98.7% for bagasse delignification. The isolated lignin was characterized in detail by surface functionality, nature, and morphology. The diffusivity coefficients values for bagasse delignification were in the range of 1.43×10^{-13}–3.43×10^{-13} m^2/s which suggest that intraparticle mass transfer resistance is the limiting factor for the delignification of sugarcane bagasse.

Thus, the above literature reports on hydrotropic delignification suggest that the solubilization of lignin is due to hydrophobic interactions between the aromatic ring of phenolic lignin with the aromatic ring of the hydrotrope which is leading to preferential solubilization of lignin compared to cellulose and hemicellulose. Sodium xylene sulfonate was the most commonly reported hydrotrope for delignification of lignocellulosic biomass, and the use of another hydrotrope remains unexplored.

5. Characterization of lignin recovered from hydrotropic solution

Ansari and Gaikar (2014) have characterized the lignin extracted from the sugarcane bagasse using 30% aqueous sodium xylene sulfonate solution. Different techniques were used to characterize lignin. The elemental analysis of the lignin showed 42.12% C, 5.27% H, and 0.64% N with no detectable sulfur in the material. The absorbance of the spectral region of 241 nm and 292 nm indicated that the dissolved lignin contained a majority of

Table 4
Chemical composition of corn stover after pretreatment and the effects of different pretreatment (source: Mou et al., 2014).

Pretreatment	Glucan	Xylan	Arabinan	AIL	ASL	$R_{delignification}$ (%)	R_{glucan} (%)	R_{xylan} (%)	Y_{solid} (%)
Raw corn stover	31.22(0.53)	17.66(0.09)	1.91(0.02)	14.20(0.23)	0.85(0.01)	ND	100	100	100
H_2O	39.46(0.14)	22.70(0.06)	2.09(0.03)	20.29(0.21)	1.13(0.01)	ND	100.46(0.38)	100.26(0.31)	83.43
SXS	48.83(0.06)	19.23(0.08)	2.06(0.01)	15.61(0.46)	0.85(0.02)	33.25(1.79)	95.48(0.11)	66.46(0.27)	61.04
MHP(PAA + SXS)	64.22(0.14)	15.83(0.15)	0.56(0.00)	9.39(0.19)	0.81(0.00)	65.58(0.64)	99.45(0.23)	45.52(0.43)	50.78

H_2O: water pretreatment, SXS: hydrotropic pretreatment, MHP (PAA + SXS): Modified hydrotropic pretreatment. Standard deviation was given in parentheses. The extractives and ash content of corn stover are 22.61% and 6.89% respectively. ND: not detected.

(1) Y_{solid} (%) = pretreated biomass (g)/original biomass (g) × 100
(2) $R_{glucan/xylan}$ (%) = Y_{solid} $C_{glucan/xylan\ of\ pretreated\ biomass}/C_{glucan/xylan\ of\ original\ biomass}$
(3) $R_{delignification}$ (%) = Y_{solid} $C_{lignin\ of\ pretreated\ biomass}/C_{lignin\ of\ original\ biomass}$

unconjugated phenolic compounds rather than conjugated phenols. The unconjugated phenols showed the peaks between 250 and 300 nm while conjugated phenols showed the maximum close to 370 nm (Aulin-Erdtman and Sanden, 1968; Goldschmid, 1954). The absorbance at 292 nm indicated the presence of phenolic hydroxyl groups of lignin (Vallejos et al., 2011). The absorbance at 241 nm also showed the presence of *p*-hydroxy-phenyl, guaiacyl, and sinapyl structures of lignin (Saariaho et al., 2003).

The IR spectra of lignins recovered from different aqueous hydrotrope solutions were apparently similar indicating similar nature of the lignin extracted by various hydrotropes. A peak at 3405 cm^{-1} was observed indicating the stretching of phenolic and aliphatic units of lignin (Boeriu et al., 2004; Faix, 1992; Singh et al., 2005). Cyclic hydrocarbons of the lignin structure were identified by the peaks at 2922–2852 cm^{-1}. The peaks at 1643 cm^{-1}, 1515 cm^{-1}, and1463 cm^{-1} indicated the carbonyl group stretching for methoxyl group of lignin, aromatic ring stretching and the methyl group (CH_2/CH_3) stretching respectively (Lisperguer et al., 2009). However, the absorbance of the guaiacyl structure of the lignin was found at 1383–1322 cm^{-1}. The *p*-hydroxy phenyl structure of lignin gave the peaks at 1261 cm^{-1} and 1162 cm^{-1} (Lisperguer et al., 2009). The ether linkage of the lignin structure was seen at1020 cm^{-1}.The XRD measurement of lignin revealed a weak peak at 22.37 (2θ value), indicating that lignin was mostly amorphous in nature. The gel permeation chromatography analysis showed the weight-average (MW) and number-average (MN) molecular weights of lignin to be 3791 g/mole and 3695 g/mole respectively. The molecular weight of lignin was comparable with the lignin extracted from bagasse using dioxane (3405–3868 g/mole, Sun et al. (2011), from alkali treatment (1680–3020 g/mole, Sun et al. (2003), and using soda process (2160 g/mole, Mousavioun and Doherty (2010).

Gabov et al. (2014) have characterized lignin extracted from birch wood using modified hydrotrope process. Hydrotropic lignins were obtained in a 10 L rocking digester using a 36% aqueous hydrotropic solution. Two treatments were performed, namely, a conventional reference method and one with the addition of formic acid and hydrogen peroxide. Lignins obtained with these treatments were designated R and M lignins, respectively. A dosage of hydrogen peroxide for the modified treatment was 2.5% based on wood, and the amount of formic acid that was added to lower the pH to 3.5. The contents of extractives in the lignins were determined to be 0.50% and 0.44% for R and M lignins, respectively. The detected extractives were in a free form. The free extractable matter of the lignins consisted exclusively of fatty acid with palmitic acid being the most abundant. The lignins had a slight amount of residual carbohydrates owing to extensive hydrolytic reactions, which took place during the treatment. In contrast to the extractives, the residual carbohydrates are covalently bound to lignin and form so-called lignin–carbohydrate complexes. As expected, xylose was the main contaminating sugar, because it was present in the birch wood in a large quantity. In the lignins, residual xylose is present in its polymeric form, xylan. Both lignins exhibited elemental composition typical of technical lignins. Technical lignins usually have a higher content of carbon and lower contents of oxygen and hydrogen than the original lignin in wood (milled wood lignins in the literature (Fengel and Wegener, 1984). Nitrogen in both samples presumably originated from a small amount of proteins from the wood. Both the lignins contained sulfur, which indicates that the samples were possibly contaminated with the hydrotropic salt. The mass ratios of the *p*-hydroxyphenyl, guaiacyl, and sinapyl units were found to be 1:25:74 for R and 2:26:71 for M lignins. The corresponding ratio for the original birch wood lignin was 3:26:71. Hence, the results show that the relative content of the units in the lignins was almost the same as in birch wood lignin; therefore, it can be concluded that the ratio did not change over the course of the hydrotropic treatments. Also, both hydrotropic lignins were comparable with each other. A comparison was made between the characteristics of two commercial hardwood technical lignins and the hydrotropic lignins. The lignin obtained from the alkaline process has a higher content of carboxyl groups in comparison to the lignins isolated in acidic conditions which may be due to higher oxidation rate of phenolic units by air in alkaline media compared to an acidic one. Hydrotropic lignins are expected to be closer to Alcell lignin due to the similarity of the nature of the processes. Indeed, the contents of carbohydrates and carboxyl groups are quite similar. Besides, the FTIR spectra of the hydrotropic lignins were analogous to the spectrum of the Alcell lignin. However, due to the difference in the wood species and treatment agents as well as the process conditions (temperature, duration, liquor to solid ratio, etc.), the lignins also showed differences. For example, the Alcell lignin has a lower molecular weight and a lower content of phenolic hydroxyl groups than the hydrotropic lignins Also, the Alcell lignin is sulfur-free, and the hydrotropic lignins contain small amounts of sulfur, which, however, might be due to contamination of the hydrotropic agent.

Thus, it appears that hydrotropic lignin can have potential application as bio-based polymer due to its stable structure which in turn can serve as a value-added chemical in a biorefinery concept.

6. Effect of hydrotrope pretreatment on hemicellulose

In addition to lignin as physical barrier, the surface distribution of xylan on biomass materials have been reported to have an adverse influence on the enzymatic hydrolysis efficiency (Leu and Zhu, 2013; Mooney et al., 1998). Gabov et al. (2013) have studied the effect of supplementing additives in a hydrotropic process of Birchwood. The additives used to modify the hydrotrope process had two contrary effects as the results showed that the additives accelerated both desired (delignification) and undesired (carbohydrate degradation) reactions though at a different rate. Hydrotropic treatments with additives removed a significant part of the hemicelluloses from Birchwood, as a consequence of hydrolytic processes induced by liberated organic acids, mainly acetic acid and formic acid (Nelson, 1978). The reaction was enhanced by formic acid that was added to the hydrotropic solution. The pulps produced by hydrogen per oxide-assisted treatments had a slightly higher content of hemicelluloses in comparison to similar treatments without hydrogen per oxide.

Mou et al. (2013b) studied the hydrotropic pretreatment of common Reed using 30% sodium xylene sulfonate with an addition of 0.17% formic acid which resulted into residual xylan of 9.2% as seen in Table 2. It is speculated that the removal of hemicellulose could produce sufficient cellulose accessible surface to cellulase, which was even more important than the removal of lignin for effective enzymatic saccharification (Leu and Zhu, 2013). Per acetic acid treatment before hydrotropic pretreatment studied by Mou et al. (2014) on corn stover revealed lower residual xylan and Arabinan content in the biomass as compared to hydrotrope treatment without per acetic acid (Table 4).

However, after evaluating the above results on the effect of hydrotrope on hemicelluloses, it seems that the addition of additives like formic acid, acetic acid and hydrogen per oxide is predominantly influencing the removal of hemicelluloses from the biomass. It is very evident from the reported literature that dilute acid pretreatment leads to removal of hemicelluloses (Sindhu et al., 2010). The role of hydrotrope in the removal of hemicelluloses seems to be feeble. Solubilization of hemicelluloses in the hydrotrope solution is possibly due to auto hydrolysis taking place at a higher temperature that too to a lower extent. Organic acids

liberated from biomass, mainly acetic and formic catalyze the hydrolysis reaction (Procter, 1971).

7. Effect of hydrotrope pretreatment on cellulose

Besides lignin hindrance, the crystallinity of cellulose is another important factor to affect enzymatic saccharification (Zhu et al., 2008). XRD was the most common technology for crystallinity determination. Mou et al. (2014) have studied and compared the XRD data of SXS; MHP and PFI pretreated corn stover with the untreated one, the increase of crystallinity index (CrI) after pretreatment (particularly for SXS pretreatment and MHP) was due to the removal of the amorphous substances, such as lignin and hemicelluloses. The CrI increased by 23% after MHP compared to the raw corn stover. Also, there was a slight decrease of CrI after PFI refining, which is because of the modification of the crystalline regions of cellulose (Leu and Zhu, 2013). The relative cellulose crystallinity value was calculated by FTIR, and it was observed that there is a slight decrease of total CrI after pretreatment, which is associated with the destruction of cellulose.

8. Physiochemical characterization of hydro tropically pretreated biomass

Physiochemical characterization studies provide valuable insights into the impact of hydrotrope treatment on the morphology of the biomass. Morphology of the hydrotropically pretreated corn stover was studied by Mou et al. (2014). The imaging of corn stover before and after treatment was carried out by the use of SEM**.** The morphology of the fiber supplies evidence on the influence of pretreatment on the fibers. The pores, particularly in parenchyma cells were created by PAA treatment, and similar observation was also found in corn stover pretreated with liquid hot-water (Zeng et al., 2012). Because of the pore generation, SXS solution penetration into the corn stover and the efficient degradation of lignin and hemicelluloses could be further strengthened. Mou et al. (2013a) have studied the Field-emission scanning electron microscopy (FE-SEM) analysis of birch and pine wood after hydrotrope treatment. In the case of hydrotropic pretreatment, no surface degradation was detected in any of the wood samples, and there was no swelling. Fractures in the pretreated pine, presumably following the direction of the fibril aggregates of the secondary cell wall were observed. The reason for the fractures was assumed to be changed lignin distribution in the cell wall after 2 h pretreatment with SXS because the surface coverage by lignin was lower than that of the reference pine (Table 1). Surface morphology of Reed after SXS pretreatment was studied by Mou et al. (2013b) along with alkaline and alkaline peroxide pretreatment. After alkaline peroxide pretreatment, the damaged outer layer was peeled off from Reed surface compared with the coarse surface of reference which had more broken outer layer due to grinding process. Apparently, the layer of lignin deposited was detected on alkaline peroxide pretreated sample and fiber walls were with sparse, broken cell wall residues on the surface. The fiber appearance became smoother than the reference Reed, and long cells were clearly visible. For the samples RN (alkaline pretreated), platode mastoid epidermal were found by FE-SEM and the exterior surface was dense, and cell wall showed delamination tendency. Similar findings were also reported previously by Corrales et al. (2012). Same platode mastoid epidermal located along vertical fiber direction were detected on the hydrotropic treated Reed. Some of them were broken to pores on fiber leading to the exposure of the internal layer of fibers. The generation of broken pores is usually favorable for accelerating enzymatic hydrolysis efficiency. They had also investigated the surface chemical characterization of common Reed after enzymatic hydrolysis by the time of flight secondary ion mass spectrometry (TOF-SIMS). The signal from lignin and carbohydrate were detected. The ratio value was calculated by dividing the sum of the peak intensities of the characteristic mass fragments of carbohydrates (pentoses and hexoses) by the sum of the peak intensities of the characteristic mass fragments of lignin according to literature (Fardim and Durán, 2003; Koljonen et al., 2003; Saito et al., 2005). The ratio value between carbohydrates and lignin was higher in the pretreated samples than in reference Reed (Table 5). Carbohydrates distributed on fiber surface were increased by alkaline, alkaline-peroxide, and hydrotropic pretreatment. But the lignin removal by alkaline and alkaline peroxide pretreatment was not as efficient as the hydrotropic pretreatment. Also, the hexose ratio was increased after the pretreatments, compared to the untreated reference. The biggest increase was detected in the SXS pretreated sample. Hexoses mainly from cellulose (glucose) were not sufficiently exposed on Reed surface after alkaline and alkaline- peroxide pretreatment. TOF-SIMS detection could give the evidence on the outstanding surface delignification of hydrotropic pretreatment which in turn enhanced the distribution of cellulose on the fiber surface, thus facilitating the downstream enzymatic hydrolysis. Further, lignin degradation was estimated by the peak intensity ratio of guaiacyl units (G) to the total lignin (sum of counts from G, S, H, and aromatic unit). The ratio was increased after hydrotropic pretreatment because guaiacyl lignin is more recalcitrant to chemical solvent (Mou et al., 2013a). Thus, residual guaiacyl lignin in Reed after hydrotropic pretreatment resulted in the G/total lignin ratio of R_{sxs} higher than that of R_{ref} as presented in Table 5. With pretreatment, lignin ether bonds were broken, and more aromatic groups generated which was shown in the G/aromatic ratio of RH, which is lower than that of reference. In addition, the surface chemical characterization of enzymatically hydrolyzed samples was also studied and the results are showed in Table 5. The decrease of hexose compared to reference was due to the hydrolysis of cellulose to glucose monomers in liquid phase especially for RN and R_{sxs}. The reduction of carbohydrates/lignin ratio indirectly refers that the surface of fiber contained relatively more lignin after hydrolysis. According to the results presented in Table 5, the hydrolysis efficiency order was R_{sxs} > RN > RH. During enzymatic hydrolysis, the G/aromatic ratio and G/lignin ratio of RN samples was increased while that of RH and R_{sxs} has no difference with E_{ref} which may be due to the removal of redeposited lignin on RN during the hydrolysis. They had also studied Attenuated total reflectance (ATR)–Fourier transform infrared spectroscopy (FTIR) analysis of Reed after pretreatments. The peak assignment was as per literature (Cao et al., 2012; Corrales et al., 2012; Galkin et al., 1997; Guo et al., 2011; Shen et al., 2011; Sun et al., 1995; Todorciuc et al., 2009). The broad peak at 3340 $cm^{-1}$is associated with O–H vibration which could be found in cellulose, hemicellulose and lignin for agriculture

Table 5
TOF-SIMS characterization of Reed samples after pretreatment and after enzymatic hydrolysis (source: Mou et al. (2013b)).

Reed	Carbohydrates/ lignin	Guaiacyl/total lignin	Guaiacyl/ aromatic	Hexose/total * 10^3
R_{ref}	0.63(0.08)	0.10(0.03)	0.16(0.08)	0.9(0.3)
RN	0.82(0.44)	0.11(0.02)	0.17(0.04)	1.0(0.7)
RH	1.09(0.09)	0.08(0.02)	0.11(0.04)	1.0(0.4)
R_{sxs}	1.05(0.30)	0.14(0.04)	0.25(0.10)	2.2(0.9)
E_{ref}	0.73(0.12)	0.06(0.01)	0.12(0.02)	2.5(0.6)
ERN	0.41(0.05)	0.17(0.02)	0.34(0.08)	1.3(0.1)
ERH	0.67(0.09)	0.06(0.01)	0.13(0.02)	2.8(0.5)
ER_{sxs}	0.44(0.02)	0.05(0.01)	0.09(0.02)	1.0(0.1)

E: enzyme hydrolyzed Reed. Enzyme hydrolysis conditions: 20 FPU/g (dry matter at 50 °C, pH 5.0, 72 h).

materials, and it is also possible from aliphatic fraction of waxes (Shen et al., 2011; Sun et al., 1995). The peak at 2926 cm^{-1} is the C–H stretching deformation of CH_3, and CH_2 respectively in untreated and treated Reed which was reported from cellulose, hemicelluloses and lignin (Corrales et al., 2012; Guo et al., 2011; Sun et al., 1995). The peak at 2847 cm^{-1} is assigned to —OCH3 commonly present in lignin (Corrales et al., 2012). The intensities of both of the two peaks were lowest in R_{sxs} and highest in RN. It is because of the more efficient hemicelluloses and lignin removal by hydrotropic pretreatment, while the increasing intensities of CH_2, CH_3 and OCH_3 groups, it was speculated to represent breaking of the aryl–ether bonds especially β–O-4 bonds in alkaline pretreatment. Stretching at 1735 cm^{-1} is assigned to the ester bond from hemicelluloses, and the intensities order is R_{ref} > RN > RH > R_{sxs}. At 1640 cm^{-1}, the C=C bond of aromatic lignin is not detected in R_{sxs} samples. The peak at 1460 cm^{-1}indicated the presence of cellulose and lignin (Sun et al., 1995). The peaks at 1700 cm^{-1} and 900 $cm^{-1}$are the characteristic region especially for the guaiacyl (G) and sinapyl (S) units (Todorciuc et al., 2009), but the spectra for all samples were similar in this area. Peaks at 1357 cm^{-1} for sinapyl with C–O stretching were not clear, but peaks at 1430 cm^{-1} and 1323 cm^{-1}were characteristics of guaiacyl lignin (Todorciuc et al., 2009). Also, peak at 1036 $cm^{-1}$was attributed to C–O–C bond from polysaccharides. The peak at 897 $cm^{-1}$was related with the β-glycosidic bond of polysaccharides (Corrales et al., 2012), while the peak at 805 cm^{-1} is speculated to be C–H out of plane bending which is from *p*-substituted benzenes only existing in the untreated Reed. As detected by ATR–FTIR, no S bond was found in R_{sxs}.

In another study, Mou et al. (2013a) have investigated the effect of the hydrotropic treatment and enzyme hydrolysis on the wood powder by comparing the TOF-SIMS spectra of the treated samples to that of the untreated samples. The peak intensities of the characteristic lignin (Saito et al., 2005), pentose (xylan), and cellulose peaks (Fardim and Durán, 2003) were normalized against the total counts. The ratios of the lower mass region count from polysaccharides and lignin (Goacher et al., 2012) were compared, and the ratios of guaiacyl and sinapyl units were also studied. During hydrotropic extraction for birch, the lignin ratio was first increased and then decreased (B REF compared to B1 and to B2) as seen in Table 6. Further, the proportion of polysaccharide peaks of the sum of the polysaccharide peaks and lignin peaks (PS/(PS + L) was slightly reduced irrespective of the treatment time. The result was suggesting that hydrotropic method was not only removing the lignin but also led to carbohydrate loss, probably from low mass hemicelluloses, and it mainly happened during the initial 30 min of the pretreatment process. The pentose ratio from fiber surface was reduced with extensive pretreatment, but cellulose was retained well. Thus, SXS had good selectivity for lignin extraction from wood species with lower cellulose removal.

For pine, some more evident effects were observed in the surface lignin, compared to those in birch. Hydrotropic treatment seemed first to decrease and then to increase the proportional surface lignin, which probably is a sign of polysaccharide degradation in a longer treatment time. The S/G peak ratios were, in general, higher than what is known about the bulk proportion of the lignin units in pine. The fragmentation inclination of the two in TOF-SIMS could be different. Hydrotropic treatment degraded syringyl groups, and enzymatic hydrolysis affected the guaiacyl groups in lignin, clearly decreasing the lignin peak intensities. The polysaccharide.ratio, as well as the cellulose ratio, was also declining during hydrotropic treatment and further during enzymatic hydrolysis.

9. Effect of hydrotrope pretreatment on enzyme hydrolysis

Enzymatic hydrolysis is the second step in the production of ethanol from lignocellulosic materials. It involves cleaving the polymers of cellulose and hemicellulose using enzymes. Consequently, the primary hydrolysis product of cellulose is glucose, whereas the hemicellulose gives rise to several pentoses and hexoses (Taherzadeh and Niklasson, 2004).

Mou et al. (2013b) studied the enzyme hydrolysis of hydrotropically pretreated common Reed (RSXS) along with alkaline (RN) and alkaline peroxide pretreatments (RH). Maximum yield of glucan from the samples RN, RH and R_{sxs}, were 89%, 85% and 93% respectively, after 72 h enzyme hydrolysis. During the first 6 h of enzyme hydrolysis, the glucan yield for the hydrotropic pretreated sample was lower than that for the alkaline and alkaline peroxide pretreated samples which correlated with the remained total lignin amount (Table 2). A total lignin removal can enhance the accessibility of enzymes (Li et al., 2012). However, as the hydrolysis time was longer than 6 h, the glucan yield of R_{sxs} increased rapidly compared to that of RN and RH. It could be speculated that the enzyme adsorption behavior to the RN and RH fiber was negatively affected by the high content of surface lignin, as the surface coverage by lignin for RN (about 100%) and RH (91.5%) was higher than that of R_{sxs} (16.5%). In another study, they have evaluated the glucan and xylan yield of pretreated corn stover after enzyme hydrolysis. Hydrotropic pretreatment significantly increased the glucan yield compared to the samples only treated by water and the order of the glucan yield observed from pretreatments was MHP > SXS > H_2O, which is in agreement with the delignification results shown in Table 4. PAA treatment improves the hydrotropic pretreatment efficiency as the MHP pretreated corn stover was more accessible to the enzyme. After PAA treatment, the possible changes in fiber

Table 6
Surface chemical composition of birch (B) and pine (P) by TOF-SIMS after hydrotropic pretreatment in 30 min (1) or 120 min (2) and enzyme hydrolysis (E). REF stands for untreated wood. PS = polysaccharides, L = lignin, S = sinapyl, G = guaiacyl (source: Mou et al., 2013a).

Samples	PS/(PS + L)[a]	S/G	Cellulose[b]/total	Pentoses[b]/total	Lignin[c]/total
B1	0.29(0.01)	0.83(0.06)	3.7(0.1)	3.2(0.6)	5.7(1.2)
EB1[d]	0.29(0.01)	0.80(0.06)	2.3(0.2)	1.7(0.2)	3.2(0.3)
B2	0.30(0.09)	0.77(0.02)	3.3(1.3)	2.2(0.3)	4.7(1.7)
EB2[d]	0.31(0.03)	0.83(0.14)	2.1(0.2)	1.6(0.1)	3.0(0.7)
B REF	0.34(0.03))	0.79(0.46)	3.9(0.6)	3.3(0.5)	3.9(0.9)
P1	0.26(0.02)	0.82(0.22)	4.3(0.1)	3.2(0.2)	2.1(0.1)
EP1[d]	0.21(0.00)	1.13(0.09)	3.1(0.3)	2.6(0.1)	1.3(0.1)
P2	0.26(0.01)	0.40(0.02)	3.8(0.3)	3.1(0.2)	4.1(0.5)
EP2[d]	0.21(0.01)	0.84(0.11)	3.1(0.5)	2.4(0.4)	1.8(0.4)
P REF	0.30(0.02)	0.80(0.20)	4.8(0.4)	3.9(0.4)	3.4(0.2)

[a] Goacher et al. (2012).
[b] Fardim and Durán, 2003.
[c] Saito et al. (2005).
[d] Enzyme hydrolysis conditions: Cellulase 50 FPU/g (dry matter) and 300 nkat/ml β-glycosidase at 50 °C, pH 5.0, 48 h.

morphology and fundamental chemical structure could enhance the efficiency of hydrotropic treatment. The opening of the cell wall by MHP treatment was caused by the removal of lignin and xylan (Table 4), and this could help enzyme access into fibers, thus enhancing the hydrolysis of cellulose to glucose. Also, beating could further improve the enzyme hydrolysis performance because of the increase in specific surface area. As a result of all positive changes of the MHP sample, the glucan yield increased to about 90% after 48 h of enzymatic hydrolysis and post-refining which corresponds to nearly 20% improvement compared to hydrotropic pretreatment under the same conditions.

In yet another study, they have carried out the enzyme hydrolysis of birch and pine wood after hydrotropic pretreatment using commercial cellulase mixture. Celluclast 1.5 at a dosage of 10 FPU/g (dry matter), 20 and 50 FPU/g fortified with Novozyme 188, 300 nkat/g (dry matter) β-glucosidase were used at 50 °C in pH 5.0, for 2, 6, 24, and 48 h, respectively. Hydrotropic pretreatment of birch for 120 min residence time was easier to hydrolyze than 30 min pretreated birch, expectedly, and that the hydrolysis efficiency was obviously higher on both of the pretreated samples than on birch without pretreatment. With an increasing dosage of enzyme and prolonging time, the glucose yield in the liquid increased expectedly. During the first 6 h, the enzyme hydrolysis rate was rapid but became slower after that. Birchwood pretreated with hydrotrope for 120 min had a maximum glucose conversion of 83.9%, after 48 h with 50 FPU/g d.m. cellulose hydrolysis. Intensifying the hydrotropic pretreatment such as increased temperature and prolonged treatment time is probably helpful to boost the enzyme hydrolysis. Severe conditions of sulfite pretreatment on pine wood have been demonstrated to be able to improve the glucose saccharification as while as certain amount of the inhibitors furfural and hydroxyl methyl furfural was produced (Lan et al., 2013). In case of pine as well, the hydrotropic treatment improved the enzymatic hydrolysis. Pine treated for 120 min resulted in better hydrolysis than determined from pine treated for 30 min with different dosages of cellulase mixture. However, the hydrolysis level with pretreated pine remained much lower as compared with birch. Even after 24 h hydrolysis, for pine with 120 min pretreatment, the glucose yield was mere 15.5%. The enzyme hydrolysis efficiency was improved with hydrotropic pretreatment; however, the glucose yield remained rather modest, and more glucose monomers were hydrolyzed from birch than from pine at the same conditions.

Thus from the above literature, it is apparent that hydrotrope pretreatment indeed enhances the enzymatic conversion of cellulose to glucose. However, due to recalcitrant nature of lignin, it may limit the enzyme hydrolysis efficiency as observed in the case of pine. Most of the studies on the hydrotropic process of lignocellulosic material have been limited to delignification and not extended to enzymatic hydrolysis and fermentation. Substantial data is lacking to have clear insights into the factors affecting the enzyme hydrolysis after hydrotrope pretreatment into different lignocellulosic biomass to have comparative evaluation.

10. Conclusion

Hydrotropic pretreatment seemingly leads to reduction and relocalization of lignin from the fiber surface. Surface chemical compositional studies of biomass by XPS and TOF-SIMS provide valuable insights on surface coverage by lignin. It appears that there is no independent chemical or structural factor that exclusively controls biomass recalcitrance as altering one structural feature is accompanied by the change of additional ones. Hydrotrope pretreatment can mitigate the environmental impact of chemical pretreatment with promising green chemistry operation. However, the study of the hydrotropic method as a pretreatment for successful fermentolysis in the biofuels technology has not received enough consideration so far.

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