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FRACTIONATION OF PROCESSED SPRUCE WOOD OBTAINED IN THE PRODUCTION OF ETHANOL

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Fuel ethanol can be produced from pretreated spruce wood through enzymatic hydrolysis and fermentation. Processed spruce wood samples (acid-catalyzed steam hydrolysis followed by enzymatic hydrolysis and fermentation) were fractionated into water-soluble products and residual solids. The dioxane/water soluble portions of the solids were fractionated by liquid-liquid extraction. A substantial portion of the processed wood (20-70 %) was insoluble in both water and dioxane/water. An almost pure lignin fraction, corresponding to about 20 % of the total lignin of the wood, was isolated. Examinations by ^{1}H NMR spectroscopy showed that the processes led to extensive cleavage of arylglycerol β-aryl ethers in the lignin. The lignin content of other fractions could be estimated very roughly by IR spectroscopy. Purified lignin from SO_2-catalyzed steam hydrolysis contained approx. 0.2 % sulphur.

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INTRODUCTION

Softwood is an abundant raw material in the northern hemisphere, and therefore it has the potential of being a renewable feedstock in the production of fuel ethanol. The hemicelluloses and cellulose in the wood can be hydrolyzed to monosaccharides using enzymes and or an acid catalyst. The monosaccharides, in turn, can be fermented with yeast. No full scale plant for ethanol production from wood is in operation as yet. The complexity of the processes has so far thwarted the development of full scale production of ethanol from wood. Lignin is the structural wood constituent that has the highest specific energy value; the mass and energy balances of the ethanol production are therefore affected by the solubility of lignin in the hydrolysis liquor.

Processes involving dilute or concentrated acid hydrolysis of wood give only low yields of monosaccharides. Processes in which a preliminary mild acidic treatment of the wood, such as SO_2-catalyzed steam hydrolysis is followed by enzymatic hydrolysis, are favourable in that the yields of monosaccharides obtained are comparatively high (Galbe and Zacchi 2002; Taherdazeh and Karimi 2007a,b). The conditions prevailing during SO_2-catalyzed steam hydrolysis have similarities to those applied in sulphite pulping, even though the reaction times are shorter and temperatures are higher (Glennie 1971). It is therefore probable that lignin structures are sulphonated to some extent during SO_2-

catalyzed steam hydrolysis. Incorporation of hydrophilic groups, such as sulphonic acid groups, leads to increased solubility.

In this work, processed spruce wood (*Picea abies*) obtained in the production of ethanol in bench-scale experiments was fractionated. Similar fractionations of steam hydrolyzed aspen wood (Bardet et al. 1985) and wheat straw (Montan et al. 1998) have been reported in the literature. Spruce wood pretreated with either SO_2 or H_2SO_4 catalyzed steam hydrolysis was examined. Products from SO_2-catalyzed steam hydrolysis were subjected to enzymatic hydrolysis or a combination of enzymatic hydrolysis and fermentation (Simultaneous Saccharification and Fermentation: SSF) and fractionated. Fractionation based on solubility (Lundquist et al. 1977) aimed at separation and characterization of the major constituents. The obtained fractions were analyzed for methoxyl and sulphur and also examined by IR spectroscopy and ^{1}H NMR spectroscopy. The distribution of lignin in the fractions was studied.

EXPERIMENTAL

Materials

Three different pretreatment conditions and two enzymatic hydrolysis and fermentation alternatives were investigated in the production of ethanol from spruce wood (*Picea abies*). A flow-sheet describing the conversion products of spruce wood obtained in ethanol production is shown in Fig. 1.
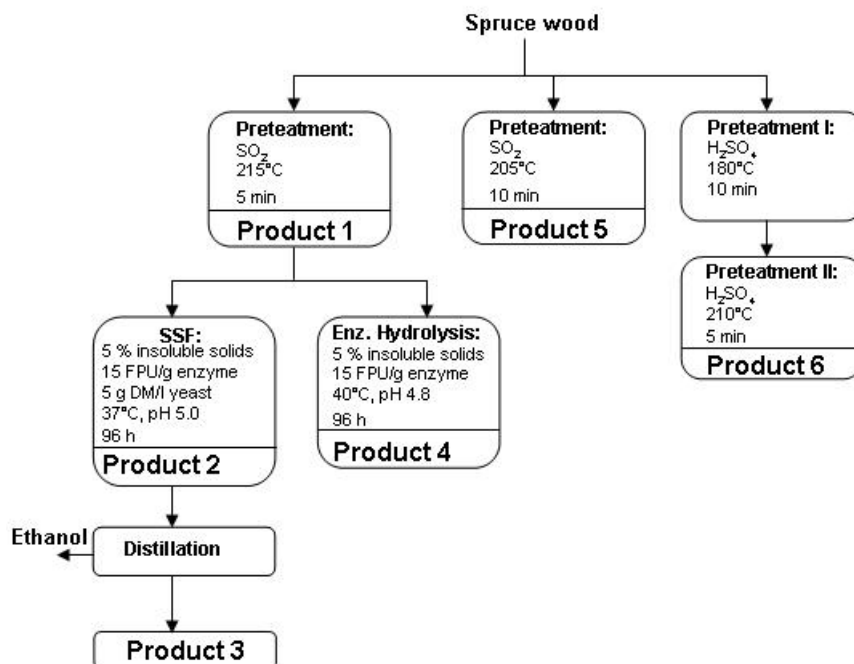

Fig. 1. Conversion products of spruce wood (Products 1-6) obtained in the production of ethanol in bench-scale experiments.

These conversion products (Products 1-6, see Fig. 1) were produced in bench-scale experiments at the Department of Chemical Engineering, Lund University, Sweden. Products 1 and 5 were prepared using SO_2 as the acid catalyst, whereas H_2SO_4 was used for Product 6. The severity factor (Overend and Chornet 1987) was used to obtain similar harshness for the different pretreatment conditions used.

This factor is a function of time, t (min), and temperature, T (ºC):

$$\log(R_0) = \log\left(t \cdot e^{\frac{T-100}{14.75}} \right) \tag{1}$$

The severity factor of the pretreatment was 4.1 for Products 1 and 5, and 3.9 for Product 6. The input weights and yields of Products 1-6 are given in Table 1.

Table 1. Weights of Spruce Wood, Processed Spruce Wood, and Final Products (Products 1-6), see Fig. 2.

Spruce wood or treated spruce wood (dry mass, g)	Product (dry mass, g)
7500	6590 (Product 1)
1510[a]	990 (Product 2)
160[b]	160 (Product 3)
1510	1570 (Product 4)
4500	3880 (Product 5)
2500	1600 (Product 6)

[a] Pretreated spruce wood
[b] SSF product

Description of the procedures applied in the preparation of Products 1-6

Product 1: Spruce chips (7.5 kg dry mass) were impregnated with 3 % (w/w) SO_2, based on the moisture content (41 %) of the wood; approximately 90 % of the SO_2 was absorbed. The impregnated wood was then transferred to a 10 liter reactor in which the prehydrolysis was initiated by introducing steam at a temperature of 215 ºC. After 5 minutes the content of the reactor was blown into a flash tank.

Product 2: Product 1 (1.5 kg dry mass) was suspended in water to a solid content of 7.5 % (w/w), which corresponded to a water-insoluble solid content of 5 % (w/w), and transferred to a fermenter tank, which was then sterilized at 140 ºC. 15 FPU/g cellulase (Novozymes A/S, Denmark), 5 g (dry weight)/l Baker's yeast (Jästbolaget AB, Sweden), 0.5 g/l $(NH_4)_2HPO_4$, 0.025 g/l $MgSO_4 \cdot H_2O$ and 1 g/l yeast extract were added to the fermenter tank. The total weight was 20 kg. The pH was adjusted by adding NaOH and $Ca(OH)_2$ and the foam level was lowered by adding an anti-foaming agent. Further data is given in Fig. 1. This step is denoted SSF (Simultaneous Saccharification and Fermentation).

Product 3: Product 3 was obtained on evaporation (at 85 ºC) of ethanol from 3 kg of Product 2.

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Product 4: Product 4 was produced in a similar manner as Product 2, but only cellulase was added. The temperature and pH differed somewhat from those applied in the Product 2 experiment (see Fig. 1).
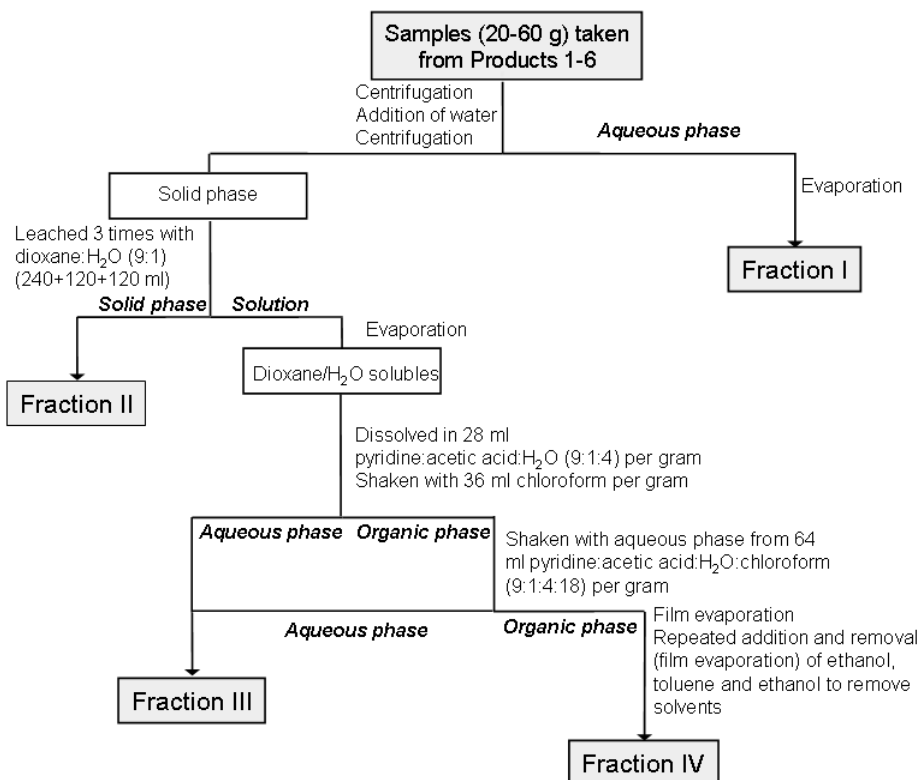

Fig. 2. Conversion products of spruce wood (Products 1-6) obtained in the production of ethanol in bench-scale experiments.

Product 5: Spruce chips (4.5 kg dry mass) were treated essentially according to the procedure adopted for the preparation of Product 1, but a lower temperature and a longer residence time (severity factor 4.1).

Product 6: Spruce chips (2.5 kg dry mass) were impregnated overnight with 0.5 % (w/w) H_2SO_4, based on the water content (1.1 kg) of the sample, in the first pretreatment step. After this treatment the product was washed with water. In a second pretreatment step the product was impregnated overnight with 1 % (w/w) H_2SO_4 based on the water content (1.5 kg). Further data is given in Fig. 2.

Fractionation of the products

Each one of the Products 1-6 was divided into four fractions (Fractions I-IV) according to Fig. 2. Solvents were removed by film evaporation. Each fraction was freeze dried and dried over P_2O_5 and KOH *in vacuo* prior to weighing. The dioxane/water soluble portion of the solids obtained on centrifugation (see Fig. 2) was fractionated into Fractions III and IV according to Lundquist et al. (1977). Solubilized lignin accumulates in Fraction IV.

Analytical Methods

Methoxyl and sulphur contents of the fractions were determined by Analytische Laboratorien GmbH, Lindlar, Germany. Infrared (IR) spectra were recorded on a Perkin-Elmer (1600 Series) FTIR spectrometer. The IR spectra were taken in KBr discs. ^{1}H NMR (400 MHz) spectra of acetate derivatives (Lundquist 1992) were recorded with a Varian Unity 400 instrument at 293 K. Solvent: $CDCl_3$. Internal reference: $(CH_3)_4Si$.

The lignin content was calculated based on an assumed molecular weight of a phenyl propane unit of 184.7 and 0.96 methoxyl groups per phenylpropane unit (Björkman and Persson 1957) (equivalent to a content of 16.1 % methoxyl in lignin), and that all methoxyl groups originate from the lignin constituents. Partial sulphonation and lignin reactions during the pretreatments will alter the weight of the phenylpropane units. However, this is not expected to change the average molecular weight of the phenyl-propane units dramatically.

The Klason lignin content (acid-insoluble part) in Fraction I of Products 1, 5, and 6 was determined according to Dence (1992).

Samples taken from Product 3 were washed with cold (20 ℃) and warm (80 ℃) water. The samples were centrifuged, decanted, slurried in wash water twice, freeze-dried and, finally, analyzed for sulphur.

RESULTS AND DISCUSSION

Composition of Products 1-6

The results obtained from the fractionations and analyses of Products 1-6 are summarized in Table 2. The content of lignin can be estimated indirectly by determining the methoxyl content. In this work, milled wood lignin (MWL) (Björkman and Persson 1957) has been used as a reference. Methoxyl groups are also found in arabino-4-O-methylglucurono-xylan. The methoxyl groups in xylan are, however, partly removed during acidic conditions: acidolysis of 4-O-methylglucuronic acid with 0.2 M HCl in dioxane:water (9:1) for 4 h liberated 77 % of the methoxyl groups as methanol (Lundquist and Ericsson 1971). Hence, the influence of methoxyl groups in xylan can be expected to be reduced on acidic treatment.

Judged by the methoxyl content, the lignin in Products 1-6 was distributed in Fractions I-IV in the following manner: 2-14 % in Fraction I, 60-80 % in Fraction II, 1-5 % in Fraction III, and 14-26 % in Fraction IV (Table 2). Most of the lignin was found in the dioxane/water insoluble Fraction II: this can be explained by the high molecular weight of the lignin, lignin carbohydrate linkages and condensation reactions during the processes. However, the processes resulted in a solubilization of a rather large portion of the lignin (approx. 20 %). The solubilized lignin accumulated in Fraction IV, and the lignin nature of this fraction is evident from the analytical data in Table 2 and from IR and ^{1}H NMR spectroscopic investigations (see below).

Lignin is often analyzed as Klason lignin (Dence 1992). Proteins interfere with Klason lignin determinations (Lai and Sarkanen 1971). Therefore lignin cannot be analyzed in samples containing enzymes and yeasts by the Klason method. The Klason lignin (acid-insoluble portion) content in Fraction I of Products 1, 5, and 6 was 20-35 %

higher than the lignin content calculated from the methoxyl content. This discrepancy is probably related to the low lignin content of these fractions.

Table 2 Methoxyl, Sulphur, and Lignin Analyses of Fractions I-IV (Fig. 2) obtained on Fractionation of Products 1-6 (Fig. 1).

Product 1 Fraction #	Amount g (%)	OCH$_3$ %	S %	Lignin g (%[a])	Product 4 Fraction #	Amount g (%)	OCH$_3$ %	S %	Lignin g (%[a])
I	5.8(25)	1.1	1.87	0.4(6.5)	I	40.3(71)	0.8	0.96	2.1(5.1)
II	12.9(56)	6.4	0.14	5.1(40)	II	10.8(19)	14.2	0.26	9.5(88)
III	2.2(9.4)	1.1	0.85	0.2(6.9)	III	1.7(3.1)	6.0	0.32	0.6(37)
IV	2.2(9.6)	10.9	0.21	1.5(68)	IV	3.8(6.7)	18.4	0.21	4.3(114)
Total	23 (100)	5.0	0.65	7.1(31)	Total	56.6 (100)	4.7	0.76	16.5(29)
Product 2	Amount	OCH$_3$	S	Lignin	Product 5	Amount	OCH$_3$	S	Lignin
I	10.0(45)	2.5	2.04	1.6(16)	I	6.7(23)	1.0	1.78	0.4(6.0)
II	8.8(40)	12.3	0.3	6.7(76)	II	18.2(62)	6.2	0.16	7.0(38)
III	0.3(1.4)	2.8	0.72	0.06(17)	III	2.0(7.0)	3.7	1.37	0.5(23)
IV	3.2(14)	13.8	0.15	2.7(86)	IV	2.3(8.0)	12.4	0.24	1.8(77)
Total	22.3 (100)	8.0	1.07	11.0(50)	Total	29.2 (100)	4.7	0.62	9.7(33)
Product 3	Amount	OCH$_3$	S	Lignin	Product 6	Amount	OCH$_3$	S	Lignin
I	13.6(44)	2.7	2.58	2.2(17)	I	4.4(17)	0.7	4.38	0.2(4.1)
II	13.3(43)	11.7	0.69	9.6(73)	II	19.1(72)	7.4	0.42	8.8(46)
III	0.7(2.4)	6.8	1.05	0.3(42)	III	1.1(4.0)	4.7	< 0.1	0.3(29)
IV	3.6(11)	16.9	0.18	3.7(105)	IV	2.0(7.6)	12.3	< 0.1	1.6(77)
Total	31.2 (100)	8.2	1.46	15.9(51)	Total	26.6 (100)	6.6	1.04	10.9(41)

[a] Proportion of the fraction as calculated based on the methoxyl content.

IR spectroscopy was used to characterize and analyze the constituents of Fractions I-IV of Products 1 and 2 (Fig. 3); a spectrum of MWL is included in the figure for comparison. Fractions I-III of Product 1 contained cellulose and other polysaccharides, as is demonstrated by the strong absorption bands around 1000 cm^{-1} (Fengel and Wegener 1989) (C-O stretching vibrations). As expected, these bands were considerably weaker in the spectrum of Product 2 due to enzymatic hydrolysis of the cellulose and fermentation of the hexoses (see Fig. 1). Both Fraction IV spectra exhibit pronounced similarities to the MWL spectrum. The band at 1510 cm^{-1} (aromatic skeletal vibrations) is a clear indication of the presence of lignin. Consequently, the presence of this band in the Fraction IV spectra is strong evidence that these fractions contained lignin. Furthermore, the small deviations of the Fraction IV spectra from the MWL spectrum suggest that they consisted primarily of lignin. In addition to this, Fractions I and II of Product 1 and Fractions II and III of Product 2 exhibitted a band at 1510 cm^{-1}, implying that lignin was also present in these fractions. The IR spectrum of Fraction I of Product 2 exhibited a strong absorbance at 1590 cm^{-1}, which was absent in Fraction I of Product 1. This absorption may be caused by the additives and fermentation products in the SSF step.

^{1}H NMR spectroscopy of the acetate of Fraction IV of Products 1 and 2 corroborated the lignin nature of these fractions. A strong signal at δ 2.30 attributed to aromatic acetate (Lundquist 1992) showed that extensive cleavage of arylglycerol β-aryl ether bonds had occurred during the pretreatment (*cf.* Bardet et al. (1985)). Quantitative estimates suggest that roughly 60 % of the lignin units are phenolic. It is notable that phenolic groups have an inhibitory effect on enzymatic hydrolysis of cellulose (Pan 2008) and that phenols have a negative effect on the fermentation of carbohydrates (Martín et al. 2002). Based on experiments with model compounds (Li et al. 2000) there are three possible reaction routes (A, B and C) for the cleavage of the β-ether bonds (Fig. 4), leading to the liberation of phenolic groups.
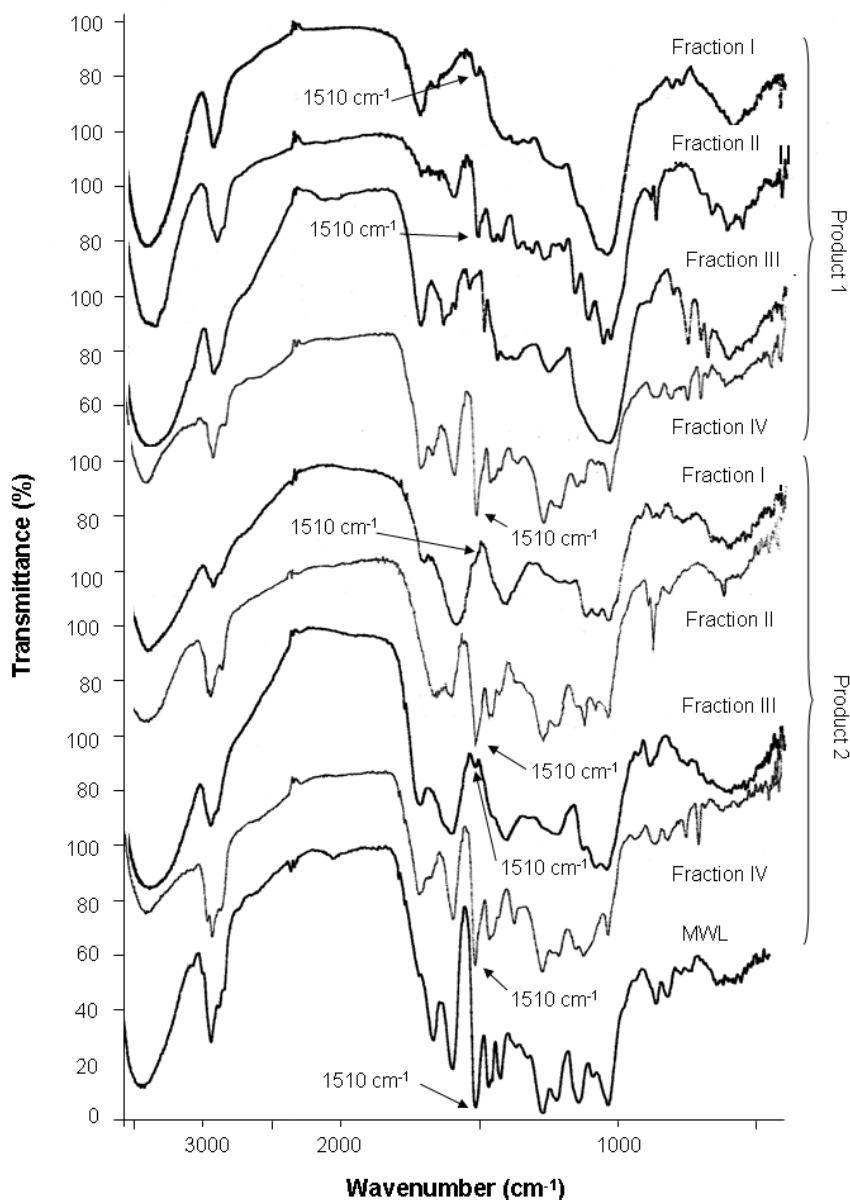

Fig. 3. Infrared spectra of milled wood lignin (MWL) and Fractions I-IV of Products 1 and 2 (cf. Fig. 1).
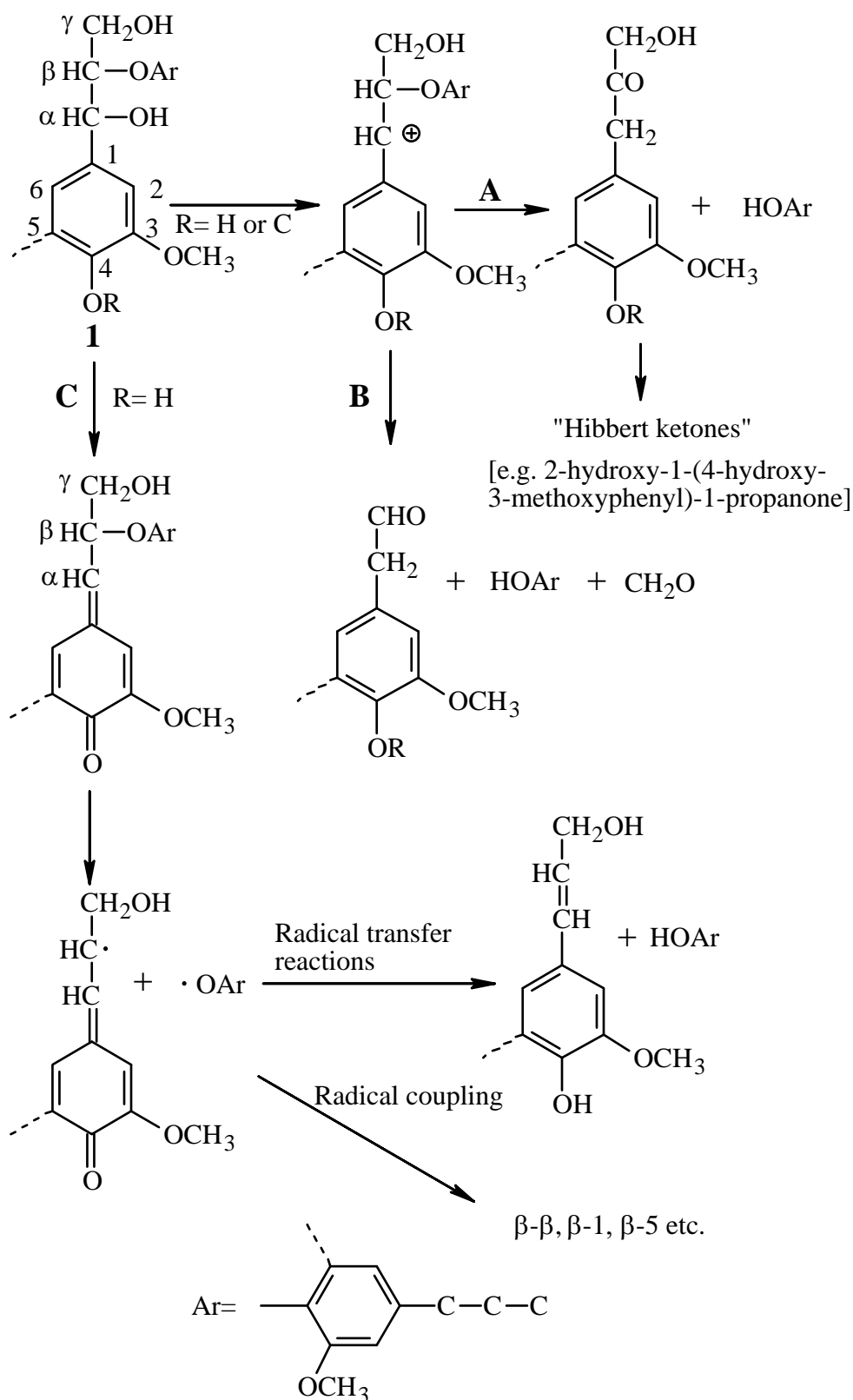
γ CH$_2$OH

β HC —OAr

α HC —OH

1

6

5

2

3 OCH$_3$

4

OR

1

R= H or C

CH$_2$OH

HC —OAr

HC ⊕

OCH$_3$

OR

A

CH$_2$OH

CO

CH$_2$

OCH$_3$

OR

+ HOAr

C | R= H

B

"Hibbert ketones"

[e.g. 2-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-1-propanone]

γ CH$_2$OH

β HC —OAr

α HC

OCH$_3$

O

CHO

CH$_2$

OCH$_3$

OR

+ HOAr + CH$_2$O

CH$_2$OH

HC·

HC

OCH$_3$

O

+ ·OAr

Radical transfer reactions

CH$_2$OH

HC

CH

OCH$_3$

OH

+ HOAr

Radical coupling

β-β, β-1, β-5 etc.

Ar=

C —C —C

OCH$_3$

Fig. 4. Reactions of arylglycerol β-aryl ethers (**1**) under acid and neutral conditions.

The water-soluble lignin in Products 1-6 accumulated in Fraction I of these products (Fig. 2 and Table 2). The amount of water-soluble lignin is of interest in the energy and mass balances of the ethanol production processes: the water is evaporated and recirculated, and the solid residue is burnt. The proportion of the total lignin content in Products 1-6 that was water-soluble appears in Fig. 5. This figure illustrates the water-solubility of the lignin in the conversion products obtained with three different pretreatments (Products 1, 5 and 6), after enzymatic hydrolysis (Product 4) and in the SSF process (Products 2 and 3). When SO_2 was used as the acid-catalyst (Products 1 and 5), 4-5 % of the lignin was soluble in water. The use of H_2SO_4 as the acid-catalyst (Product 6) resulted in considerably lower solubilization of the lignin, but this result is jeopardized by the fact that the product was washed after the first pretreatment step (Fig. 1). Washing was carried out to withdraw solubilized carbohydrates to avoid by-product formation in the second pretreatment step. Based on the methoxyl content the amount of water-soluble lignin increased from 5 % to 12-14 % with enzymic hydrolysis (Product 4) or in the SSF process (Product 2), and remained constant after distillation (Product 3). The increasing water solubility after enzymic hydrolysis and/or fermentation may be related to the high temperature (140 ºC) prevailing during sterilization in the fermenter tank. However, there is an alternative explanation for the increased methoxyl content: the enzymatic treatment may release water-soluble xylan fragments with attached 4-O-methylglucuronic acid units. The comparatively large fraction of water-soluble lignin in Products 1-5 is probably due to sulphonation reactions, which increase the hydrophilicity of lignin.
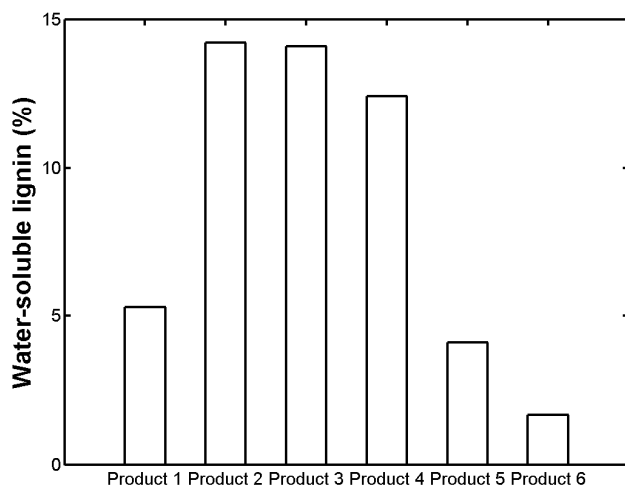

Fig. 5. Amount of lignin present in Fraction I (Table 2 of Products 1-6 (Fig. 1) compared to the total lignin content in these products. The lignin content is calculated based on the methoxyl content.

Sulphur Content

The solid lignin residue can be used as a fuel in non-industrial burners. Low sulphur content is necessary, since sulphur is detrimental when the solid residue is burnt. Sulphur in this residue can be explained by sulphonation of lignin structures. The sulphur content in Fractions IV of Products 1-6 are shown in Table 2. The sulphur analyses of

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these fractions indicate that the products pretreated with SO_2 (Products 1-5) had a higher sulphur content than the product that was pretreated with H_2SO_4 (Product 6). This can be explained by sulphonation of the lignin structures by the SO_2. The presence of bound sulphonate in the solid phase of Product 2 (Fig. 2) was confirmed using a spectrophotometric method described in Westermark and Samuelsson (1993).

To investigate if the sulphur contents could be lowered, Product 3 (Fig. 1) was washed with cold (20 ℃) and warm (80 ℃) wash water. The sulphur content of the solid phase decreased from 0.60 % to 0.40 % by the cold wash, and the warm wash decreased the sulphur content to 0.27 %.

CONCLUSIONS

1. The processing of the wood (acid catalysed steam, enzymatic hydrolysis, and fermentation) resulted in a solubilization of a substantial portion of the lignin, and arylglycerol beta-aryl ethers in the lignin were cleaved to a large extent.
2. The sulphur in the solid residue from SO_2-catalyzed steam hydrolysis, enzymatic hydrolysis, fermentation, and distillation can only be partially removed by washing with water.
3. The major constituents of the products obtained on processing of spruce wood can be separated based on water solubility and the application of liquid-liquid extraction. The isolated lignin fractions can be used to elucidate the lignin reactions occurring during the processes.

ACKNOWLEDGMENTS

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