Supporting information

Autohydrolysis pretreatment of softwood – Enhancement by phenolic additives and the effects of other compounds

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Additional information on tested compounds

Table S1 Purity and supplier of the different compounds added to pretreatment.

Compound	*Purity / %*	*Supplier*
Methanol	≥99.8	Fluka
Ethanol	≥99.8	Fluka
2-Propanol	≥99.8	Scharlau
Phenol	≥99	Fluka
Hydroquinone	≥99	Sigma-Aldrich
Catechol	≥99	Sigma-Aldrich
Resorcinol	98	Chemie Brunschwig
Phloroglucinol*	97	Aldrich
Dimethylphloroglucinol	≥95	Green Pharma
2-Naphthol	98	Sigma-Aldrich
Propylamine	≥99	Aldrich
Butylamine	99.5	Sigma-Aldrich
Diethylamine	≥99.5	Sigma-Aldrich
Ethylendiamine	≥99.5	Fluka
Diethylhydroxylamine	≥98	Aldrich
Aniline	≥99.5	Sigma-Aldrich
m-Phenylendiamine	≥99	Aldrich
Furan	≥99	Aldrich
2-Methylfuran	99	Aldrich
Furfural	99	Sigma-Aldrich
Benzofuran	99	Aldrich
Pyrrole	98	Aldrich
2,4-Dimethylpyrrole	97	Aldrich
Indole	99	Aldrich
2-Methylindole	98	Aldrich
Thiophene	99	Aldrich
2-Methylthiophene	98	Aldrich
L-Methionine	≥98	Sigma-Aldrich
Pentamethylbenzene	97	Sigma-Aldrich
Naphthalene	99	Chemie Brunschwig
Anthracene	98	Sigma-Aldrich
Pyrene	≥99	Sigma-Aldrich
Thiophenol	97	Aldrich
Anthraquinone	97	Aldrich
Butylhydroxytoluol	≥99	Aldrich

*Dihydrate

Comparing the effect of 2-naphthol for the pretreatment of spruce and pine wood. A different biomass was used for this study. Spruce and pine wood were cut in spring 2012 in Villigen (canton of Aargau, Switzerland), debarked, split with an axe, chipped and knife milled (SM200 cutting mill; Retsch) through a 1 mm screen size and further prepared as described in the experimental section. Spruce composition: glucan 44.49 ± 0.51%, mannan 18.33 ± 0.12%, acid soluble lignin (ASL) 5.00 ± 0.08%, acid insoluble lignin (AIL) 26.79 ± 0.17%, extractives 5.13% and ash 0.28% ± 0.01 (total 100.02%); dry matter 93.35 ± 0.18%. Pine composition: glucan 40.18 ± 0.20%, mannan 19.77 ± 0.19%, acid soluble lignin (ASL) 4.90 ± 0.02%, acid insoluble lignin (AIL) 28.46 ± 0.38%, extractives 4.59% and ash 0.08% ± 0.03 (total 97.98%); dry matter 92.79 ± 0.79%. The pretreatment was carried out as described in the experimental section with varying pretreatment times (0/10/20/60/120/240 min) corresponding to severities between $\log R_0$=4.5-5.4. 118.5 mg and 123.6 mg of 2-naphthol were added to the spruce and the pine pretreatment, respectively, corresponding to an equal concentration of 0.205 mol/mol lignin C_9 unit.

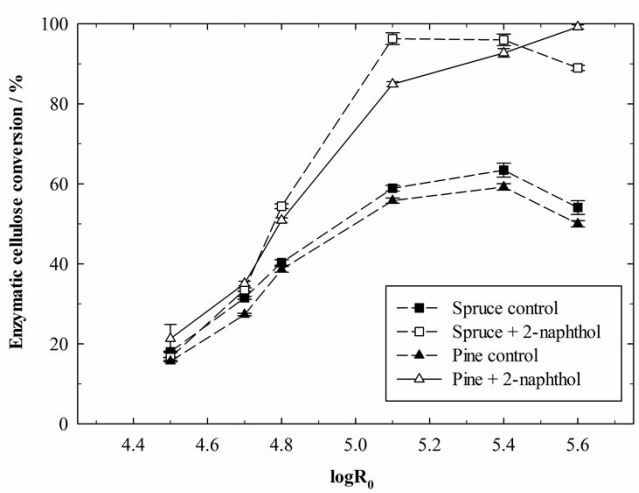

Fig. S1 Cellulose conversion/glucose yield in the enzymatic hydrolysis of spruce and pine wood after autohydrolysis pretreatments at different severities with 2-naphthol and without additive (control). Pretreatment conditions: 2.5 g spruce, 39.2 g H_2O, 0.205 mol additive/mol lignin C_9 unit. Hydrolysis conditions: 1%w/w cellulose, 60 FPU g^{-1} cellulose.

Experimental details: Alcohol additives

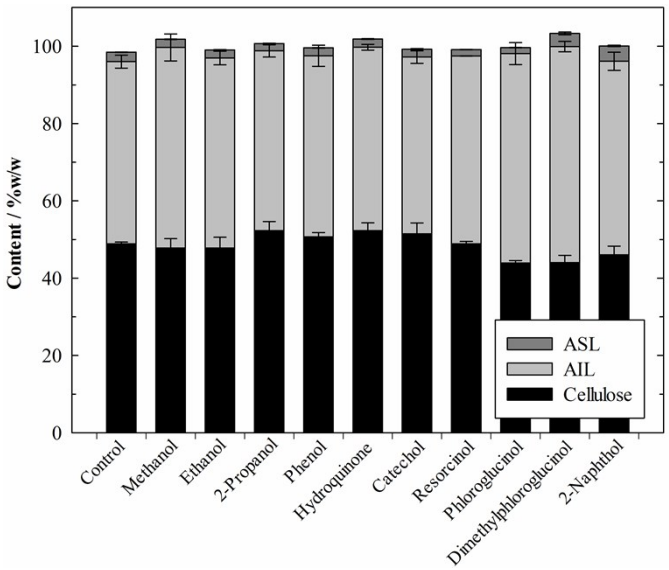

Fig. S2 Composition of spruce wood pretreated without (control) and with alcohol additives.

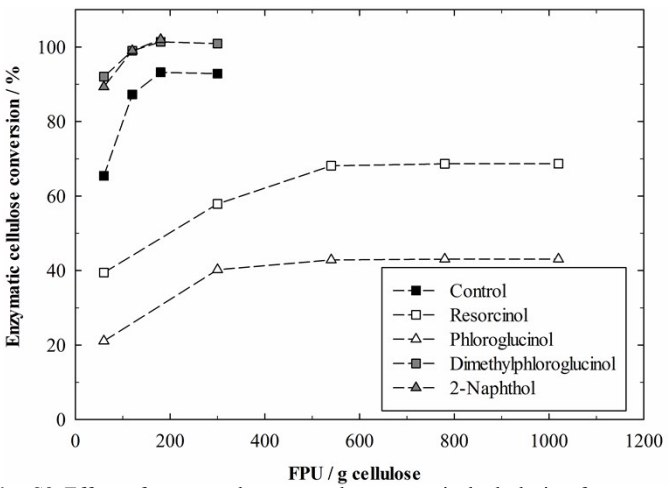

Fig. S3 Effect of enzyme dosage on the enzymatic hydrolysis of spruce pretreated with resorcinol, phloroglucinol, dimethylphloroglucinol, 2-naphthol and without additive (control). Hydrolysis conditions: 1%w/w cellulose; the enzyme dose was increased successively, whereupon at least 24 h were waited before addition of the follow-up dose.

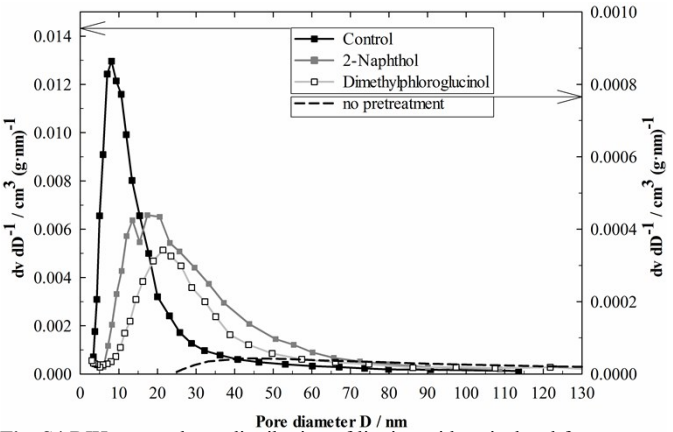

Fig. S4 BJH pore volume distribution of lignin residues isolated from spruce pretreated with resorcinol, 2-naphthol, dimethylphloroglucinol and without additive (control). The pore volume distribution of lignin isolated from non-pretreated spruce is shown as well (secondary axis of ordinate). Lignins were isolated by enzymatic hydrolysis with an excessive enzyme dosage of at least 180 FPU g^{-1} cellulose (compare Fig. S4). Details of the experimental procedure are provided elsewhere[1].

Sample	*BET specific surface area / m^2 g^{-1}*
Non-pretreated	3.7 ± 0.39
Control	38.2 ± 1.4
Dimethylphloroglucinol	22.0 ± 1.4
2-Naphthol	30.0 ± 0.4

Table S2 M_w and PDI of lignins as determined by SEC. The extraction yields of the lignins in the $NaOH_{aq}$ solvent are shown as well. Shown are the samples of spruce pretreated without additive (control) and spruce pretreated with phloroglucinol, dimethylphloroglucinol and 2-naphthol.

Sample	*Extraction yield / %*	*M_w / g mol^{-1}*	*PDI*
Control	91	55k	16
Phloroglucinol	87	652k	251
Dimethylphloroglucinol	95	9k	5
2-Naphthol	93	6k	5

Table S3 BET specific surface area of lignins isolated from non-pretreated spruce, from spruce pretreated without additive and from spruce pretreated with dimethylphloroglucinol and 2-naphthol. Lignins were isolated by enzymatic hydrolysis with an excessive enzyme dosage of at least 180 FPU g^{-1} cellulose (compare Fig. S4). Details of the experimental procedure are provided elsewhere[1].

Experimental details: Amine additives

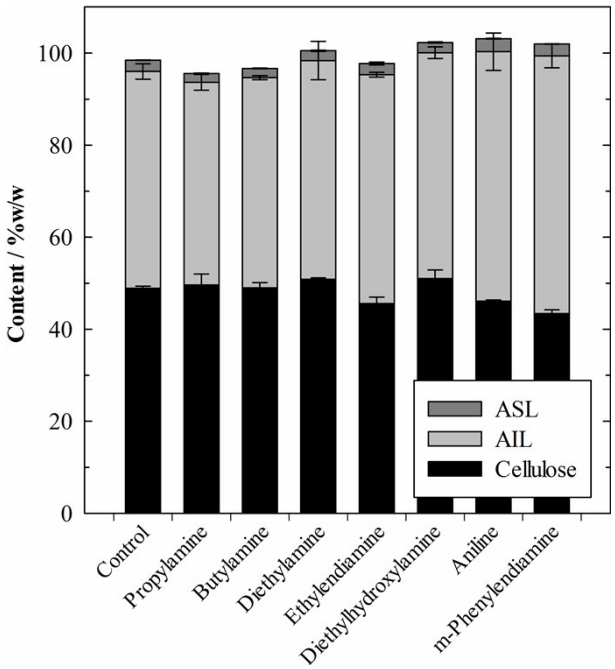

Fig. S5 Composition of spruce wood pretreated without (control) and with amine additives.

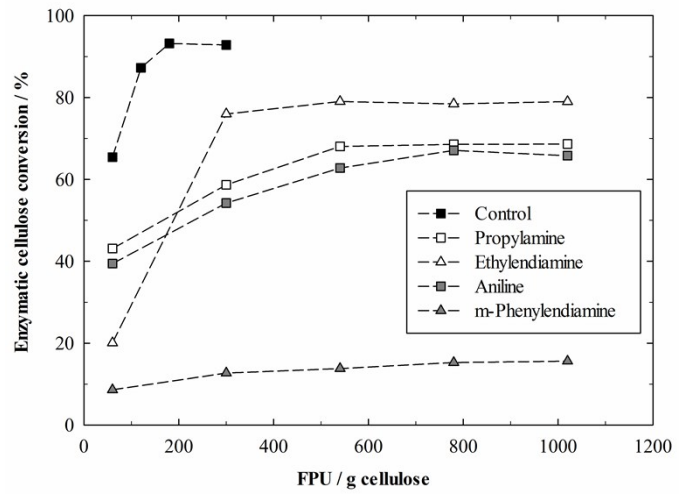

Fig. S6 Effect of enzyme dosage on the enzymatic hydrolysis of spruce pretreated with propylamine, ethylendiamine, m-phenylendiamine, aniline and without additive (control). Hydrolysis conditions: 1%w/w cellulose; the enzyme dose was increased successively, whereupon at least 24 h were waited before addition of the follow-up dose.

Table S4 pK_a values of the amines' corresponding ammonium ions.

Amine	*pK_a of R_3NH^+*
Propylamine	10.54
Butylamine	10.60
Diethylamine	10.84
Ethylendiamine	(1) 9.92, (2) 6.86
Diethylhydroxylamine	5.73
Aniline	4.87
m-Phenylendiamine	(1) 5.11, (2) 2.50

Table S5 M_w and PDI of lignins as determined by SEC. The extraction yields of the lignins in the $NaOH_{aq}$ solvent are shown as well. Shown are the samples of spruce pretreated without additive (control) and spruce pretreated with ethylendiamine and m-phenylendiamine,

Sample	*Extraction yield / %*	*M_w / g mol^{-1}*	*PDI*
Control	91	55k	16
Ethylendiamine	98	302k	81
m-Phenylendiamine	93	320k	143

Heterocyclic additives. Several furans, pyrroles and thiophenes - five-membered-ring aromatic compounds with oxygen, nitrogen respectively sulfur as heteroatoms - were evaluated as additives (Fig. S8). All three compound types are much more reactive than benzene towards electrophilic aromatic substitution due to a higher electron density over each carbon atom, with the reactivity being pyrrole > furan > thiophene > benzene.[2] Pyrrole is particularly active; its reaction rate towards a nucleophilic attack and substitution on an aromatic ring has been reported to be $5.3x10^7$ times higher than thiophene and $3.8x10^5$ times higher than furan.[3] Heterocyclic compounds undergo substitution preferably on position 2 adjacent to the heteroatom.[2] Because unsubstituted rings have two such positions available, it is possible that they could promote the bridging of lignin molecules. Therefore, five-membered heterocycles with substituents on position 2 (e.g. 2-methylfuran) were also included in the screening. It is relevant to note that, unlike amines, the nitrogen atom of pyrroles is non-basic, because the unbound electron pair is part of the aromatic π-electron system. However, pyrrole is unstable in strongly acidic solutions because it polymerises readily.[2] Indole and benzofuran are aromatic compounds that contain a benzene ring fused with a pyrrole or a furan ring, respectively. In general, reactions on such compounds occur at free positions on the heterocyclic ring in preference to the benzene ring, with substitution occuring preferably on position 2 in benzofuran and on position 3 in indole.[4] Indole shows a much larger difference in reactivity between positions than pyrrole, but both indole and benzofuran are in general less reactive than their monocyclic counterparts.[4]

It can be observed that, with the exception of 2,4-dimethylpyrrole, the final pH of the pretreatment was practically not changed compared to the control, consistent with the non-basic nature of the substances (Table 1). The composition of the pretreated biomass is shown in Fig. S9. Hemicellulose was completely removed and samples show similar amounts of cellulose and lignin.

The glucose yields obtained in the enzymatic hydrolysis are shown in Fig. S10. All compounds derived from furan and thiophene had a negligible effect on glucose yields. As in the case of some compounds discussed earlier (e.g. phenol), it is likely these substances are not reactive enough to compete with the aromatic rings in lignin and play a role in lignin depolymerisation/repolymerisation reactions. It is interesting to note that pyrroles/indoles did show a large effect on glucose yields. This is consistent with the high differences in reactivity of this type of compounds as described earlier. The fact that the glucose yields were worse than the control indicates that, as in previous cases, crossing of lignin fragments might have taken place due to multiple substitutions on the compound. All of these compounds have more than one available position for substitution. For example, as mentioned before, the most reactive positions of the pyrrole ring are those adjacent to the nitrogen atom. Pyrrole has two such positions (out of a total of four free positions), while 2,4-dimethylpyrrole only one (out of a total of two). It would be expected for 2,4-dimethylpyrrole to become substituted on this free activated position first, but given the fact that the molecule as a whole is more reactive than

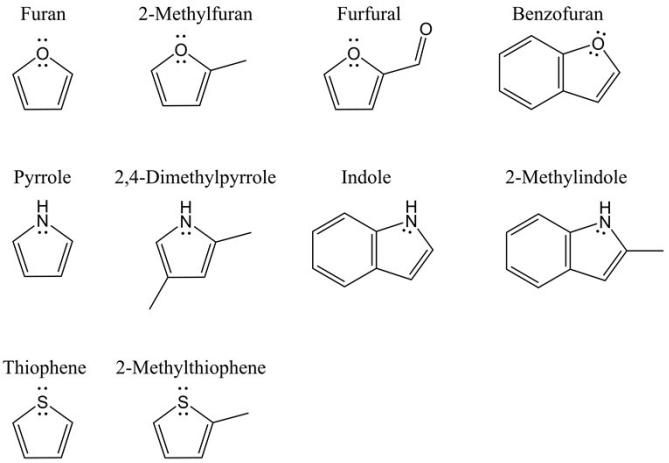

Fig.S7 Structures of heterocyclic additives (furans, pyrroles, thiophenes).

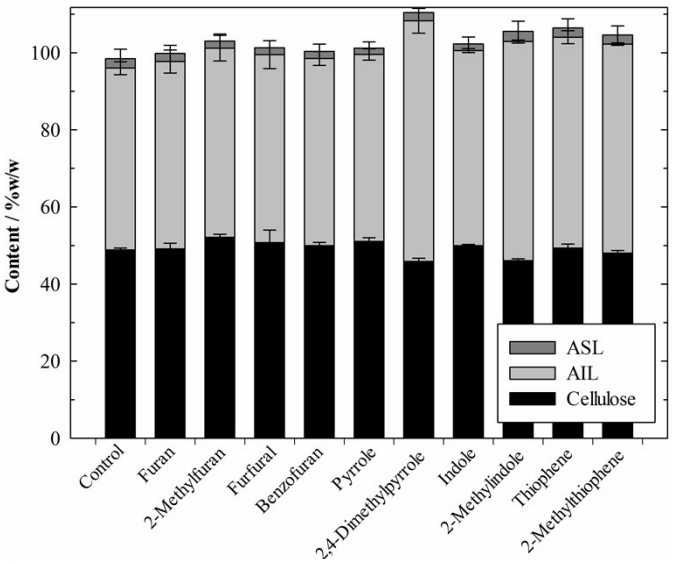

Fig. S8 Composition of spruce wood pretreated without (control) and with heterocyclic additives.

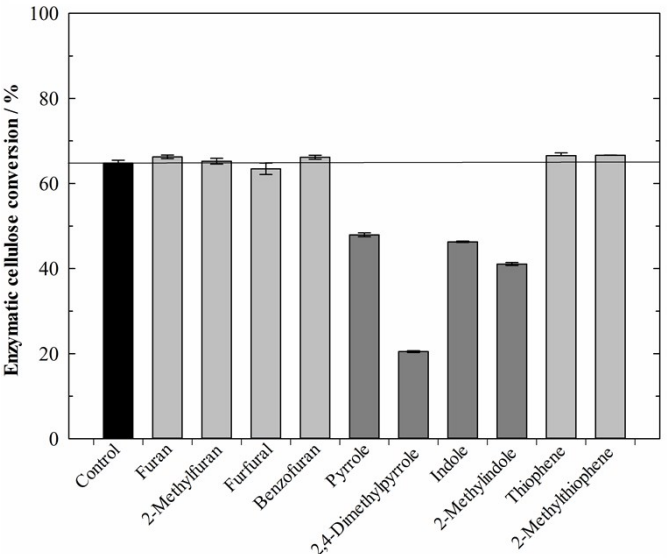

Fig. S9 Cellulose conversion/glucose yield in the enzymatic hydrolysis of spruce after autohydrolysis pretreatments without additive (control) and with heterocyclic additives (furans, pyrroles, thiophenes). Pretreatment conditions: 2.5 g spruce, 39.2 g H_2O, 0.205 mol additive/mol lignin C_9 unit. Hydrolysis conditions: 1%w/w cellulose, 60 FPU g^{-1} cellulose.

plain pyrrole (due to the electron-donating nature of the methyl substituents), it is possible it could have become substituted even on the position not "directed" by the heteroatom. A similar effect could have occurred in the case of indole/2-methylindole. The compound with the largest impact on hydrolysis (2,4-dimethylpyrrole) also showed the highest increase in AIL content (Fig. S9), indicating a considerable amount of the additive was integrated into the lignin structure.

Similarly as shown for the other additives, heterocyclic compounds that decreased sugar yields reduced the amount of accessible cellulose as well, shown exemplarily for pyrrole and 2,4-dimethylpyrrole (Fig. S11).

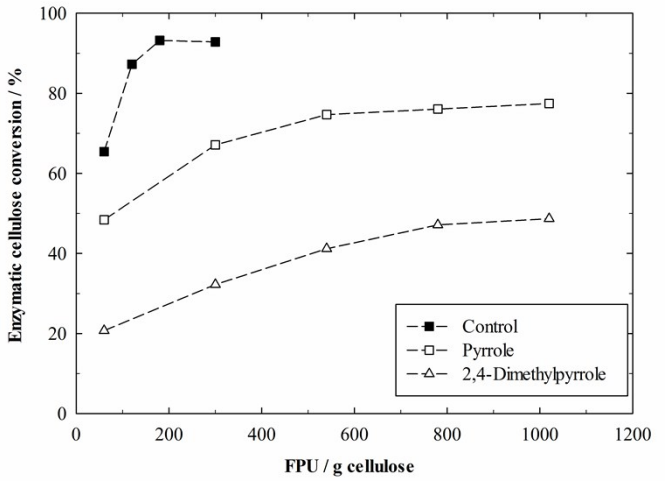

Fig. S10 Effect of enzyme dosage on the enzymatic hydrolysis of spruce pretreated with pyrrole, 2,4-dimethylpyrrole and without additive (control). Hydrolysis conditions: 1%w/w cellulose; the enzyme dose was increased successively, whereupon at least 24 h were waited before addition of the follow-up dose.

Other additives. The compounds in this category (one aliphatic and seven aromatic) do not belong to any of the previously described chemical groups. Their structures are shown in Fig. S12 and the corresponding glucose yield of the enzymatic hydrolysis in Fig. S13.

Methionine is an aminoacid containing a thioether group, the sulfur atom is nucleophilic. In fact, methionine is one of the aminoacids most likely to be attacked by carbocations in solid peptide synthesis.[5] The acid-base behavior of aminoacids in solution is not straightforward, but it appears the amine group of methionine had some effect in raising the final pH of the pretreatment (Table 1). Methionine had a large negative effect on enzymatic hydrolysis (Fig. S13). As a primary amine, many of the factors previously discussed for this type of compounds come into play for explaining this result. As the decrease in the yield was actually more pronounced than that caused by the other primary aliphatic amines propylamine and butylamine (compare Fig. 9), this might reflect a higher reactivity of methionine compared to these two substances.

Pentamethylbenzene is an aromatic compound with only a single ring position available for electrophilic substitution, the other positions being occupied by methyl groups. In that way it might act as a blocking agent. The free ring position is also activated by the combined inductive effect of the five methyl groups. Pentamethylbenzene improved the glucose yield in hydrolysis by 5.3% (Fig. S13), its potential as a blocking agent likely accounting for the positive effect. However, the ring activation by the methyl groups seems to be too weak to make it a very effective additive, since their activating effect is much lower than e.g of hydroxy groups.[6]

Three polycyclic aromatic hydrocarbons (PAHs) were included in the screening as well. The reactivity of PAHs is similar to the reactivity of benzene in electrophilic aromatic substitutions.[6] Their higher electron density might however activate them further for a reaction with lignin carbocations. Naphthalene had no effect at all on digestibility, while anthracene and pyrene increased yields by 15.7% respectively 6.4% (Fig. S13). Next to that, the lignin contents of the biomass pretreated with pyrene and anthracene was increased compared to naphthalene (Fig. S14), further indicating naphthalene was not reactive enough so as to be considerably integrated into the lignin structure. Possibly the higher electron density or a higher ability to stabilise the positive charge in the transition state make anthracene and pyrene more reactive. It remains unclear why anthracene had a more positive effect than pyrene, though one has to bear in mind that lignin crossing reactions can also play a role.

Thiophenol is the sulfur analogue of phenol and is also activated towards electrophilic aromatic substitution. Thiophenol has been used as carbocation scavenger to suppress unwanted reactions in solid-phase peptide synthesis.[5, 7] Thiophenol had a positive effect on the glucose yield, enhancing it by 7.6%. Thiophenol is more reactive than phenol but not as much as aniline[8], so it possible that it strikes the necessary "middle ground" to act as a blocking agent but not as a bridging agent. Nonetheless, the small improvement in yield along with the fact that thiophenol is highly toxic means that in practice it would not be a suitable carbocation scavenger.

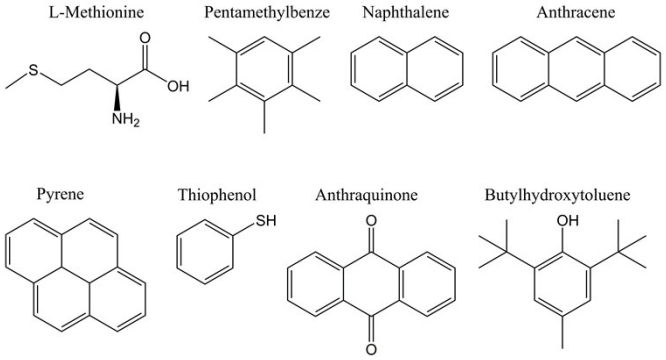

Fig. S11 Structure of methionine and several aromatic additives tested in pretreatment.

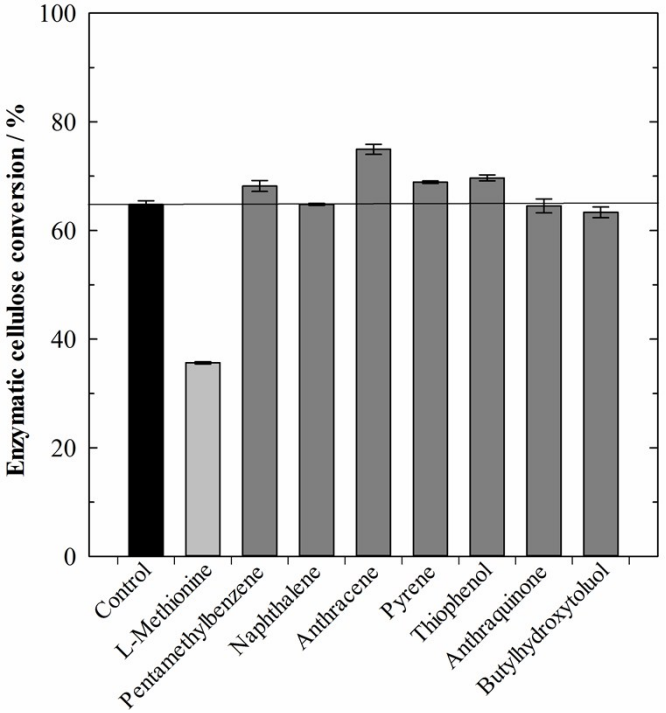

Fig. S12 Cellulose conversion/glucose yield in the enzymatic hydrolysis of spruce after autohydrolysis pretreatments without additive (control), with methionine and with several aromatic additives. Pretreatment conditions: 2.5 g spruce, 39.2 g H_2O, 0.205 mol additive/mol lignin C_9 unit. Hydrolysis conditions: 1%w/w cellulose, 60 FPU g^{-1} cellulose.

Anthraquinone is an additive used in alkaline pulping processes, where it acts as a redox catalyst. Its reduced counterpart (anthrahydroquinone) reacts with lignin causing the breaking of ether bonds and thus promoting delignication.[9] It was tested to determine whether a similar positive effect could be observed under acidic conditions. The fact that anthraquinone had no effect indicates that, in contrast to its effect during alkaline pulping, it may not play a role in the breaking of lignin molecules under acidic conditions. Its structure does not particularly activate it towards electrophilic substitution neither, which is why no effect is observed at all.

Butylhydroxytoluol (BHT) is an effective and widely used scavenger for radicals.[10, 11] Other molecules can transfer their

radical on its phenolic oxygen atom to give a delocalised and stabilised phenoxy radical.[10] Due to the additional steric hindrance by the adjacent tert-butyl groups (compare Fig. S12) the BHT radical is practically unreactive[10], in that way stopping further radical polymerisation. Lignin can repolymerise via the formation of radicals as well, next to the ionic repolymerisation via carbocations.[12] BHT seems unlikely to also act as a carbocation scavenger and was therefore used as an additive to specificly test the blocking of the radical repolymerisation pathway. The use of BHT however had no effect on the yield in hydrolysis (Fig. S13), suggesting that repolymerisation was dominated by the ionic pathway involving carbocations.

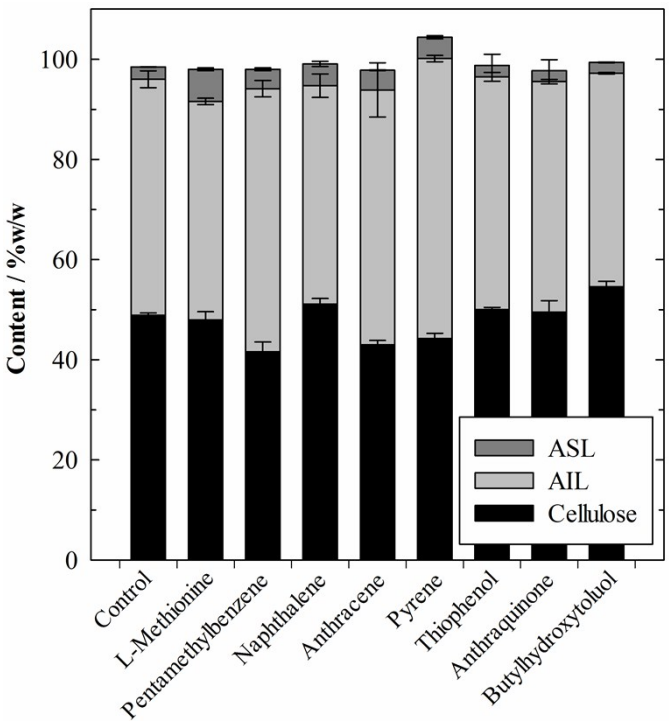

Fig. S13 Composition of spruce wood pretreated without (control) and with other additives.

References

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